

**CONSTITUTIVE AND INDUCED RESISTANCE TO INSECT  
PESTS IN GROUNDNUT AND THEIR POTENTIAL FOR PEST  
MANAGEMENT**

**THESIS**

*Submitted to the*

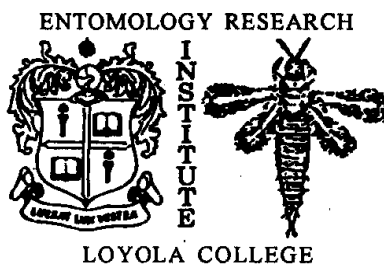
**UNIVERSITY OF MADRAS**

*in partial fulfillment for the award of the degree of*

***DOCTOR OF PHILOSOPHY***

*By*

**Abdul Rashid War**



**Entomology Research Institute,  
Loyola College, Chennai- 600 034  
India**

**April 2012**

*Dedicated to  
My Family,  
My Teachers and  
Little Tehniyat*

## **DECLARATION**

I hereby declare that the thesis entitled, “**Constitutive and induced resistance to insect pests in groundnut and their potential for pest management**”, submitted for the award of the Ph. D. to the **University of Madras, Chennai**, has not been submitted by me to any other University for a degree or diploma. The material obtained from other sources has been duly acknowledged in this thesis.

**Date: 20-4-2012**

**Abdul Rashid War**

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## LIST OF ABBREVIATIONS

µg	Microgram
µl	Microlitre
µM	Micromolar
12-OPDA -	12-oxophytodienoic acid
AD	Approximate digestibility
APX	Ascorbate peroxidase
ASAL	<i>Allium sativum</i> leaf agglutinin
ASC-GSH	Ascorbate- glutathione
BA <sub>p</sub> NA	<i>N</i> - α- Benzoyl-DL-arginine 4-nitroanilide
BBI	Bowman-Birk protease inhibitor
BPH	Brown planthopper
BSA	Bovine serum albumin
BXs	Benzoxazinoids
CaCl <sub>2</sub>	Calcium chloride
Capx	Cytosol ascorbate peroxidase
CAT	Catalase
CDPK	Calcium-dependent protein kinases
CI	Consumption index
CO11	CORONATIN-INSENSITIVE 1
ConA	Concavalin A
DAT	Days after treatment
DGR	Directorate of groundnut research
DIMBOA	2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one
DTT	Dithiothreitol
ECD	Efficiency of conversion of digested food
ECI	Efficiency of conversion of ingested food
EDTA	Ethylene diamine tetra acetic acid
EFN	Extra floral nectar
EST	Esterase
FACs	Fatty acid-amino acid conjugates
GLL	Groundnut leaf lectin
GNA	<i>Galanthus nivalis</i> agglutinin
GST	Glutathione-S-transferase
h	Hour
HIN	<i>Helicoverpa armigera</i> infestation
HIPVs	Herbivore induced plant volatiles
IPM	Integrated pest management
IU	Unit activity of enzyme
JA	Jasmonic acid
JAZ	Jasmonate ZIM-domain
kDa	Kilo Dalton
KPIs	Kunitz type proteinase inhibitors
LD	lethal dose
LOX	Lipoxygenase
LSD	Least significant difference
MAPKs	Mitogen activated protein kinases
Mapx	Microbody APX
MDA	Malondialdehyde
MeBA	Methyl benzoate

MeJA	Methyl jasmonate
MeSA	Methyl salicylate
mg	Milligram
min	Minute
mL	Millilitre
mM	Millimolar
OD	Optical Density
OPR3	OPDA reductase 3
OS	Oral secretions
PAGE	Polyacrylamide gel electrophoresis
PAL	Phenylalanine ammonia lyase
PIN2	Proteinases inhibitor II
PIs	Proteinase inhibitors
PJA	Pretreatment with jasmonic acid
POD	Peroxidases
ppm	Parts per million
PPO	Polyphenol oxidase,
PSA	Pretreatment with salicylic acid
PUFA	Polyunsaturated fatty acids
PVP	Polyvinyl pyrrolidone
ROS	Reactive oxygen species
rpm	Revolution per minute
SA	Salicylic acid
SCFCOII	Skip/Cullin/F-box-COII
SD	Standard deviation
SIN	<i>Spodoptera litura</i> infestation
SOD	Superoxide dismutase
SPIs	Serine proteinase inhibitors
VOCs	Volatile organic compounds



# *Chapter 1*

## *Introduction*

## INTRODUCTION

Plants face innumerable challenges from biotic and abiotic stresses such as insect attack, pathogen infection, temperature fluctuations and drought. The greatest threat the plants face is from insect herbivores as they take away a heavy toll of crop yields. The total pest associated losses in agricultural production has been estimated as US\$243.4 billion worldwide annually (Oerke 2006). In addition, the annual pesticide application costs about US\$10 billion. Among these, insects alone cause losses of about US\$90.4 billion. Although synthetic insecticides provide an effective control of many pests, their indiscriminate and injudicious use has led to many adverse effects such as harmful effect on non-target organisms, pesticide residues in food, development of pesticide resistance in insects, pest-resurgence, health hazards in human beings and environmental pollution (Sharma and Adlakha 1981; Isman 2006; Sharma 2009). *Helicoverpa armigera* (Hubner) and *Spodoptera litura* (Fab.) are the important polyphagous pests in many countries including India. *H. armigera* is a serious pest of cotton, vegetables, legumes and cereals; it has developed resistance to many insecticides (Kranthi et al. 2002; Wu et al. 2004). Similarly, *S. litura* is a serious pest of groundnut, cotton, cauliflower and tobacco; it has developed resistance to many insecticides (Kranthi et al. 2002; Prasad and Gowda 2006; Ahmad et al. 2007).

Due to the unwanted effects of synthetic chemical pesticides, there is an urgent need to develop alternative crop protection technologies that will minimize the use of dreadful pesticides for sustainable crop production. At present greater efforts are being made to develop crop cultivars with enhanced resistance to insect pests (Sharma et al. 2003; Smith 2005; Sharma 2009). Host plant resistance is one of the most economic and environment friendly methods of controlling plant damage by herbivores, of which induced resistance forms a key component. Improving host plant defense to insects will result in reduced losses

due to the herbivores, less insecticide use, better crops yields, and a safer environment to live (Sharma 2009).

### **1.1. Insect pest problems in groundnut (*Arachis hypogaea*)**

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop cultivated in tropical and subtropical regions. It is valued for high quality edible oil and easily digestible protein in its seeds. In India, groundnut is one of the major oil seed crops with an area of 6.21 million hectare, production of 6.74 million tons and an average yield of 1081 kg ha<sup>-1</sup> (DGR 2011). A large number of insect pests damage different stages and parts of groundnut crop. The major pests of groundnut include thrips [*Frankliniella occidentalis* (Pergande) and *Thrips palmi* Karny]; aphids [*Aphis craccivora* Koch]; white grubs [*Holotrichia consanguinea* (Blanch.)]; leaf miner [*Aproaerema modicella* (Dev.)]; leafhoppers [*Empoasca dolichi* Paoli]; armyworm, *S. litura* and cotton bollworm, *H. armigera* (Sharma et al. 2005).

The Asian armyworm, *S. litura* is an economically important polyphagous pest of many agricultural crops including groundnut, and is widely distributed in Asia, North Africa, Japan, Australia, and New Zealand. It feeds on more than 150 plant species (Wightman and Ranga Rao 1997; Sharma et al. 2003, 2005; Prasad and Gowda 2006). It has become a serious pest of groundnut, because of groundnut cultivation in the postrainy season in rice fallows (Sharma et al. 2003).

Cotton bollworm/legume pod borer, *H. armigera* is a polyphagous pest, and is widely distributed in Asia, Africa, southern Europe, and Australia (Sharma et al. 2003; Sharma 2005). It is a major pest of cereals, grain legumes, cotton, vegetable, and fruit crops, including groundnut (Manjunath et al. 1989; Sharma 2005; Sharma et al. 2005). In semi-

arid tropics, *H. armigera* causes an estimated loss of over US\$2 billion annually, despite US \$500 million worth of pesticides applied for controlling this pest (Sharma 2005).

Cowpea aphid or groundnut aphid, *A. craccivora* is a polyphagous pest, and feeds on a number of crops in America, Europe, Africa, Australia, and Asia (Wightman and Ranga Rao 1994; Minja et al. 1999; Palumbo and Tickes 2001). It causes severe damage to groundnut by sucking plant sap and by acting as a vector of at least seven viral diseases including groundnut rosette virus in Africa and peanut stripe in Asia (Padgham et al. 1990; Reddy 1991; Grayer et al. 1992).

## **1.2. Perception of herbivore damage by the plants**

Plants perceive herbivore attack through chemical cues in insect oral secretions (OS) and compounds in the oviposition fluids (Halitschke et al. 2001; Spiteller and Boland 2003; Zavala et al. 2004; Wu and Baldwin 2010). The defenses generated by various elicitors differ based on the type of the elicitor and the biological processes involved (Kessler and Baldwin 2002; Howe and Jander 2008; Bruinsma et al. 2009; Wu and Baldwin 2010). Fatty acid-amino acid conjugates (FACs) are the major components and the best studied elicitors in the oral secretions of insects. The first FAC elicitor identified was volicitin, N-(17-hydroxylinolenoyl)-L-glutamine, detected in the OS of beet armyworm larvae, *Spodoptera exigua* (Hub.) (Alborn et al. 1997). Since then, many elicitors have been identified from various lepidopteran insects (Pohnert et al. 1999; Halitschke et al. 2001; Spiteller and Boland 2003). Plants also perceive oviposition fluids and mount defense against oviposition through different mechanisms (Blaakmeer et al. 1994; Seino et al. 1996; Doss et al. 2000; Petzold-Maxwell et al. 2011).

### **1.3. Host plant defense against insects**

The evolutionary arms race between plants and insects has resulted in development of an elegant defense system in plants that has the ability to recognize the non-self molecules or signals from damaged cells, much like the animals, and activates the plant immune response against the herbivores (Verhage et al. 2010; Hare 2011). About 4-6 million insect species are present in the world, of which 50% are herbivorous (Novotny et al. 2002). Plants have developed various strategies to counter attack/defend against insect pests. These strategies occur in plants either constitutively or are induced in response to the insect attack. Induced plant resistance is an important defensive mechanism as it makes the plants phenotypically more plastic and thereby unpredictable to the insects. Induced resistance to insects can be deployed as one of the components for reducing the losses due to insect pests. Host plant resistance not only helps in reducing the insect damage, but also reduces the crop protection costs by the farmers. It is an important component of pest management, particularly in cereals, grain legumes, and oilseed crops grown in the semi-arid tropics.

Overall, plant defense against insect herbivores can be classified into three categories; tolerance, antibiosis and antixenosis. Tolerance is the resistance in which the plant can withstand or recover from insect damage without adversely affecting the growth or survival of the attacking herbivore. In antibiosis mechanism of resistance, a wide variety of defensive compounds (allelochemicals) are produced by the plants against herbivory, which are toxic to insects and reduce growth, inhibit reproduction, alter physiology, and cause several physical or behavioral abnormalities in herbivores. In antixenosis, there is a non-preference reaction of herbivore to a resistant plant, when biophysical or allelochemical factors adversely affect herbivore behavior, and lead to the delayed acceptance and possible outright rejection of host plant for feeding and/or oviposition.

### 1.3.1. Induced resistance in plants against insect pests

Insect-plant interaction is possibly one of the most important multidisciplinary endeavors in plant biology. Plants are capable of perceiving the presence of herbivore non-self, deciphering the signal perceived, and of mounting defense responses even at the cellular level (Fig. 1.1). They perceive the insect damage and translate this “perception” into a precise, reliable and successful defensive response (Dangl and Jones 2001; Howe and Jander 2008). Plant defenses against insect pests have been known to change from time to time. These defenses were generally believed to be expressed constitutively. However, after Green and Ryan reported for the first time in 1972 that Colorado potato beetle, *Leptinotarsa decemlineata* (Say) wounding generated the rapid accumulation of proteinase inhibitors (PIs) in potato and tomato leaves, this impression was changed, and a new area of “induced resistance” came into existence. These PIs were proposed to defend plants against insect pests, because of their ability to interfere with protein digestion. Now it is widely accepted that plant defense against insects could be constitutive or induced.

Constitutive defense is always present in the plant irrespective of any external stimuli and forms the first barrier to herbivorous insects, whereas induced resistance is stimulated in response to the herbivore attack, pathogen infestation, mechanical wounding and/or elicitor application and defends plants from further damage (Kessler and Baldwin 2002; Arimura et al. 2008; Howe and Jander 2008; Sethi et al. 2009; Wu and Baldwin 2010). Even if an insect manages to overcome the constitutive defense present in a plant, it may be subjected to the induced defense. Although constitutive resistance in plants has its own role to protect plants in the absence of the induced resistance, it cannot go long, because of the metabolic cost involved for setting up the constitutive resistance. Since constitutive defenses are always present in the plant and are maintained even in undamaged plants, they are thought to attain more cost as compared to induced resistance (Karban and

Baldwin 1997; Agrawal 1999, 2000; Agrawal et al. 2002). Even though induced resistance would confer some metabolic reallocation, they are thought to be less costly as they are evoked only in presence of the attack and not in absence of it. Thus, induced resistance allows plants to respond to the invader dynamically and makes them unpredictable to it. The main limitation of induced resistance is that it becomes effective only when the plant has already been attacked and there is possibility of delay in setting up the induced resistance. Moreover, the induced resistance cannot be much effective, when the initial damage is too severe and rapid. However, induced resistance is still considered as more effective and flexible as compared to the constitutive resistance as it can save the reallocation cost and optimize them in growth, reproduction and resistance, since it is established only when demanded. They play a potent role in plant defense when aimed at the stress of immediate concern (Miranda et al. 2007; Karban 2011). Moreover, induced resistance depends on wound-detection pathways, defense precursors, and storage vesicles, which require energy and resources allocation away from growth and reproduction (Purrington 2000).

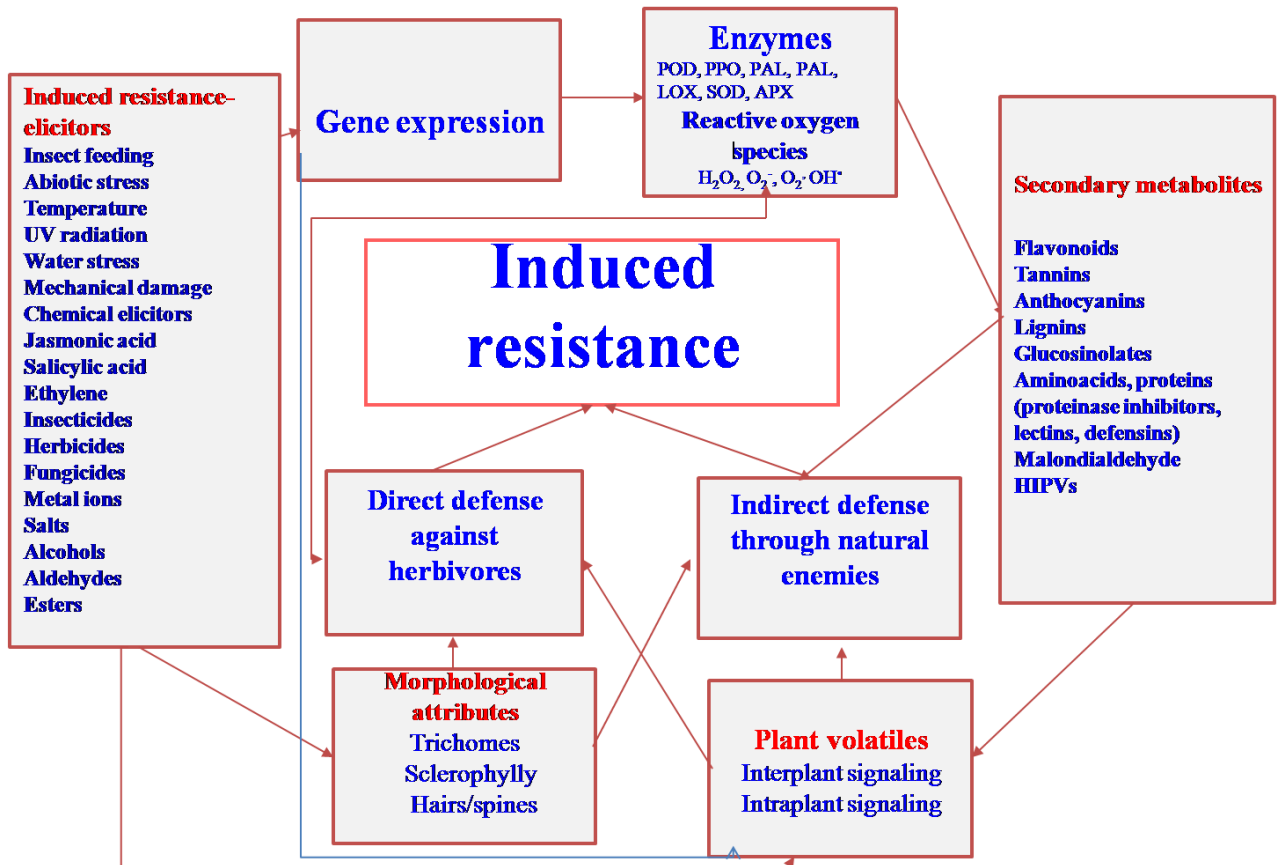
Research on induced resistance has gained high momentum worldwide due to its wide ranging nature as a cascade of biochemical changes occur in plants in response to insect herbivory. For example, in wheat against *Sitobion avenae* (Zhao et al. 2009; Han et al. 2009), rice against many insect pests (Usha Rani and Jyothsna 2010), cucumber against *Bemisia tabaci* (Gen.) (Zhang et al. 2008), chrysanthemum against aphids (He et al. 2011) etc. Induced resistance is of higher energy utilization efficiency and more economic (Zhao et al. 2009). Induced resistance is manifested by the dynamic change in transcriptomics, proteomics and metabolomics of the host plant in response to herbivory (Kessler and Baldwin 2002; Zhu-Hanley et al. 2007; Howe and Jander 2008; Usha Rani and Jyothsna 2010; Karban 2011). Induced defense can be either direct or indirect. Direct resistance aims at the accumulation of substantial amounts of defense proteins and/or production of noxious

chemicals in damaged plants that reduce feeding, oviposition, and growth and development of herbivores (Felton et al. 1992, 1994a; Heng-Moss et al. 2004; Karban and Chen 2007; Zhao et al. 2009; Usha Rani and Jyothsna 2010; Karban 2011), while, the indirect defense aims at attracting the natural enemies of the insect herbivore through the emission of blend of volatile compounds (Dudareva et al. 2006; Arimura et al. 2009; Hare 2011; War et al. 2011c). Induced resistance defends the plants both at the insect's egg and larval stages. It defends plants directly from egg deposition by; a) repelling the egg-laying female via oviposition-induced plant compounds (Blaakmeer et al. 1994); b) neoplasma formation at the egg site due to which eggs are raised from the site and fall off (Doss et al. 2000); c) killing the eggs by production of ovicidal substances (Seino et al. 1996; Suzuki et al. 1996); and/or d) disintegrating/detaching the eggs from the plant surface by hypersensitive responses such as necrosis (Balbyshev and Lorenzen 1997).

Direct defenses are mediated by plant characteristics that affect the herbivore's biology such as mechanical protection on the surface of the plants, e.g. hairs, trichomes, thorns, spines, and thicker leaves or production of toxic chemicals such as terpenoids, alkaloids, anthocyanins, phenols, and quinones that either kill or retard the growth and development of the herbivores (Kessler and Baldwin 2002; Hanley et al. 2007; Usha Rani and Jyothsna 2010; He et al. 2011; Smith and Clement 2012). Indirect defenses against insects are mediated by the release of a blend of volatiles that specifically attract natural enemies of the herbivores and/or by providing food [e.g., extra floral nectar (EFN)], and housing to enhance the effectiveness of the natural enemies (Dudareva et al. 2006; Arimura et al. 2009; Agrawal 2010). Induced response that occurs very early is of great benefit to the plant, and reduces the subsequent herbivore and/or pathogen attack, besides improving overall fitness of the plant (Agrawal 2010). While direct defense has its own role to play in plant defense against herbivorous insects, indirect defense also forms an important



component in insect pest control through the attraction of carnivores (Dudareva et al. 2006; Kessler and Baldwin 2002; Rasmann and Agrawal 2009; Arimura et al. 2009; von Mérey et al. 2011). Herbivore induced plant volatiles (HIPVs) not only communicate between the infested plant and natural enemies of the attacking insects, but also warn off the neighboring undamaged plants of the forthcoming danger, besides communicating between different parts of the infested plant (inter plant and intraplant signaling, respectively) (Frost et al. 2008; Arimura et al. 2009; Karban 2011). HIPVs also act as feeding and/or oviposition deterrent to the insect pests (De Moraes et al. 2001; Dudareva et al. 2006; Arimura et al. 2009; von Mérey et al. 2011).



**Fig. 1.1. Mechanism of induced resistance to herbivory in plants**

POD= peroxidase; PPO= polyphenol oxidase; PAL= phenylalanine ammonia lyase; TAL= tyrosine alanine ammonia lyase; LOX= lipoxygenase; SOD= superoxide dismutase; APX= ascorbate peroxidase; HIPVs= Herbivore induced plant volatiles

### **1.3.1.1. Morphological structures**

Plant structures are the first line of defense against herbivory, and play an important role in host plant resistance to insects. Structural defenses include morphological and anatomical traits that confer a fitness advantage to the plant by directly deterring the herbivores from feeding (Hanley et al. 2007; Agrawal et al. 2009), and range from prominent protuberances on a plant to microscopic changes in cell wall thickness as a result of lignification and suberization (Hanley et al. 2007; Sharma et al. 2009). Structural traits such as spines and thorns (spinescence), trichomes (pubescence), toughened or hardened leaves (sclerophylly), incorporation of granular minerals into plant tissues, and divaricated branching (shoots with wiry stems produced at wide axillary angles) play a leading role in plant protection against herbivory (Hanley et al. 2007; Sharma et al. 2009; Chamarthi et al. 2010). Amongst all these structural defenses, trichomes play a great role in plant protection against insect herbivores and mediate both physical and chemical defenses (Smith 2005; Hanley et al. 2007; Chamarthi et al. 2010; Sharma et al. 2009; He et al. 2011).

### **1.3.1.2. Secondary metabolites and plant defense**

Induced biochemical defenses are manifested through the production of anti-digestive proteins or toxic secondary metabolites. The secondary metabolites do not affect the normal growth and development of plant, but reduce the palatability of the plant tissues to the herbivores (Boerjan et al. 2003). Among the secondary metabolites, plant phenols constitute one of the most common and widespread group of defensive compounds, which play a major role in host plant resistance against herbivores, including insects (Sharma et al. 2009; Usha Rani and Jyothsna 2010; Ballhorn et al. 2011). Qualitative and quantitative alterations in secondary metabolites and the elevation in activities of oxidative enzyme in plants in response to insect attack is a general phenomenon (Maffei et al. 2007; Barakat et

al. 2010; He et al. 2011). Phenols mediate both direct and indirect defenses. The direct defenses are mediated by their toxic or deterrent activity against insect pests and the indirect defenses by attracting the natural enemies of insect pests (Barakat et al. 2010; Johnson et al. 2009; Sharma et al. 2009). Condensed tannins are the potent plant defensive compounds implicated against insect pests and have been reported to reduce the growth and survivorship in many insect pests (Grayer et al. 1992; Sharma et al. 2009; Barbehenn et al. 2009). Flavonoids play a central role in various facets of plant life especially in plant-environment interactions (Simmonds and Stevenson 2001; Treutter 2006). These defend plants against biotic and abiotic stresses, including ultraviolet radiation, pathogen infection and herbivore damage (Simmonds and Stevenson 2001; Simmonds 2003; Treutter 2006). Flavonoids are divided into various classes that include anthocyanins, flavones, flavonols, flavanones, dihydroflavonols, chalcones, aurones and flavans (Simmonds 2003; Treutter 2006). There are a number of flavonoids in plants with additional groups such as hydroxyl, methoxyl, glycosyl etc., which have a vital role in host plant defense against various stresses (Treutter 2006; Nuessly et al. 2007).

#### **1.3.1.3. Plant defensive proteins**

The expression of plant defensive proteins is altered in response to herbivore damage, wounding and/or elicitor application. The protein based defense is an important component of plant resistance against insects as they directly affect the insect physiology through antibiosis and regulate insect growth and development. The most important ones are proteinase inhibitors (PIs), lectins, and the antioxidative enzymes. The PIs reduce the digestibility of the proteins in insect gut and thus devoid the insects from amino acids and other essential compounds. Lectins constitute another group of important plant defensive proteins involved in plant resistance against insect pests (Stoger et al. 1999; Saha et al. 2006; Macedo et al. 2007; Chakraborti et al. 2009). Lectins are carbohydrate binding

proteins, which bind to the glycosyl groups of membrane innning the insect gut and thus disrupts the metabolic processes in insects, and depriving the insects from essential nutrients (Saha et al. 2006; Chakraborti et al. 2009; Vandenborre et al. 2011).

#### **1.3.1.3.1. Plant defensive enzymes**

Insect damage causes oxidative stress in plants that leads to induced expression of various plant defensive enzymes, which in turn mediate the production of several defensive compounds. The important oxidative plant enzymes induced in response to insect herbivory include; peroxidases (POD), polyphenol oxidase (PPO), superoxide dismutase (SOD), phenylalanine ammonia lyase (PAL), lipoxygenase (LOX), catalase (CAT) and ascorbate peroxidase (APX) (Felton et al. 1994a,b; Chen et al. 2009; Zhao et al. 2009; Usha Rani and Jyothsna 2010; He et al. 2011). Recent progress in understanding the physiological, biochemical and molecular aspects of herbivore induced plant defense has contributed to a more comprehensive picture of the biology of defensive enzymes.

Peroxidase (POD) plays diverse ecological and physiological roles in plants, including plant resistance to insect pests, pathogens and wounding (Duffey and Stout 1996; Heng-Moss et al. 2004; Gulsen et al. 2010; He et al. 2011). PODs are monomeric hemoproteins distributed as soluble, membrane-bound and cell wall-bound within the cells, and include several isozymes whose expression depends on tissue developmental stage and environmental stimuli (Heng-Moss et al. 2004; Gulsen et al. 2010; He et al. 2011). A number of process are regulated by PODs that have direct or indirect role in plant defense, including lignifications, suberization, somatic embryogenesis, auxin metabolism, and wound healing (Bi et al. 1997; Sethi et al. 2009; He et al. 2011). The PPOs are important antinutritional enzymes in plants that regulate feeding, and growth, and development of insect pests, and play a leading role in plant defense against different stresses (Mahanil et al.

2008; Bhonwong et al. 2009; He et al. 2011). PPOs are metallo-enzymes that catalyze the oxidation of monophenols and *O*-diphenols to quinines that crosslink the proteins and reduce the nutritional quality of the food (Zhang et al. 2008; Bhonwong et al. 2009). Phenylalanine ammonia lyase (PAL) is an important enzyme of phenylpropanoid pathway that catalyzes the first step involved in the synthesis of phenols including lignins (Ritter and Schulz 2004; Zhao et al. 2009). The PAL catalyzes the synthesis of phenylpropanoids and the production of phenylpropanoid-derived metabolite salicylic acid, which in turn mediates the levels of phenylpropanoid by-products such as chlorogenic acid (Felton et al. 1994a,b). Lipoxygenase (LOX) constitutes an important class of enzymes that defend plants against a variety of stresses (Kessler et al. 2004; Liavonchanka and Feussner 2006; Mao et al. 2007; Bruinsma et al. 2009). LOX is a key enzyme of jasmonic acid pathway, which is the most important defensive pathway against insect herbivores. LOX produce conjugated unsaturated fatty acid hydroperoxides from polyunsaturated fatty acids. The former are enzymatically and/or chemically degraded to unstable and highly reactive aldehydes,  $\gamma$ -ketols, epoxides (Bruinsma et al. 2009), and reactive oxygen species (ROS) such as hydroxyl radicals ( $\text{OH}^\cdot$ ), singlet oxygen ( $\text{O}^\cdot$ ), superoxide ion ( $\text{O}_2^\cdot$ ) and peroxy, acyl and carbon-centered radicals (Maffei et al. 2007; Bruinsma et al. 2009). The SOD is involved in plant defense against various stresses including pathogens and insect pests (Khattab and Khattab 2005; Usha Rani and Jyothsna 2010). It plays a key role in plant protection against oxidative stresses. The SOD is a scavenging enzyme that reduces the toxic radicals such as superoxide ( $\text{O}_2^\cdot$ ) produced during stress by dismutating them to produce less toxic and freely diffusible  $\text{H}_2\text{O}_2$  (Bowler et al. 1992). APX isozymes eliminate  $\text{H}_2\text{O}_2$  and are distributed in distinct cell compartments, the stroma (sAPX) and thylakoid membrane (tAPX) in chloroplasts, the microbody (mAPX), and the cytosol (cAPX) (Asada 1999; Ishikawa et al. 1997). They regulate cell expansion and redox state of the apoplastic space

in cells during growth and stress responses (Kato and Esaka 1999; Pignocchi et al. 2006). The CAT is another important antioxidative enzyme involved in plant defense against various biotic and abiotic stresses including insect herbivory (Chen et al. 1993; Heng-Moss et al. 2004; Divol et al. 2007; Boyko et al. 2006). It scavenges the excessive H<sub>2</sub>O<sub>2</sub>, resulting in the production of water and molecular oxygen, and is localized in mitochondria and peroxisomes (del Rio et al. 2002; Khattab and Khattab 2005; Maffei et al. 2006) to avoid the autotoxicity in plants.

#### **1.3.1.4. Phytohormones and induced resistance in plants**

An enormous variety of insect-associated products, referred to as ‘general elicitors’, which trigger plant species-specific defense responses upon damage to the leaf tissue are inducers of defense responses in plant–insect interactions (Cipollini and Redman 1999; Walling 2000; Heng-Moss et al. 2004; Gulsen et al. 2010; Kawazu et al. 2012). Although many plant hormones are involved in plant defense, the most important and widely used phytohormones, which protect plants from insect pests and other stresses are jasmonic acid (JA) and salicylic acid (SA; Moran and Thompson 2001; Moran et al. 2002; De Vos et al. 2005; Halitschke and Baldwin 2005; Zhao et al. 2009; Sethi et al. 2009; Moreira et al. 2009; Venu et al. 2010; Kawazu et al. 2012). The use of these phytohormones in inducing plant resistance against insect pests has raised the possibility of using induced resistance to insects for pest management (Hamm et al. 2010). The JA application results in the induction of plant responses that are similar, although not identical, to herbivore feeding. The JA mediated octadecanoid pathway works well against insects and mediates the expression of both direct and indirect defenses (Arimura et al. 2008; Scott et al. 2010). It accumulates in plants near the site of herbivore attack (Moreira et al. 2009), and stimulates the production of many defensive components such as, defense proteins, oxidative enzymes, glandular trichomes, alkaloids, volatiles, etc. (Wasternack 2007; Scott et al. 2010). Salicylic acid

(SA), a benzoic acid derivative, is an important phytohormone involved in the regulation of plant defense. It is an endogenous plant growth regulator that generates a wide range of metabolic and physiological responses in plants involved in defense in addition to their impact on plant growth and development (Vicent and Plasencia 2011). SA also induces greater plant defense against insect pests (Moran and Thompson 2001; Moran et al. 2002; Peng et al. 2004; Maffei et al. 2007; Zhao et al. 2009; Kawazu et al. 2012).

### **1.3.2. Insect response to plant defense**

Plant's defense against insect pests is mediated through many toxic proteins and other secondary metabolites that affect the insect development and metabolism. Plant's defenses affect the activity of digestive enzymes such as serine proteinases, amylases, trypsin, chymotrypsin, etc., which are the important enzymes involved in insect growth and development (Johnston et al. 1993; Jongsma et al. 1996; Bown et al. 1997, 2004). They also alter the activity of detoxifying enzymes such as esterases, monooxygenases and GSTs, thereby affecting the insect's ability to adapt to host plant defenses (Berenbaum 1995; Smirle et al. 1996; Mukanganyama et al. 2003).

### **1.3.3. Tritrophic interactions**

Tritrophic interactions play a potent role in insect plant interaction. Tritrophic interaction is the indirect defense implicated by the plants against the insect pests. The recruitment of natural enemies is mediated through plant volatiles (more preferably herbivore induced plant volatiles or HIPVs) that attract the natural enemy of the particular insect pest. Natural enemies of insect pests (parasitoids and predatory insects) are the central players of the biological control methods of many insect pests. Parasitoids are fascinating insects whose adult females lay their eggs in or on other insects, and immature larvae develop by feeding on host bodies resulting in death of the host. Parasitoids are used



on a large scale to control insect pests in variety of crops. Plant emitted chemical cues play a major role in insect host selection by parasitoids (Bai et al. 2011; Tamiru et al. 2011). The successful parasitization of the parasitoid depends on the attraction of parasitoid to the pest infested plants by HIPVs.

The *Campoletis chloridae* Uchida (Hymenoptera: Ichneumonidae) is an important indigenous endo-larval parasitoid that dramatically reduces the population of two major lepidopteran pests in India – *S. litura* and *H. armigera* (Bhatnagar et al. 1982; Kumar et al. 1994; Romeis and Shanower 1996; Bajpai et al. 2005). It mainly attacks the second- and third-instar larvae, thus potentially suppresses the larval population before significant damage is caused. Larvae of *C. chloridae* emerge from the fourth-instar hosts to pupate and spin a cocoon (Nikam and Gaikwad 1989) and the total developmental period lasts for about 15–16 days (Nandihalli and Lee 1995).

*Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) is a minute wasp very small in size and is frequently used against lepidopteran insect in integrated pest management of crops and vegetables (Nagarkatti and Nagaraja 1977; Boo and Yang 1998; Romeis et al. 2005; Hou et al. 2006).

## **Aims and objectives**

Screening of germplasm for resistance to insect pests has received considerable attention; however, there is limited progress in characterization of physiological and biochemical mechanisms conferring resistance to insects (Heng-Moss et al. 2004; Sharma et al. 2009). Although the response of groundnut to drought, pathogens and other stresses has been well documented (Sathiyabama and Balasubramanian 1998; Cardoza et al. 2003; Sankar et al. 2007; Chitra et al. 2008; Usha Rani and Jyothsna 2009), there is no information on the magnitude and mechanisms of induced responses in groundnut as a component for controlling insect pests, which is the most important oilseed crop grown in the tropic under rainfed conditions in Asia and Africa. Therefore, the present studies were carried out to understand the defensive responses of groundnut genotypes with differential levels of resistance to insect pests with different modes of feeding, and their implications for pest management.

The present studies were focused on the constitutive and induced resistance against *S. litura* and *H. armigera* - the two important leaf defoliators, and the sap sucking insect, *A. craccivora*, and the role of JA and SA in induced resistance against insect pests to gain an understanding of the possible use of induced plant responses for pest management.

## **Objectives**

The present investigation was carried out with following objectives:

- To study the response of groundnut genotypes with different levels of resistance to chewing (*Helicoverpa armigera* and *Spodoptera litura*) and sucking (*Aphis craccivora*) type of insects.
- To identify the secondary metabolites, induced in plants in response to feeding by the insects (HPLC fingerprinting).

- To find out the role of phytohormones (salicylic acid and jasmonic acid) in induced resistance to insect pests.
- To study the orientation behavior of parasitoids (*Trichogramma chilonis* and *Campoletis chloridae*) of *Helicoverpa armigera* and *Spodoptera litura*.
- To study the effect of flavonoids, lectins and phenyl- $\beta$ -D-glucoside on growth, development and gut enzyme activities of *Helicoverpa armigera* and *Spodoptera litura*.

# *Chapter 2*

## *Review of Literature*

## Review of Literature

### 2.1. Groundnut

Groundnut (*Arachis hypogaea* L.), also known as peanut, is an annual herbaceous plant belonging to the family Fabaceae. It occupies about 9% of the world's oilseed crop area, and contributes to about 5% vegetable oil production (Birtal et al. 2010). It is cultivated mostly in the semi-arid tropical and sub-tropical regions (Naidu et al. 1999; Sharma et al. 2003; Prasad and Gowda 2006). Groundnut is the principal source of digestible proteins, cooking oil and vitamins to the poor people in Asia, Africa and South America (Savage and Keenan 1994), and plays a significant role in food and nutritional security, and in reducing poverty, especially in the developing countries (Smartt 1994).

### 2.2. Insect pests of groundnut

Insect pests are one of the major constraints in groundnut production. In India, the annual yield losses by insect pests in groundnut are about 15%, which accounts for about 1.6 million tonnes and 25.27 billion rupees (Dhaliwal et al. 2010). Groundnut is damaged by more than 350 species of insects (Stalker and Campbell 1983; Wightman and Amin 1988; Sahayaraj and Raju 2003). Armyworms [*Spodoptera litura* (Fab.), *Spodoptera littoralis* Bois., and *Spodoptera frugiperda* (J.E. Smith)], cotton bollworm/corn earworm [*Helicoverpa zea* Bois., and *Helicoverpa armigera* (Hub.)], white grubs [*Holotrichia consanguinea* Blanch., *Holotrichia serrata* F., *Eulepida mashona* Arr.], aphid [*Aphis craccivora* Koch], thrips [*Scirtothrips dorsalis* Hood, *Caliothrips indicus* Bag., *Frankliniella schultzei* (Try.), and *Thrips palmi* Karny], leafhoppers [*Empoasca kerri* Pruthi], red hairy caterpillars [*Amscata moorei* Butler and *Amsacta albistriga* (Walk.)], and leaf miner [*Aproaerema modicella* Dev.] are the most important pests worldwide (Wightman and Amin 1988; Sahayaraj and Raju 2003). Aphids and thrips act as vectors for viral diseases. Infestation by insects at an early stage of the crop leads to severe losses in

yield (Panchabhavi and Nethradani Raj 1987; Wightman and Ranga Rao 1994). The major insect pests of groundnut are described below.

### **2.2.1. Tobacco armyworm, *Spodoptera litura***

The tobacco armyworm, *S. litura* is a polyphagous pest (Hill 1975; Shivayogeshwara 1991; Singh and Jalali 1997; Prasad and Gowda 2006), and feeds on more than 200 crop species including groundnut, brinjal, tobacco, cotton, jute, maize, rice, soybean and vegetables (Shivayogeshwara 1991; Singh and Jalali 1997; Prasad and Gowda 2006) (Fig. 2.1a,b). In India, *S. litura* is endemic to southern states and causes severe yield loss (Panchabhavi and Nethradani Raj 1987; Wightman and Ranga Rao 1997; Sahayaraj and Raju 2003).

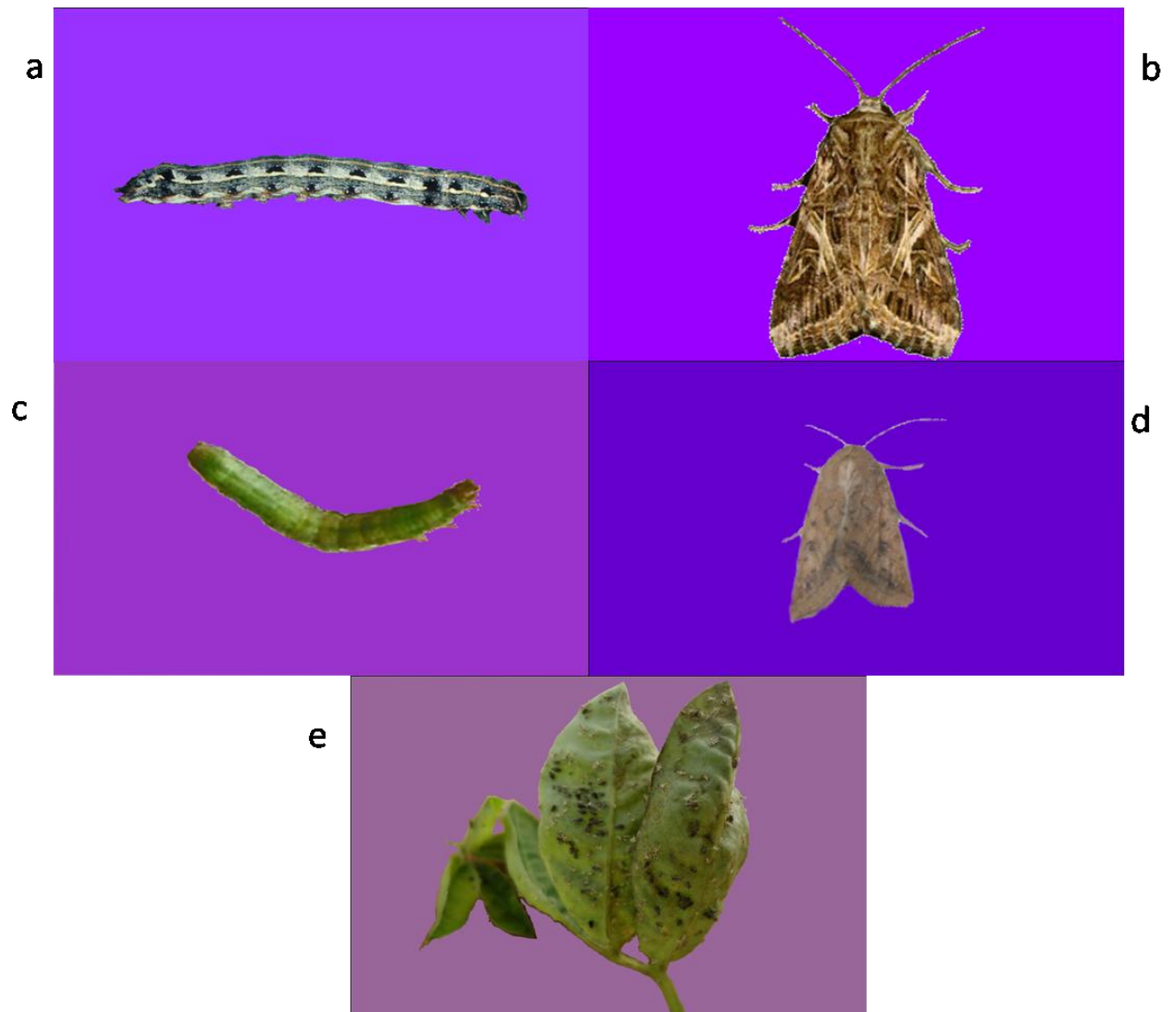
### **2.2.2. Cotton bollworm/legume pod borer, *Helicoverpa armigera***

The cotton bollworm/legume pod borer, *H. armigera* is one of the most important crop pests, has a wide host range and is distributed in Australia, Asia, and Africa (Zalucki et al. 1994; Wightman and Ranga Rao 1994; Cunningham et al. 1999; Sharma 2005). It exhibits high fecundity and has the ability to escape adverse conditions through diapause (Reed 1965; Eger et al. 1981; Sharma 2005) (Fig. 2.1c,d). It feeds on many crop plants including chickpea (*Cicer arietinum* L.), pigeonpea [*Cajanus cajan* (L.)], tomato (*Lycopersicon esculentum* Mill.), okra [*Abelmoschus esculentus* (L.) Moe.], cotton (*Gossypium* spp.), sorghum (*Sorghum bicolor* Moench), pearl millet [*Pennisetum glaucum* (L.)], maize (*Zea mays* L.), tobacco (*Nicotiana tabacum* L.), and groundnut (*A. hypogaea*) (Manjunath et al. 1989; Sahayaraj and Raju 2003; Sharma 2005; Sharma et al. 2003, 2005).

### **2.2.3. Aphid, *Aphis craccivora***

*Aphis craccivora* is a polyphagous sap sucking insect. It is 2 mm long, soft-bodied, shiny and black, with a dull grayish color (Fig. 2.1e). The alates (winged females) are mostly dispersed by wind and reproduce parthenogenetically producing viviparous colonies of apterae (wingless females). These later on develop the wings and disperse to avoid overcrowding and deterioration of the host plant. It shows distinct preference for legumes including cowpea, groundnut, and beans (Grayer et al. 1992; Wightman and Ranga Rao 1997; Padgham et al. 1990; Minja et al. 1999; Palumbo and Tickes 2001). It also attacks citrus, okra, coffee and cocoa. At earlier stage, *A. craccivora* mostly feeds on stems, terminal shoots and petioles, while at the latter stages of the crop, it also feeds on pods and flowers. This pest penetrates the plant tissues through epidermal and mesophyll cells with a stylet and feeds on photo-assimilates that translocate through the phloem, thereby causing substantial effect on fitness in many crop plants (Pollard 1972; Dixon 1998; Tjallingii and Hogen Esch 1993; Tjallingii 2006). Removal of sap leads to plant weakness, poor and stunted growth, leaf curling and distorted leaf growth and wilting, which in turn result in yield losses. Furthermore, heavy damage by aphids can lead to delayed flowering, and infestation at flowering stage results in pod shriveling and yield loss (Grayer et al. 1992).

The serious attack of *A. craccivora* on young plants may lead to death of the plant, and reduces the growth and development of older plants. In addition to damage by feeding, aphids transmit many viral pathogens (Davies 1972; Reddy 1991; Minja et al. 1999). The honey dew secretion of aphids causes growth of black sooty mould on the plant and interferes with photosynthesis.



**Fig. 2.1: (a) *Spodoptera litura* larva; (b) *S. litura* adult; (c) *Helicoverpa armigera* larva; (d) *H. armigera* adult; (e) *Aphis craccivora* on groundnut leaf.**



### 2.3. Host plant resistance against insect herbivores

Plants have coevolved with phytophagous insects, and have developed a multitude of defense strategies against the insect pests, and respond to herbivore attack through an intricate, but erratic defense system that includes structural barriers to feeding, toxic chemicals, and attraction of natural enemies of the infesting herbivore (Kessler and Baldwin 2002; Rasmann and Agrawal 2009; Arimura et al. 2009). Host plant resistance is one of the most important and environmental friendly method of controlling insect pests, including *H. armigera* (Sharma et al. 2003, 2005, 2009). To counter attack the attacking insect, the plants produce myriad specialized metabolites and proteins that have toxic, repellent, or antinutritional effects on the attacker (Duffey and Stout 1996; Ryan 2000; Wittstock and Gershenzon 2002; Zhu-Salzman et al. 2008; Usha Rani and Jyothsna 2010; He et al. 2011). Induced response is quite rapid, highly dynamic, and plays an important role in protecting the plants from further damage (Karban and Baldwin 1997, 2002; Howe and Jander 2008; Stout et al. 2009; Karban 2011). Kogan and Paxton (1983) defined induced plant resistance as the “quantitative or qualitative enhancement of a plant’s defense mechanism against pests in response to extrinsic physical or chemical stimuli”. Induced resistance in plants was first reported by Green and Ryan (1972), showing that Colorado potato beetle, *Leptinotarsa decemlineata* (Say), damage induces proteinase inhibitors (PIs) expression in potato and tomato plants. These PIs inhibited the digestive proteases activity in insect gut. Since then, induced resistance got high momentum and many examples of plants’ inducible plant defenses have been discovered; plants express proteins such as PIs and a number of defensive enzymes and secondary metabolites, which contribute to plant defense either directly or indirectly (Johnson et al. 1989; Howe et al. 1996; Constabel et al. 2000; Kessler and Baldwin 2002). Plants confront the herbivores either directly by affecting host plant preference or survival and reproduction success or indirectly by recruiting natural enemies

of the insect pests (Felton et al. 1994a,b; Heng-Moss et al. 2004; Howe and Jander 2008; Dudareva et al. 2006; Arimura et al. 2009). Recently, studies at physiological, biochemical and molecular levels have made this area more interesting and increased our understanding of insect-plant interactions. Induced resistance makes plants phenotypically plastic, and thereby, decreases the chances of the attacking insects to adapt to the induced chemicals (Ananthakrishnan 1997; Kessler and Baldwin 2002; Howe and Jander 2008; Agrawal 2010).

Plants utilize several strategies against insect pests. These include morphological features, secondary metabolites, defensive proteins, ROS, and HIPVs (Bi et al. 1997; Felton et al. 1994a,b; Bi and Felton 1995; Bowers 1991; Kessler and Baldwin 2002; Biere et al. 2004; Maffei et al. 2007; Sethi et al. 2009; He et al. 2011). Once a plant is under stress by herbivory, pathogen infection and/or abiotic factors, several signal transduction pathways are activated that lead to plant defense against these stresses (Ryan and Moura 2002; Zhao et al. 2009). There is a group of evidences that suggest the role of jasmonic acid (JA), salicylic acid (SA), systemin and volicitin in signal transduction against insect herbivores (Creelman and Mullet 1997; Ryan 2000; Ryan and Moura 2002; Zhao et al. 2009). Further understanding of how plants communicate with their neighbors, symbionts, pathogens, herbivores, and with their personal “bodyguards”- the natural enemies, both above and below ground via chemical signals is still in its infancy. However, this is an enthralling area from an ecological point of view, and has a great potential for utilization in crop protection. There is an increasing appreciation that induced resistance could form an important component in insect pest management.

### **2.3.1. Plant morphology and resistance against insect pests**

Structural defenses include morphological and anatomical traits that confer a fitness advantage to plants by directly deterring the herbivores from feeding (Smith 2005; Hanley et al. 2007; Agrawal et al. 2009), and range from prominent protuberances on a plant to microscopic changes in cell wall thickness as a result of lignification and suberization (Peter et al. 1995; Hare and Elle 2002; Handley et al. 2005; Hanley et al. 2007; Sharma et al. 2009; He et al. 2011). Structural traits such as spines and thorns (spinescence), trichomes (pubescence), toughened or hardened leaves (sclerophylly), incorporation of granular minerals into plant tissues, and divaricated branching (shoots with wiry stems produced at wide axillary angles) play a leading role in plant protection against herbivory by reducing the palatability and digestibility of the tissues, thereby, reducing the herbivore damage (Handley et al. 2005; Hanley et al. 2007; Agrawal et al. 2009; Sharma et al. 2009; Chamarthi et al. 2010; He et al. 2011). However, trichomes are the most important structural features implicated in plant defense against herbivores.

#### **2.3.1.1. Trichomes**

Trichomes are the hairy structures extending from the epidermis of the above ground plant parts including stem, leaves, and even fruits, and occur in several forms such as straight, spiral, stellate, hooked, and glandular structures (Agrawal 1999; Elle and Hare 2000; Hanley et al. 2007). Trichomes form the important physical barrier to the insects and restrict their movement and feeding (Levin 1973; Elle and Hare 2000; Peter et al. 1995; He et al. 2011). Trichomes could be glandular or non-glandular. The non-glandular trichomes mainly provide the physical defense against insects, while glandular trichomes provide both physical as well as chemical defense. Glandular trichomes secrete secondary metabolites including flavonoids, terpenoids, and alkaloids that are either toxic or repellent to insect

pests and can even serve as EFN for natural enemies of insect pests, thus forming a combination of structural and chemical defense (Elle and Hare 2000; Peter et al. 1995; Peter and Shanower 1998; Rudgers et al. 2004; Handley et al. 2005). In addition, trichomes also defend plants from abiotic stresses such as drought by reducing the absorbance of solar radiation including UV light (Benz and Martin 2006).

Increase in number and density of trichomes in plants in response to herbivore feeding and elicitor application has been reported in many plants (Baur et al. 1991; Traw 2002; Traw and Dawson 2002; Dalin and Bjorkman 2003; Agrawal 1999; Bjorkman and Ahrne 2005). Dalin and Bjorkman (2003), and Bjorkman and Ahrne (2005) reported that adult leaf beetle, *Phratora vulgatissima* L. damage in *Salix cinerea* L. increased trichome density in new leaves developing thereafter. Increase in trichome density after insect damage has also been established in *Lepidium virginicum* L. and *Raphanus raphanistrum* L. (Agrawal 1999). In black mustard, trichome density and glucosinolate levels were elevated after feeding by *Pieris rapae* (L.) (Traw and Dawson 2002). Induction of trichome densities up to 76% in leaves of *Brassica nigra* L. by *P. rapae* in seventh leaf and by *Trichoplusia ni* (Hub.) by 113% in ninth leaf has been reported by Traw and Dawson (2002). Trichome density increased in *Alnus incana* (L.) Moench damaged by beetles (Baur et al. 1991). The increase in trichome density in response to herbivory is typically between 25 to 100%; however, there are cases where 500 to 1000% increase in trichome density has been reported. Changes in trichome density occur within days or weeks after insect damage (Agrawal 1999; Traw and Dawson 2002; Dalin and Bjorkman 2003; Bjorkman and Ahrne 2005). However, the response is delayed for up to one year in some woody perennials (Valkama et al. 2005). Furthermore, change in relative proportion of glandular and non-glandular trichomes is also induced by herbivory (Agrawal 1999). In addition to herbivory, trichome production has been found to be induced by JA (Traw and Bergelson 2003). Plants

treated with JA and methyl jasmonate (MeJA) showed greater number of trichomes than the untreated plants (Traw and Bergelson 2003; Li et al. 2004; Boughton et al. 2005). Infestation of willows with adult leaf beetles (*Phratora vulgatissima* L.) showed increased density of trichomes after 10–20 days of infestation (Bjorkman et al. 2008). Furthermore, a positive correlation has been observed between natural enemies' abundance and/or their effectiveness and trichome density. Predation by fire ants was greater on soybean isolines with dense trichomes (Styrsky et al. 2006). Apple trees with dense trichomes harbor large numbers of predatory mites than those with low density of trichomes (Roda et al. 2003), and has been associated with the fact that higher pubescence captures more pollen and fungal spores that serve as alternative food for the predators. Gonzales et al. (2008) observed that the density of trichomes increased in *Madia sativa* Molina in response to mechanical damage and drought stress. They found that both glandular and non-glandular trichomes were induced under plant damage and drought stress.

### **2.3.2. Secondary metabolites and plant defense**

Induced biochemical defenses in plants are manifested through the production of anti-digestive proteins and toxic secondary metabolites (Bowers 1991; Kessler and Baldwin 2002; Biere et al. 2004; Sethi et al. 2009; Usha Rani and Jyothsna 2010). Secondary metabolites have diverse roles in plants and are involved in plant defense against biotic and abiotic stress responses and in hormonal regulation (Sharma and Nwanze 1997; D'Auria and Gershenzon 2005; Chamarthi et al. 2011). This field has been explored tremendously over the last decade. Out of the estimated more than 400,000 unique metabolites in plant kingdom (Oksman-Caldentey and Inze 2004; Saito and Matsuda 2010), only a handful have been discovered so far. Plants with high variability in defensive chemicals exhibit a better defense compared to those with moderate variability (Walling 2000; Kessler and Baldwin 2002; Chen et al. 2009). The secondary metabolites have been reported to reduce the

palatability of the plant tissues to insects in which they are produced and thereby, prevent the tissue from insect damage (Kessler and Baldwin 2002; Boerjan et al. 2003; Bernards and Bastrup-Spohr 2008; Mazid et al. 2011). The defensive secondary metabolites can be either constitutively stored as inactive forms or induced in response to the insect or microbe attack and/or elicitor application. The former are known as phytoanticipins and the latter as phytoalexins. The phytoanticipins are mainly activated by  $\beta$ -glucosidase during herbivory, which in turn mediate the release of various biocidal aglycone metabolites (Morant et al. 2008). The classic examples of phytoanticipins are glucosinolates that are hydrolyzed by myrosinases (endogenous  $\beta$ -thioglucoside glucohydrolases) during tissue disruption. Other phytoanticipins include Benzoxazinoids (BXs), which are widely distributed amongst Poaceae. Hydrolyzation of BX-glucosides by plastid-targeted  $\beta$ -glucosidases during tissue damage leads to the production of biocidal aglycone BXs that are involved in plant defense against insects (Morant et al. 2008; Ahmad et al. 2011). Phytoalexins include isoflavonoids, terpenoids, alkaloids, etc., which influence the performance and survival of the herbivore (Walling 2000). It has been reported that maize host plant resistance to *H. zea* is mainly due to the presence of the secondary metabolites C-glycosyl flavone maysin [2''-O-  $\alpha$ -L-rhamnosyl-6-C-(6-deoxy-xylo-hexos-4-ulosyl) luteolin] and the phenylpropanoid product chlorogenic acid (Nuessly et al. 2007). Phenolic compounds such as 4,4- dimethyl cyclo-octene, protocatechuic acid,  $p$ -hydroxybenzaldehyde, cinnamic acid, luteolin, apegenin, syringic acid and lonol - 2 have been found to be responsible for shoot fly *Atherigona soccata* (Rondani) resistance in *S. bicolor* (Panday et al. 2005; Chamarthi et al. 2010, 2011). Wild radish plants infested by *P. rapae* exhibited higher levels of glucosinolate that lead to the reduced performances by insect pests such as, Lepidoptera, aphids, and leaf miner (Agrawal 1999). Secondary metabolites also act as signal compounds for attracting the pollinating or seed dispersing animals, and also protect the plants from ultraviolet radiation

and oxidants (Boerjan et al. 2003). Secondary metabolite production has been associated with quick, transient increase in activities of enzymes of the phenylpropanoid/flavonoid and/or octadecanoid pathways such as POD, PPO, PAL, APX, SOD, CAT and LOX (Gundlach et al. 1992; Boerjan et al. 2003; Bernards and Bastrup-Spohr 2008). In addition to their role in plant defense, secondary metabolites increase the fitness of the plants (Wink 2003).

#### **2.3.2.1. Phenols**

Phenolic compounds are widely produced in plants and have been implicated in many important “secondary” ecological roles, including plant interaction with insects and microbes (Matsuki 1996; Cooper-Driver and Bhattacharya 1998; Ballhorn et al. 2011). Plant phenols act as antifeedant (Wrubel and Bernays 1990; Bernays and Chapman 2000), digestibility reducers (Feeny 1968; Isman and Duffey 1982; Sharma and Norris 1991), and toxins (Bernays 1981; Steinly and Berenbaum 1985; Stevenson et al. 1993). Phenols are involved in both physical and chemical defense of plants against insect pests. For example, phenols such as cell wall bound phenolics, lignins, suberin, and cuticle-associated phenolics give physical defense to plants and restrict the insect damage, and also act as antifeedant as well as insecticidal compounds against insect herbivores (Walling 2000; Sharma et al. 2009; Barakat et al. 2010). Induced defenses have both local as well as systemic effect. The biochemical defense mediated by phenols also serves as the main component of phenolic based plant protection against insects (Sharma and Norris 1991; Sharma et al. 2009; Ballhorn et al. 2011). The toxicity of phenols is mainly due to the production of oxidative products such as semiquinone radicals and ROS upon oxidation (Appel 1993; Barbehenn et al. 2001). They are also involved in cyclic reduction ROS, which activate a cascade of reactions leading to the activation of defensive enzymes (Johnson and Felton 2001; Maffei et al. 2007). Qualitative and quantitative alterations in phenols and elevation in activities of

oxidative enzymes in response to insect attack is a general phenomenon (Usha Rani and Jyothisna 2010; Barakat et al. 2010; He et al. 2011). Lignin, a phenolic heteropolymer acts as a central component of physical plant defense against insects and pathogens (Barakat et al. 2010). It limits the entry of pathogens by blocking physically or increasing the leaf toughness that reduces the feeding by herbivores, and also decreases the nutritional content of the leaf (Johnson et al. 2009). Lignin synthesis has been found to be induced by herbivory or pathogen attack and its rapid deposition reduce further growth of the pathogen and herbivore fecundity (Johnson et al. 2009). Phenolics, tannins, indole alkaloids and non-protein amino acids are the main components of plant resistance to insect pests including aphids (Ciepiela and Sempruch 1999; Mallikarjuna et al. 2004; Chen et al. 2009; Usha Rani and Jyothisna 2010). The secondary metabolites of various plants have been reported to affect the Lepidopteran growth and development (Isman and Duffey 1982; Ortego et al. 1998; Felton et al. 1992; Barakat et al. 2010). Corn borer, *Ostrinia furnacalis* (Guenee) showed reduction in growth and efficiency of conversion of the ingested food when fed on diet treated with 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA; Ortego et al. 1998). Usha Rani and Jyothisna (2010) observed production of various phenolic compounds in rice infested with insects. Plants infested with pests and pathogens resulted in increased expression of lignin associated genes (CAD/CAD-like genes) (Bhuiyan et al. 2009; Barakat et al. 2010). Malformations in *H. armigera* have been associated to the altered levels of phenols and phenolic acids in chickpea and red gram (Ananthakrishnan et al. 1990). Moreover, the incorporation of phenolic compounds leads to the reduced growth and development of insect pests (Elliger et al. 1980; Isman and Duffey 1982; Felton et al. 1992; Akhtar et al. 2012). PPO when added to the diet increases the oxidation of chlorogenic acid and the orthoquinones- amino acid and protein linkage, thereby, reduces their nutritional quality and/or bioavailability (Felton et al. 1992; Bhonwong et al. 2009). Arnold et al.



(2004) reported the induction of total phenols in poplar infested with gypsy moth, mechanical wounding and treatment with JA. Higher levels of phenolic acids such as caffeoylmalic, feruloylmalic and *p*-coumaroylmalic acids have been found to be induced by SA in *Thunbergia alata* Bojer ex Sims (Housti et al. 2002).

#### **2.3.2.2. Flavonoids**

Flavonoids are the major plant defensive phenolic compounds utilized against a variety of stresses (Sharma and Norris 1991; Stevenson et al. 1993; Treutter 2006). More than 5,000 flavonoids have been reported in plants, which play a central role in various facets of plant life, especially in plant-environment interactions (Treutter 2006). Flavonoids and isoflavonoids protect the plants against insect pests by influencing their behavior, growth and development (Sharma and Norris 1991; Stevenson et al. 1993; Widstrom and Snook 2001; Simmonds 2003). These serve an important role in plant defense, pigmentation, and many diverse host-plant interactions. Flavonoids alter the growth and reproduction of herbivores by directly interacting with steroid hormone systems (Oberdorster et al. 2001). Molting, reproduction, feeding, and behavior of insects have been reported to be affected by flavonoids (Stevenson et al. 1993; Simmonds and Stevenson 2001; Onyilagha et al. 2004). A number of flavonoids such as genistein, wighteone, 2-hydroxygenistein, luteone, licoisoflavone have been investigated as feeding deterrents against many insect pests (Harborne 1993). Flavones, 5-hydroxyisoderricin, 7-methoxy-8-(3-methylbutadienyl) – flavanone and 5-methoxyisoronchocarpin isolated from *Tephrosia villosa* (L.), *T. purpurea* (L.), and *T. vogelii* Hook.f., respectively, have been found as feeding deterrents against *S. exempta* (Walk.), and *S. littoralis* (Simmonds et al. 1990). Some flavonoids have been found as toxic to insects such as, western corn rootworm (Mullin et al. 1992), the corn earworm (Widstrom and Snook 2001), and the common cutworm (Morimoto et al. 2000). The flavonoids such as daidzein, glyceollins, sojagol and

coumestrol in soybean have suggested to be associated with the resistance against *T. ni* (Sharma and Norris 1991). They further suggested that these flavonoids exhibit antifeedant and/or antibiotic activities against insect pests. Summer and Felton (1994) reported the midgut toxicity in insect pests fed on the diet containing chlorogenic and caffeic acid. Flavonoids have been reported to protect plants against *H. zea* and the *Ostrinia nubilalis* (Hub.) larvae (Elliger et al. 1980; Isman and Duffy 1982; Abou-Zaid et al. 1993). Various flavonoids including isorhamnetin-3-sophoroside-7-glucoside and kaempferol-3,7-diglucoside have been reported as feeding deterrents against *Mamestra configurata* (Walk.) (Onyilagha et al. 2004).

Flavonoid production has been found to confer resistance in *Arabidopsis thaliana* (L.) Heynh against *S. frugiperda* (Johnson and Dowd 2004). Isoflavonoids (judaicin, judaicin-7-*O*-glucoside, 2-methoxyjudaicin, and maackiain) isolated from the wild relatives of chickpea act as antifeedants against *H. armigera* at 100 ppm. Judaicin and maackiain were also found deterrent to *S. littoralis* and *S. frugiperda*, respectively (Simmonds and Stevenson 2001). Cyanopropenyl glycoside and alliarinoside strongly inhibit feeding by *Pieris napi oleracea* L., while isovitexin-6'-*D*- $\beta$ -glucopyranoside acts as feeding deterrent to the late instars (Renwick et al. 2001). Piubelli et al. (2003) observed greater induction of daidzin and genistin in soybean plants infested with *Nezara viridula* L. Rutin (quercetin 3-*O*-glucosyl rhamnoside) and genistin in foliage-fraction of soybean negatively affected the behavior and physiology of *H. zea* and *T. ni* (Hoffmann-Campo 1995; Hoffmann-Campo et al. 2001). Rutin when incorporated in artificial diet resulted in poor growth and development of a number of insect pests (Duffey and Isman 1981; Mallikarjuna et al. 2004; Ateyyat et al. 2012) and *Anticarsia gemmatalis* Hubner (Hoffmann-Campo et al 2006; Salvador et al. 2010). Rutin has also been reported to interfere in molting by effecting prothoracicotrophic hormone and ecdysteroid activity (Nijhout and Williams 1974; Horwath

and Stamp 1993). Larval survival and development and pupal weights of *T. ni* are considerably affected by rutin (Hoffmann-Campo et al. 2001). The flavonoids, quercetin dehydrate, rutin hydrate and naringine at 1000 ppm showed the mortality of 85, 93 and 86%, respectively; in *Eriosoma lanigerum* (Haus.) in a twig dip assay (Ateyyat et al. 2012). Exogenous application of SA and MeJA induced phenolic and flavonoid content in lettuce and resistance against pill bugs (Tierranegra-Garcia et al. 2011). The role of flavonoids in plants against insect herbivory has been well documented (Treutter 2006; Erb et al. 2009). There is a group of evidences depicting the role of phenylpropanoids, flavonoids and lignans as antifeedant agents (Morimoto et al. 2000). In addition, flavonoids scavenge the free radicals including ROS, and reduce their formation by chelating metals (Treutter 2006). However, some flavonoids have been found to act as feeding stimulants (van Loon et al. 2002).

#### **2.3.2.3. Tannins**

Tannins are astringent (mouth puckering) bitter polyphenols. They are divided into two groups; condensed and hydrolysable tannins. Hydrolysable tannins are formed from glucose and phenolic acids, such as gallic acid, while condensed tannins are polymerized flavonoids. Condensed tannins (also known as proanthocyanidins) are the important and highly effective plant secondary metabolites involved in defense against herbivory (Sharma and Agarwal 1983; Bi et al. 1997; Felton et al. 1994a,b; Ananthakrishnan 1997; Rao et al. 1998; Rao 2003; Forkner et al. 2004; Sharma et al. 2009; Barbehenn and Constabel 2011). Depending on the stereochemistry and the flavonoid groups' hydroxylation pattern, the condensed tannins are divided into several sub-types. These are the oligomers or polymers of flavan-3-ols such as catechin and epicatechin, and the corresponding trihydroxylated gallo catechins. The two common condensed tannins are procyanidins and the prodelphinidins, which are linear chains of flavan-3-ols linked through C4–C8 bonds.

Condensed tannins defend plants against insect herbivores by deterrence and/or toxicity (Nomura and Itioka 2002; Kranthi et al. 2003; Stevens and Lindroth 2005; Barbehenn et al. 2009). Earlier it was hypothesized that plant defensive role of tannins against insects is due to their ability to precipitate plant proteins (also the digestive enzymes of herbivores), by hydrogen bonding or covalent bonding of protein  $-NH_2$  groups, and thereby, reducing the nitrogen mineralization and/or digestion in the midgut, and the growth and survivorship in many insect pests (Ayres et al. 1997; Nomura and Itioka 2002). It is now believed that the anti-herbivory effect of tannins against insects is due to their oxidative activation and disruption of the peritrophic membrane (Karowe 1989; Galati et al. 2002; Barbehenn et al. 2001, 2009). However, in insect-plant interaction, tannins are still considered as the protein binding agents that reduce the protein digestion (Ossipov et al. 2001; Rossi et al. 2004; Marquis and Lill 2010). Tannins are oxidized in insect's gut having high pH, producing highly toxic semiquinone radicals, quinones and other ROS, thereby inhibiting insect growth and development (Stevens and Lindroth 2005). These oxidative products have direct toxicity on the insect gut and/or react with the essential amino acids in plants, thereby reduce the nutritional quality of the plant tissue (Felton et al. 1996). However, protein digestion efficiencies did not show any alteration in caterpillars fed on the tannic acid containing diet (Karowe 1989). Similarly, Barbehenn et al. (2009) did not observe any effect on the protein digestion of *Lymantria dispar* (L.) caterpillars fed on leaves coated with tannins. Although tannins have been found to inactivate the proteases under *in vitro* conditions, there are limited studies on *in vivo* inactivation of proteases by tannins (Blytt et al. 1988; Juntheikki and Julkunen-Tiitto 2000). Furthermore, tannins have been reported to reduce the protein digestion and utilization in some mammalian herbivores (Robbins et al. 1987; McArt et al. 2009), probably due to the higher content of cell wall bound proteins or protein precipitation. Moreover, tendency of tannins to bind to carbohydrates *in vitro* has

been reported (Takechi and Tanaka 1987; De Veau and Schultz 1992), where they alter the levels of fatty acids.

In addition to their midgut toxicity, tannins also chelate the metal ions, and thus, reducing their bioavailability to herbivores. Induction of tannins in plants in response to insect infestation has been well documented. Induction of tannins in *Quercus* species in response to insect damage has also been observed (Schultz and Baldwin 1982; Hunter and Schultz 1995; Rossi et al. 2004). Accumulation of tannins in *Populus* spp. infested by several insects has been reported (Arnold and Schultz 2002; Peters and Constabel 2002; Stevens and Lindroth 2005). It has been suggested that increase in insect developmental period in *Earias vittella* (Fab.) by gossypol, tannins and anthocyanins could be utilized as a source of biochemical resistance against insects as this could expose the larvae to natural enemies and other unfavorable conditions (Sharma and Agarwal 1983). Condensed tannins such as, (+) - catechin, (+) - gallo catechin, and vanillin in leaves of *Quercus robur* L. inhibited winter moth larvae, *Operophtera brumata* (L.) (Feeny 1968). In contrast, no significant differences in tannin content were found in *Quercus serrata* Murray (Hikosaka et al. 2005) and silver birch (*Betula pendula* Roth) in response to insect herbivory (Keinanen et al. 1999).

Induction of tannins in *Populus tremuloides* Michx. leaves in response to wound- and herbivore occur by transcriptional activation of the flavonoid pathway (Peters and constable 2002). Genes responsible for the production of tannins in response to wounding have been identified and are activated by the expression of a condensed tannins regulatory gene, *PtMYB134*, which is itself induced by damage (Mellway et al. 2009). Some polyphagous insect species have the ability to tolerate gallotannins, e.g., *Schistocerca gregaria* (Forsk.) by hydrolyzing them rapidly to avoid any damaging effects (Harborne 1993), or by restricting the passage of tannins by adsorbing them on the thick peritrophic

membrane, and by inhibiting the tannin protein complex formation by surfactants in the midgut (Bernays and Chapman 2000). Progress in molecular biology has eased the understanding of the induction of tannins in response to herbivory.

### **2.3.3. Plant defensive proteins**

The nutritional requirements of insects are similar to other animals and any imbalance in digestion and utilization of plant proteins in insects will have drastic effects on insect growth and development. Alteration of gene expression under stress leads to qualitative and quantitative changes in proteins, which in turn play an important role in signal transduction, oxidative defense, toxicity and anti-pathogenesis (Chen et al. 2009). On account of biotic stresses, plants undergo an alteration in levels of various proteins, and also produce new entities (Usha Rani and Jyothsna 2010; Gulsen et al. 2010). Many plant proteins ingested by insects are stable, and remain intact in midgut, and also move across the gut wall into the hemolymph. An alteration in the protein's amino acid content or sequence influences the function of that protein. Likewise, anti-insect activity of a proteolysis-susceptible toxic protein can be improved by administration of protease inhibitors (PIs), which prevent degradation of the toxic proteins, and allow them to exert their defensive function.

#### **2.3.3.1. Proteinase inhibitors**

Proteinase inhibitors (PIs) are one of the most abundant defensive proteins in plants. Higher concentrations of PIs occur in storage organs such as seeds and tubers, and 1 to 10% of their total proteins comprises of PIs, which inhibit different types of enzymes (Lawrence and Koundal 2002). They play an important defensive role against insect pests, pathogens, wounding, and environmental stresses (Koiwa et al. 1997; Browse and Howe 2008; Dunse et al. 2010). PIs have probably evolved as a defense against insect herbivores as a means of

natural crop protection. The defensive capacities of plant PIs rely on their binding to digestive enzymes in the insect gut and inhibiting their activity, thereby reducing protein digestion, resulting in the shortage of amino acids, and slow development and/or starvation of the insects (De Leo et al. 2001; Azzouz et al. 2005; Dunse et al. 2010; Parde et al. 2010, 2012). PIs bind to the enzymes and form stable complexes with proteases, thus blocking and/or preventing access to the enzyme active site. PIs reduce the digestive enzyme activities in insects (Ryan 1990; Parde et al. 2010), and the incorporation of serine and cysteine PIs in diet subdue the growth, development and reproduction of insects (Gatehouse and Boulter 1983; Broadway and Duffey 1986; Johnston et al. 1993; Kuroda et al. 2001). The defensive function of many PIs against insect pests, directly or by expression in transgenic plants to improve plant resistance against insects has been studied against many Lepidopteran (Lawrence and Koundal 2002; Dunse et al. 2010; Parde et al. 2010) and hemipteran insects (Azzouz et al. 2005). In *Nicotiana attenuata* (Torr. ex Watson), trypsin proteinase inhibitors and nicotine expression, contributed synergistically to the defensive response against *S. exigua* (Steppuhn and Baldwin 2007). The PIs from *Solanum nigrum* L. has been found to adversely affect a number of insect pests (Hartl et al. 2010). Tschardt et al. (2001) reported the induction of PIs in *Alnus glutinosa* L. infested with *Agelastica alni* (L.) and treated with MeJA, JA and ethylene. The molecular weight of plant PIs varies from 4 to 85 kDa (Hung et al. 2003). The success of transgenic crops in expressing PIs against insect pests has accentuated the need to understand the mechanisms, and interactions of multiple PIs with other defenses, and the adaptive responses of the herbivores.

Many classes of PIs are induced in plants in response to stresses. The serine protease inhibitors constitute an important group of PIs that occurs throughout the plant kingdom and have been isolated from many plants. Kunitz type proteinase inhibitors (KPIs) are the serine protease inhibitors (SPIs), which are strongly up-regulated defense genes in response to

wounding or herbivore feeding on plants (Ritonja et al. 1990; Lawrence and Koundal 2002; Miranda et al. 2007). The KPIs are highly active against serine proteases; however, they can also inhibit the activity of other proteases (Ritonja et al. 1990). They are mostly present in legumes, cereals and in Solanaceae family (Laskowski and Kato 1980; Ishikawa et al. 1994; Chye et al. 2006). Progress in genome sequencing has resulted in identification of a large number of proteinase inhibitors and other defense components induced in plants on account of herbivore damage. Although most of the KPIs in plants are up-regulated in response to insect herbivory, their degree of induction varies with the nature of insect plant interactions. Various KPIs allow plants to deal with multiple generations of insects by providing a genetic storehouse of varied PIs.

Bowman-Birk inhibitors (BBIs) are mainly present in legumes and cereals including groundnut (Suzuki et al. 1987; Tanaka et al. 1997). Although, these PIs occur in seeds, they are induced in leaves on wounding and insect damage (Eckelkamp et al. 1993; Moura and Ryan 2001). Trypsin, chymotrypsin and elastase are the first reactive site in these inhibitors (Qi et al. 2005), which is stabilized by the disulfide bonds (Lin et al. 1993). The BBIs have been found to inhibit the activity of trypsin and chymotrypsin in groundnut (Suzuki et al. 1987), but the relative affinity of binding to these enzymes is altered by the presence of the other (Tur et al. 1972). However, some insects respond to PIs by constitutive or induced production of PI-insensitive proteases (Bayes et al. 2005), or by the inactivation of ingested PIs, thereby preventing them from binding to sensitive proteases (Zhu-Salzman et al. 2008). Such a feeding response by the insects negatively affects the PI activity, and may result in even greater damage to the plants (Steppuhn and Baldwin 2007). This counter defense by the insects is a major hindrance to manipulation and utilization of PIs for a longer-lasting plant defense, and there is a need to understand the in-depth mechanisms by which insects counteract the PI-based plant defense.



### **2.3.3.2. Plant lectins**

Lectins are carbohydrate-binding (glyco) proteins, ubiquitous in nature, and have protective function against a range of pests (Chakraborti et al. 2009; Vandenborre et al. 2011). The insecticidal activities of different plant lectins have been utilized as naturally occurring insecticides against insect pests (Saha et al. 2006). They act as antinutritive and/or toxic substances by binding to membrane glycosyl groups lining the digestive tract, leading to an array of harmful systemic reactions (Chakraborti et al. 2009; Vandenborre et al. 2011). Disruption of lipid, carbohydrate, and protein metabolism causes enlargement and/or atrophy of key tissues, which in turn alters the hormonal and immunological status, threatening the growth and development of insects (Saha et al. 2006; Chakraborti et al. 2009; Vandenborre et al. 2011). Lectins have been found to be promising against homopteran (Saha et al. 2006; Chakraborti et al. 2009), Lepidopteran and Coleopteran insects (Macedo et al. 2007). Insecticidal properties of *Galanthus nivalis* L. agglutinin (GNA) were the first plant lectin shown to be active against hemipteran insects (Stoger et al. 1999). Expression of lectin coding genes in transgenic plants and their defense against insects has been worked out in many plants, e.g., GNA, PSA (*Pisum sativum* L.; pea), WGA (*Triticum vulgare* Kunth; wheatgerm), ConA (*Canavalia ensiformis* (L.); jack bean), AIA (*Artocarpus integrifolia* Forst.; jack fruit), OSA (*Oryza sativa* L.; rice), ASAL (*Allium sativum* L.), and UDA (*Urtica dioica* L.; stinging nettle) (Dutta et al. 2005; Saha et al. 2006; Chakraborti et al. 2009). Plant lectins incorporated in artificial diet have been found to reduce the larval growth and development in several insects (Czapla and Lang 1990; Machuka et al. 1999; Shukla et al. 2005).

### **2.3.3.3. Plant defensive enzymes**

One of the important aspects of host plant defense against insects is the disruption of insect's nutrition. The enzymes that impair the nutrient uptake by insects through formation of electrophiles includes POD, PPO and APX, and other enzymes oxidizing mono- or dihydroxyphenols, that lead to the formation of reactive *O*-quinones, which in turn polymerize or form covalent adducts with nucleophilic groups of proteins due to their electrophilic nature (e.g. -SH or -NH<sub>2</sub> of Lys) (Green and Ryan 1972; Hildebrand et al. 1986; Felton et al. 1994a,b; Constabel et al. 2000; Chaman et al. 2001; Heng-Moss et al. 2004; Bhonwong et al. 2009; Gulsen et al. 2010). Other important defensive enzymes include LOX, PAL, CAT and SOD (Duffey and Stout 1996; Felton et al. 1994a,b; Khattab and Khattab 2005; Zhao et al. 2009; Bhonwong et al. 2009; Han et al. 2009; He et al. 2011). These enzymes have potential roles in the production of plant defensive compounds and are implicated in plant resistance against insect herbivores. In addition to reducing the digestibility and palatability of plant tissues to insect herbivores, the prevention of tissue oxidative damage is a potent mechanism of plant defense against biotic and abiotic stresses (Dat et al. 1998; Bhonwong et al. 2009; He et al. 2011). This stress tolerance in plants is mostly attributed to the increased antioxidative enzyme activities and the amounts of antioxidative compounds, which then remove the toxic free radicals produced in plants on account of stress.

A tremendous alteration in the oxidative system of soybean plants infested with *H. zea* lead to the increased levels of ROS, MDA and elevation in the activities of LOX, POD, APX and NADH oxidase (Bi and Felton 1995). Moreover, larvae fed on the previously infested plants suffered the midgut oxidative damage. Induction of antioxidative enzymes in plants following herbivory has received considerable attention (Green and Ryan 1972; Bi and Felton 1995; Constabel et al. 2000; Chaman et al. 2001; Heng-Moss et al. 2004; Chen et al. 2009).

### 2.3.3.3.1. Peroxidases

Peroxidases (PODs) are involved in diverse ecological and physiological roles in plants, which include plant resistance to insects and pathogens, and healing of wounds (Duffey and Stout 1996; Heng-Moss et al. 2004; Zhao et al. 2009; Gill et al. 2010; Gulsen et al. 2010). The POD activity has been implicated as a part of the immediate response in plants to insect damage (Chaman et al. 2001; Moloji and van der Westhuizen 2006; Gulsen et al. 2010; He et al. 2011).

Apart from mediating various signaling pathways and production of toxic secondary metabolites, PODs also produce direct gut toxicity in insects (Zhu-Salzman et al. 2008; Barbehenn et al. 2009). A number of reports suggest that the levels of POD increase with insect feeding or leaf damage, which in turn defends the plants against insect pests and other stresses (Heng-Moss et al. 2004; Usha Rani and Jyothisna 2010; Gill et al. 2010; Gulsen et al. 2010; He et al. 2011). Increase in POD activity in leaves of resistant cereal plants in response to infestation with cereal aphid was observed by Xinzhi et al. (2001). Heng-Moss et al. (2004) and Gulsen et al. (2010) reported higher induction of POD in buffalograsses, *Buchloe dactyloides* (Nuttall) infested with *Blissus occiduus* Bar. Similarly, Hildebrand et al. (1986) and Felton et al. (1994b) observed increased levels of POD activity in insect resistant genotypes of soybean in response to herbivory by mites, bean leaf beetles, and three-corned alfalfa leafhoppers. Exposure of tomato plants to insects up-regulated the POD (Stout et al. 1999). Chaman et al. (2001) demonstrated that aphid infestation induced higher activity of POD in barley. He et al. (2011) found greater induction of POD in chrysanthemum plants (*Chrysanthemum grandiflorum* L.) infested with *Macrosiphoniella sanbourni* Gillette. They further observed that resistant genotypes showed quicker and greater induction than the susceptible ones. Increase in POD activity in cucumber seedlings in response to white fly, *Bemisia tabaci* (Gen.) infestation has been demonstrated by Zhang et al. (2008). *Aphis*

*medcaginis* Koch infestation stimulated the expression of POD in alfalfa plants (Huang et al. 2007). Rangasamy et al (2009) recorded two fold increases in POD activity in chinch bug, *Blissus insularis* Barber resistant varieties of St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze after 5 and 8 days of infestation compared to the uninfested control plants. Ni et al. (2001) observed positive correlation between wheat resistance to Russian wheat aphid, *Duraphis noxia* (Mord.) and POD activity (Ni et al. 2001). Bi et al. (1997) observed higher levels of POD in cotton infested with *H. zea*. Infestation by *Spissistilus festinus* (Say) increased the activities of several oxidative enzymes including PPO, POD, LOX, and ascorbate oxidase in soybean plants (Felton et al. 1994a,b).

#### **2.3.3.3.2. Polyphenol oxidase**

The PPOs are the copper metalloenzymes, which catalyze the *O*-hydroxylation of monophenols to *O*-diphenols and oxidation of *O*-dihydroxyphenols to *O*-diquinones (Steffens et al. 1994). They regulate feeding, growth and development of insect pests, and play a leading role in plant defense against biotic and abiotic stresses (Bhonwong et al. 2009; He et al. 2011). In addition, PPOs are also involved in biosynthesis of pigments, lignans, phenolic signaling molecules and regulation of plastid oxygen levels (Mayer and Harel 1979; Ryan 2000; Cho et al. 2003; Wang and Constabel 2004). The PPOs occur in leaves, stem, roots, and flowers of plants, and are differentially regulated in response to different stresses. The young tissues have greater vulnerability to insect attack and exhibit greater induction. These are the main antinutritional enzymes that reduce the plant tissue digestibility and palatability, thereby render them unfit for the insects (Zhao et al. 2009; Gould et al. 2009). In addition to its antinutritional property, PPO also catalyzes the lignin

synthesis thereby making the tissues hard for the insects and pathogens to penetrate (Sethi et al. 2009; Bhonwong et al. 2009). The PPOs can function in following ways: (a) PPO-generated quinones could alkylate essential amino acids, decreasing plant nutritional quality; (b) quinones may produce oxidative stress in the gut lumen through redox cycling; and (c) quinones and radicals produced by phenolic oxidation could be absorbed and have toxic effects on herbivores.

Besides their role in host plant defense against insects, the PPOs have also been reported to induce resistance against pathogens such as *Fusarium oxysporum* F. sp. *albedinis* (Fao) (Jaiti et al. 2009). Elevation of PPO is mediated by increased mRNA accumulation, which is characteristic of a variety of herbivore induced defense proteins (Constabel et al. 2000). The PPO genes are differentially induced by signaling molecules and injury due to wounding, and pathogen and/or insect infestation (Bhonwong et al. 2009; Zhao et al. 2009). Correlation between induction of PPO activity and insect fitness has been reported in many plants including tomato and lettuce (Thipyapong et al. 2006; Bhonwong et al. 2009; Sethi et al. 2009).

Induction of PPOs in plants in response to insect attack and their role in plant defense against insect herbivory has been well documented. Stout et al. (1999) reported up-regulation of PPO in tomato leaves infested with *H. zea*. Induction of PPOs in *A. glutinosa* infested with *A. alni* has been reported by Tscharntke et al. (2001). Greater PPO activity was observed in cotton infested with *H. zea* (Bi et al. 1997). Bhonwong et al. (2009) observed that tomato plants with higher PPO activities were resistant to *S. frugiperda* than those with lesser PPO activity. Similarly, chrysanthemum (*C. grandiflorum*) leaves exhibited a quick induction of PPOs when infested with *M. sanbourni* (He et al. 2011). Zhang et al. (2008) reported the induction of PAL activity in cucumber seedlings infested with *B. tabaci*. Higher induction of PPO activity was observed in alfalfa plants in response

to damage by *A. medicaginis* (Huang et al. 2007). Chaman et al. (2001) and Ni et al. (2001) recorded greater induction of PPO in barley and cereal plants, respectively, infested with the Russian wheat aphid, *D. noxia* than the uninfested control ones. Moreover, greater PPO activity was observed in aphid resistant plants than in the susceptible ones (Chaman et al. 2001; Ni et al. 2001). Phytohormones also induce PPO activities in plants. Induction of PPO by JA (Tscharntke et al. 2001; Wang and Constabel 2004; Cipollini et al. 2004; Kumari et al. 2006; Zhao et al. 2009; Gould et al. 2009; Bhonwong et al. 2009), and SA (Zhao et al. 2009; Idrees et al. 2011; War et al. 2011a,b) has been reported. Induced PPO activities have also been found to reduce insect growth and development in potato, cotton, soybean, tomato, rubber tree, poplar, barley and lettuce (Felton et al. 1994a,b; Chaman et al. 2001; Wang and Constabel 2004; Bhonwong et al. 2009; Sethi et al. 2009). The PPOs confer resistance to *S. litura*, *H. armigera*, *B. tabaci*, *Tetranychus cinnabarinus* (Boisd.), *Myzus persicae* (Sulzer), *Empoasca fabae* (Harris), *Aphis medicaginis* (Koch), *S. exigua*, and *A. alni* (Tscharntke et al. 2001; Bhonwong et al. 2009; He et al. 2011). However, induced PPO levels had no or limited impact on *L. dispar*, *Orgyia leucostigma* (JE Smith) (Barbehenn et al. 2009), and *Blissus occiduus* Barber (Heng-Moss et al. 2004). Polyphenol oxidase also stimulates the biosynthesis of phenylpropanoid; the important components of SA mediated phenylpropanoid pathway and other toxic secondary metabolites that are involved in plant defense to herbivory and other stresses (Rao et al 1998; Zhao et al. 2009; Idrees et al. 2011). The role of PPO in plant defense against pests and pathogens has been widely studied by biologists, plant pathologists, and ecologists. The glandular trichomes of *Solanum berthaultii* Hawkes contains higher levels of PPO (70% of soluble protein), which catalyzes the oxidation and polymerization of phenolics on trichome breakage by insects and entraps the mobile insects. It eventually traps the insect and/or impedes their mouth parts with a sticky polymer (Kowalski et al. 1992). Moreover, quinones alkylate proteins during insect

feeding and degrade the amino acids in insect gut (Felton et al. 1992; Barbehenn et al. 2010). Under acidic conditions, quinones form semiquinone radicals that in turn give rise to ROS, while under basic conditions; quinines react with cellular nucleophiles (Bhonwong et al. 2009).

#### **2.3.3.3.3. Phenylalanine ammonia lyase**

Phenylalanine ammonia lyase (PAL) is a key enzyme that catalyzes the phenylalanine deamination to cinnamic acid that is the initial and preliminary step of phenylpropanoid pathway (Ritter and Schulz 2004). The PAL mediated pathway results in the production of phytoalexins and phenolics. It is a primary enzyme of phenyl propane biosynthesis, which is an important constituent of phenolic acids, lignins and flavonoids. The phenylpropanoid by-products alter the palatability and suitability of the plant tissues to insect pests. PAL activity is induced by various biotic and abiotic stresses including wounding, insect herbivory, and pathogen infection (Hahlbrock and Scheel 1989; Dixon and Paiva 1995; Zhao et al. 2009). Its *de novo* synthesis and increased activity is an initial defensive plant response to damage (Chaman et al. 2003; Qin et al. 2005). There are many reports showing upregulation of PAL activity in response to insect damage. For example, Ralph et al. (2006) observed over expression of PAL genes in Sitka spruce, *Picea sitchensis* (Bong.) in response to feeding by spruce budworm, *Choristoneura occidentalis* Lederer or white pine weevils, *Pissodes strobi* (Peck). Johnson and Felton (2001) showed that over-expression of PAL in *N. tabacum* is associated with reduced digestibility of leaves by the larvae of *Heliothis virescens* Fabricius. Greater induction of PAL activity in poplar plants infested with *Clostera anachoreta* Denis and Schiff., larvae was reported by Hu et al. (2009). *B. tabaci* infestation induced PAL activity in cucumber seedlings (Zhang et al. 2008). Aphid infestation increased the PAL activity in barley and cotton seedlings (Chaman et al. 2003; Qin et al. 2005). Furthermore, Zhao et al. (2009) observed an increase in PAL activity in

wheat infested by *Sitobion avenae* (F.); however, the differences were not significant. There was a considerable increase in PAL activity in alfalfa plants infested with *A. medcaginis* (Huang et al. 2007). Resistant cultivars of lettuce and barley exhibited greater induction in PAL activity in response to damage by lettuce root aphid, *Pemphigus bursarius* L. (Cole 1984) and greenbug, *Schizaphis graminum* (Rond.) (Chaman et al. 2003), respectively. Similarly, resistant cultivars of wheat showed greater induction of PAL activity in response to *S. avenae* infestation (Han et al. 2009). Sethi et al. (2009) reported negative correlation between PAL activity and banded cucumber beetle, *Diabrotica balteata* LeConte growth and development. There are many reports showing up regulation of PAL activity in response to insect damage, JA and SA application (Zhao et al. 2009; Kiselev et al. 2010). For example, Ralph et al (2006) observed over expression of PAL genes in Sitka spruce, *Picea sitchensis* Carr. in response to feeding by spruce budworms, *C. occidentalis*, or white pine weevils, *Pissodes strobe* W.D.Peck. Salicylic acid induced the expression of PAL genes (*VaPAL* and *VaPAL3*) in *Vitis amurensis* Rupr. cell culture (Kiselev et al. 2010).

#### **2.3.3.3.4. Lipoxygenase**

Lipoxygenase (linoleate: oxygen oxidoreductase) catalyzes the initial step of the JA mediated octadecanoid pathway. LOX is a non-heme iron-containing enzyme, which catalyzes the addition of molecular oxygen to linoleic acid (C18:2) and linolenic acid (C18:3) at either C9 or C13 position, and the primary products are 9S- or 13S-hydroperoxides, and are thus referred to as 9- or 13-LOX (Rosahl 1996; Feussner and Wasternack 2002; Porta and Rocha-Sosa 2002). These hydroperoxides (C9 or C13) are then metabolized into JA, MeJA, traumatin, conjugated dienoic acids, and volatile aldehydes (Anderson 1989; Creelman and Mullet 1997; Nemchenko et al. 2006), which are important players of plant defense against different stresses including insect damage (Siedow 1991; Fidantsef et al. 1999; Zheng et al. 2007; Wang et al. 2008; Bruinsma et al. 2009). LOX also



acts directly as a deterrent to insect pests (Felton et al. 1994b; Zhu-Salzman et al. 2004; Zhou et al. 2009). Oxidation of polyunsaturated fatty acids (PUFAs) such as PUFA-hydroperoxides, PUFA-hydroxides, or PUFA-ketones derived from the enzymatic action of LOXs play an important role in plant defensive response (Blee 1998; Feussner and Wasternack 2002). The hydro-peroxidelyase in association with PUFA hydroperoxides form C6 and C9 aldehydes, which mediate both direct and indirect plant defenses against insects (Matsui 2006; Maffei et al. 2007; Bhonwong et al. 2009). Chewing insects have been reported to elicit the long lasting elevation in JA activated defensive enzymes and PIs (Ryan and Pearce 1998). Fidantsef et al. (1999) observed that feeding by aphids also increased the LOX mRNA in tomato showing that aphid feeding modulates the JA synthesis pathways and plant defense. Moreover, the unstable reactive products interact with proteins resulting in protein-protein cross linking and amino acid damage that in turn affects the amino acid assimilation (Maffei et al. 2007). Furthermore, lipid peroxidation end products act as insect repellents or antixenotic agents (Bruinsma et al. 2009; Arimura et al. 2009), and are also directly toxic to insect pests (antibiosis; Felton et al. 1994b; Maffei et al. 2007; Bhonwong et al. 2009). Oxidation of linolenic acid in JA signaling pathway by LOX mediates both direct and indirect defense in plants against insects (Kessler et al. 2004; Mao et al. 2007; Bruinsma et al. 2009). Lipoxygenase pathway leads to the synthesis of some volatile organic compounds (VOCs) involved in attraction of natural enemies of insect pests (Boland et al. 1995; Kessler et al. 2004; Bruinsma et al. 2009), and expression of LOX genes are upregulated in plants in response to herbivore attack (Feussner and Wasternack 2002; Liavonchanka and Feussner 2006; Maserti et al. 2011). Induction of transcripts encoding LOX genes in tomato leaves in plants infested with *H. zea* has been reported by Fidantsef et al. (1999). In *N. attenuata* infestation by sap sucking *Myzus nicotianae* Blackman induced the expression of LOX genes (Voelckel et al. 2004). Upregulation of

cabbage *BoLOX* gene has been reported in response to several insect pests such as caterpillars [*P. rapae*, *Piers brassicae* (L.) and *Mamestra brassicae* (L.)], spider mites (*Tetranychus urticae* Koch), and locusts (*S. gregaria*) (Zheng et al. 2007). Strong induction of *OsLOX1* transcript occurs in rice upon infestation by the brown planthopper (BPH), *Nilaparvata lugens* (Stal.) (Wang et al. 2008), and of *OsHI-LOX* by BPH and striped stem borer, *Chilo suppressalis* (Walk.) (Zhou et al. 2009). Isozymes of lipoxygenase involved in synthesis of JA are induced by aphid damage in many crops including tomato, *Arabidopsis* and sorghum (Fidantsef et al. 1999; Moran and Thompson 2001; Zhu-Salzman et al. 2004). Zhao et al. (2009) observed higher LOX expression in *S. avenae* infested wheat plants than the uninfested ones. Infestation by *T. urticae* and treatment with MeJA induced higher levels of LOX in *Citrus clementina* (Hort. ex Tan) (Maserti et al. 2011). The LOX activity was greater in *H. zea* infested plants in cotton than in the uninfested plants (Bi et al. 1997). Hu et al. (2009) reported increased levels of LOX in poplar infested with *C. anachoreta* and sprayed with MeJA. Similarly, Gomi et al. (2002) observed the accumulation of LOX transcripts in *Citrus jambhiri* Lush., after wounding and infestation with *Alternaria alternata* Keissl. LOX pathway also regulates growth and development in plants (Anderson 1989; Siedow 1991; Feussner and Wasternack 2002).

#### **2.3.3.3.5. Superoxide dismutase**

The SOD plays an important role in plant defense against many biotic and abiotic stresses (Khattab and Khattab 2005; Sankar et al. 2007; Usha Rani and Jyothisna 2010). It acts as a first line of defense by catalyzing dismutation of superoxide ( $O_2^{\cdot-}$ ) to  $H_2O_2$ . The  $O_2^{\cdot-}$  is a highly reactive and unstable ROS, and is the first ROS to be formed in biological systems and the first univalent oxygen reduction product. Thus, SOD mediates the primary defense in scavenging the oxygen radicals through dismutation, which is catalyzed by metal ions such as, iron, copper or manganese. In addition, SOD also scavenges the extra  $H_2O_2$

and other toxic free radicals produced in plants on account of stresses. Induction of SOD activities in plants in response to insect damage has been reported in many plants. Khattab and Khattab (2005) demonstrated higher activities of SOD in *Eucalyptus obliqua* L'Herit infested with gall forming psyllids. SOD activity was induced in alfalfa plants when infested with *A. medcaginis* and the induction was more in resistance varieties than in the susceptible ones (Huang et al. 2007). *T. urticae* infestation and MeJA treatment induced higher levels of SOD activity in *C. clementina* (Maserti et al. 2011). Apel and Hirt (2004) observed induction of SOD in wheat infested with aphids. The *H. zea* infestation induced greater levels of SOD in tomato (Felton et al. 1994b) and soybean (Bi and Felton 1995) plants, and the induction was associated with lower plant damage and reduced larval growth and development.

#### **2.3.3.3.6. Ascorbate peroxidase**

The ascorbate peroxidase (APX) is involved in plant defense against a variety of stresses (Bi and Felton 1995; Bi et al. 1997; Felton and Summers 1993; Asada 1999; Mittler et al. 2004; Garcia-Pineda et al. 2004; Qureshi et al. 2007; Gill et al. 2010 Whitehil et al. 2011). It is regarded as an important plant defensive enzyme against insect pests and its expression is induced upon insect damage (Felton and Summers 1993; Bi and Felton 1995; Bi et al. 1997; Garcia-Pineda et al. 2004). APX reduces the excessive H<sub>2</sub>O<sub>2</sub> to water using ascorbic acid as hydrogen donor (Asada 1999; Qureshi et al. 2007). It also oxidizes phenolic compounds to quinines, which inhibit insect feeding (Felton et al. 1992; Dowd 1994), and scavenges the harmful free radicals (Kumari et al. 2006; Whitehil et al. 2011). Whitehil et al. (2011) reported that APX provides resistance to the *Fraxinus mandshurica* Rupr. against Emerald ash borer, *Agrilus planipennis* Fair. Gall forming psyllid infestation elevated the levels of APX in *Eucalyptus* (Khattab and Khattab 2005). Wei et al. (2009) reported upregulation of APX in rice leaves infested with *N. lugens*. Maserti et al. (2011)

demonstrated the induction of APX activity in *C. clementina* infested by *T. urticae*. Greater induction of APX activity in *C. grandiflorum* has been reported in response to *M. sanbourni* infestation (He et al. 2011). Increase in APX activity was reported in soybeans in response to *H. zea* infestation (Bi and Felton 1995). Felton and Summers (1993) observed that APX caused the significant loss of ascorbate from the midgut of *H. zea*, thereby affecting its growth and development. They observed greater induction of APX activity in the resistant genotypes than in the susceptible ones. In contrast, Barbehenn et al. (2008) did not find any effect on ascorbate levels in the midguts of *L. dispar* and *Melanoplus sanguinipes* (Fab.) fed on the transgenic poplar over-expressing APX.

#### **2.3.3.3.7. Catalase**

Catalase (CAT) is an important antioxidative enzyme involved in plant defense against a variety of stresses (Sankar et al. 2007; Qureshi et al. 2007; Usha Rani and Jyothsna 2010). However, studies on its role in plant defense against insects are limited. It is an important component of the oxygen-scavenging systems. It scavenges the toxic and unstable ROS and converts them into less toxic and more stable components such as H<sub>2</sub>O<sub>2</sub> and water without consuming cellular reducing equivalents (del Rio et al. 2002; Khattab and Khattab 2005; Qureshi et al. 2007; Sankar et al. 2007). Usha Rani and Jyothsna (2010) observed an increase in CAT activity in rice plants infested with *Scirpophaga incertulas* (Walk.) and *Cnaphalocrosis medinalis* (Gue.). Feeding by *H. zea* induced CAT activity in soybean (Bi and Felton 1995). Higher CAT activities were observed in *Eucalyptus* infested with gall forming psyllids (Khattab and Khattab 2005). An upregulation of genes for CAT has been observed in aphid infested resistant wheat plants (Boyko et al. 2006), and in *M. persicae* susceptible celery plants (Divol et al. 2007). Heng-Moss et al. (2004) did not find any direct correlation between CAT activity and plant resistance in buffalograsses, *B.*

*dactyloides* and damage by *B. occiduus*. No alteration in CAT activity was observed in *D. noxia* infested cereal leaves.

#### **2.3.4. Role of reactive oxygen species (ROS) in plant defense**

Oxidative state of plants is an important tactic that enables the plants to defend against various stresses. Rapid and transient generation of ROS is a common phenomenon in plants on account of oxidative stress due to biotic and abiotic factors (Maffei et al. 2007; Torres 2010). The ROS play versatile signaling functions that mediate multiple responses, and can also act directly as toxins. ROS include partially reduced forms of oxygen such as superoxide ( $O_2^-$ ), singlet oxygen ( $O^*$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl radicals ( $OH^\cdot$ ) (Ludwig et al. 2004; Maffei et al. 2007; Torres 2010). Distinct signaling pathways are activated by different types of ROS especially the ones involving mitogen activated protein kinases (MAPKs: Ludwig et al. 2004; Torres 2010). Under stress, there is a rapid accumulation of ROS, referred as the “oxidative burst” (Maffei et al. 2007). The ROS convert linolenic acid into phytoprostanes that signal transduction pathways (Maffei et al. 2007; Pieterse et al. 2009). Following insect attack, ROS accumulate in apoplastic as well as in symplastic regions, besides their main concentration in extracellular matrix, peroxisomes/mitochondria, and plasma membrane (Pei et al. 2000; Maffei et al. 2007). The ROS are directly toxic to insects and pathogens and also mediate the oxidative cross-linking of the cell wall (Lamb and Dixon 1997). In addition, ROS at lower concentration signal the transduction of various pathways that result in the production of plant defensive secondary metabolites (Orozco-Cardenas et al. 2001; Hancock et al. 2006). However, to prevent the self-toxicity of oxygen free radicals, plant cells have developed ROS scavenging systems

for removing the excess free radicals to maintain a relatively low and constant concentration (Maffei et al. 2007; Torres 2010).

Among all the ROS, H<sub>2</sub>O<sub>2</sub> plays an important role in plant defense against oxidative stress because of its high stability and freely diffusible property, and acts through signal transduction pathways, which lead to the expression of defense genes (Orozco-Cardenas et al. 2001; Foreman et al. 2003; Maffei et al. 2007). Although H<sub>2</sub>O<sub>2</sub> is produced in various ways, the oxidative burst is supposed to occur through the activation of membrane bound NADPH complex. NADPH oxidase generates superoxide anion at the plasma membrane or in the apoplast extracellularly, which is then converted to H<sub>2</sub>O<sub>2</sub> by SOD (Lamb and Dixon 1997; Orozco-Cardenas et al. 2001; Maffei et al. 2007; Torres 2010). Besides having direct effect on the pathogens and herbivores, H<sub>2</sub>O<sub>2</sub> stimulates a cascade of reactions that lead to the expression of defense genes, which prevent the plants from subsequent attack by pathogens and herbivores (Lamb and Dixon 1997; Torres 2010). H<sub>2</sub>O<sub>2</sub> acts as a second messenger in JA-mediated defense signaling that acts downstream from JA, and corresponds with oxidative damage in the midgut of insects feeding on previously wounded plants (Orozco-Cárdenas et al. 2001; Usha Rani and Jyothisna 2010). Greater accumulation of ROS has been reported in wheat and rice on infestation with Hessian fly [*Mayetiola destructor* (Say)] larvae (Liu et al. 2010). The LD50 value of H<sub>2</sub>O<sub>2</sub>, when incorporated in artificial diet has been found to be very less (< 0.05 µg mL<sup>-1</sup> or 1.7 µM) against *Drosophilla* larvae (Liu et al. 2010), suggesting that it can have detrimental effect on insect pests.

Furthermore, H<sub>2</sub>O<sub>2</sub> stimulates a cascade of events that trigger physiological and molecular plant responses to prevent or minimize the insect attack (Dangl and Jones 2001; Powell et al. 2006; Boyko et al. 2006; Maffei et al. 2006, 2007). Insects have been found to induce the accumulation of H<sub>2</sub>O<sub>2</sub> in many plants (Walling 2000; Powell et al. 2006; Maffei et al. 2006; Usha Rani and Jyothisna 2010; Barbehenn et al. 2010; He et al. 2011). Kempema

et al. (2007) reported that the mRNA levels of various genes involved in ROS scavenging were increased by *B. tabaci* feeding, which indicated that feeding by whitefly induced ROS. Moloj and van der Westhuizen (2006) reported that H<sub>2</sub>O<sub>2</sub> produced by NADPH oxidase activation is the main component of plant defense in wheat against *D. noxia* and activates the PODs involved in the wheat resistance response. A greater accumulation of H<sub>2</sub>O<sub>2</sub> was observed in *C. grandiflorum* upon infestation with *M. sanbourni* (He et al. 2011). In addition, H<sub>2</sub>O<sub>2</sub> has been found to be involved in many physiological processes, such as photosynthesis, photorespiration, senescence, cell cycle and growth and development (Noctor and Foyer 1998; Foreman et al. 2003; Torres 2010).

### **2.3.5. Response of biological control agents of *H. armigera* and *S. litura* to host plants**

More than 100 species of parasitoids have been reported on *H. armigera* and *S. litura*, some of which are being exploited as the important biological control agents (Bhatnagar et al. 1982; Wightman and Ranga Rao 1997; Sharma 2005). These parasitoids mostly belong to the families Ichneumonidae, Braconidae and Tachinidae (Ranga Rao et al. 1993). Parasitoids have developed various strategies to find their host. The parasitic phase is an important stage of the parasitoids life cycle and in this phase parasitoid completely depends on the host insect for survival and eventually kills the host. Both parasitoids and hosts are in arms race with each other. The host tries to avoid the detection by the parasitoids and the parasitoids develop new methods to locate the host. Host foraging by parasitoids has been divided by Vinson (1976) into five phases: host habitat finding, host location, host acceptance, host suitability, and host regulation. One of the major issues in plant defense against insect herbivores is parasitoid searching behavior and the efficiency to use host location cues. Parasitoids exploit both the insect host and the plant cues to locate the host (Turlings and Wackers 2004; Erb et al. 2010). Thus, olfactory perception is considered important in host selection. Genotype preference by parasitoids is very important

for the control of insect pests. The most common technique for examining host preference of natural enemies is use of olfactometer.

The parasitoid *Campoletis chloridae* Uchida has been reported on various crops including chickpea, cotton, groundnut (Nath and Rai 1999; Mishra and Shrivastava 2000; Devi et al. 2002). It is an important biological control agent of many insect pests including *H. armigera* (Bhatnagar et al. 1982; Pandey et al. 2004; Chandel et al. 2005) and *S. litura* (Battu 1977; Sathe 1987; Ranga Rao et al. 1993). The *C. chloridae* deposits its eggs singly in the host larvae, which dies in third or fourth instar. Parasitoid larvae emerge from second and fourth instar host larvae and pupate by spinning a cocoon (Pawar et al. 1989; Venkatesan et al. 1995), however, parasitization is more successful on second instar host larva. The total developmental period of *C. chloridae* on *S. litura* is about 17.4 days with longevity of 19 days and fecundity 60 eggs per female (Venkatesan et al. 1995).

The egg parasitoids, *Trichogramma* spp. are considered as the potent biocontrol agents and are being used worldwide against Lepidopteran pests in many crops (Smith 1996; Wajnberg and Hassan 1994; Hou et al. 2006; Shahid et al. 2007). *Trichogramma chilonis* Ishii is the most important egg parasitoid of *H. armigera* (Smith 1996; Romeis Shanower 1996). It is being used in augmentative-release programs for the control of *H. armigera* and *S. litura* (Bhatnagar et al. 1982; Manjunath et al. 1989; Ranga Rao et al. 1993; Wightman and Ranga Rao 1997). Bhatnagar et al. (1982) reported about 80% of parasitization of *H. armigera* eggs by *T. chilonis* in sorghum. *T. chilonis* showed 100% parasitism depending upon the availability of favorable condition (Hou et al. 2006; Shahid et al. 2007), and is an important component of integrated pest management (IPM; Smith, 1996; Romeis et al. 2005; Hou et al. 2006; Shahid et al. 2007).



Albeit direct defenses have a major role in host plant resistance against insects, indirect defense forms an important component in pest control through the attraction of carnivores (parasitoids and predators). Plants emit a greater amount of volatiles upon infestation by insect herbivores, which otherwise are released in lesser amounts. Although, parasitoids are attracted to the chemical cues from insect herbivores, which are more reliable, the herbivores are usually imperceptible, because of their small size in comparison to the host plant. Thus natural enemies of these insect herbivores often depend on the plant cues to locate the insect pest (Turlings et al. 1991; Steinberg et al. 1993; Geervliet et al. 1994). Moreover, the perception of herbivore derived cues by natural enemies is often limited, because of their low detectability, particularly when at large distances (Vet and Dicke 1992). Thus, plant derived volatiles are the important and strongly perceived cues by carnivorous insects as compared to the insect pest derived cues (Turlings et al. 1991; Steinberg et al. 1993; Geervliet et al. 1994; Dicke 1999). These HIPVs even facilitates intra- and interplant communication (Lewis and Martin 1990; Kost and Heil 2006; Arimura et al. 2009).

Attraction of insect parasitoids by volatiles emitted from damaged plants has been well documented. Methyl benzoate (MeBA), which structurally resembles MeSA, has also been detected from insect-infested plants (Chen et al. 2003). The *S. frugiperda* infestation in rice induces emission of about 30 volatiles, including MeSA and MeBA, which are highly attractant to the natural enemies including *Cotesia marginiventris* (Cres.), an important parasitoid of *S. frugiperda* and *Mythimna separata* (Walk.) (Yuan et al. 2008). The HIPVs are involved in interaction of plants with insect natural enemies, the neighboring plants, and different parts of the same plant. There are many reports of attraction of parasitoids towards the cues from host plants through olfactometer studies (Guerrieri et al. 1999; Geetha 2010; Peñafior et al. 2011). Moreover, plant chemical cues can help phoretic egg parasitoids to

locate host adults on which they can ride to locate new oviposition sites (Fatouros et al. 2007). *H. zea* infested cowpea plants strongly attracted the females of the braconid wasp, *Microplitis croceipes* (Cresson), and the ichneumonid wasp, *Netelia heroica* Tow., than the undamaged plants (Whitman and Eller 1990). Female *Cotesia rubecula* (Mar.) were attracted more towards the *Brassica oleracea* var. *gemmifera* Zen. damaged by larval feeding of *P. rapae* (Geervliet et al. 1994). Similarly, Turlings et al. (1991) observed greater preference of female *C. marginiventris* toward the *S. exigua* damaged plants.

### **2.3.6. Role of phytohormones in induced resistance in plants**

When plants are infested by insects, either by leaf chewing, phloem ingestion, or cell content feeding, phytohormone signaling pathways are activated and plant defensive response is elicited. A growing body of evidences suggests the role of plant hormones such as JA, SA and ethylene in plant defense against insect pests. The JA and SA are regarded as the most important plant defense signaling molecules that induce different antioxidative enzymes and secondary metabolites, thereby, enhance the host plant resistance against insect herbivores and other stresses (Farmer and Ryan 1990; Steppuhn and Baldwin 2007; Westernack 2007; Zhao et al. 2009; Shivaji et al. 2010). Moreover, insect damage has been reported to trigger the induction of JA and SA, which in turn signal the expression of induced defensive enzymes such as POD, PPO, PAL, LOX, PIs and secondary metabolites, and also the emission of plant volatiles (Farmer and Ryan 1990; Steppuhn and Baldwin 2007; Zhao et al. 2009; Pieterse et al. 2009; Shivaji et al. 2010). Furthermore, exogenous application of JA, SA, and their precursors and derivatives also induce the production of defensive proteins and other nonprotein compounds in plants (Thaler et al. 1996; Zhao et al. 2009; Pieterse et al. 2009; Shivaji et al. 2010), besides increasing plant fitness, increased parasitism, and reduced growth and development of insect pests (Kessler and Baldwin 2002; Verhage et al. 2010). Specific sets of defense related genes are activated by these pathways

upon wounding or by insect feeding. These hormones may act individually, synergistically, or antagonistically, depending upon the attacker. Although there are many reports suggesting the negative cross-talks between SA and JA, recent studies have shown the overlapping and even synergistic effects of these hormones (Schenk et al. 2000; Zhao et al. 2009).

#### **2.3.6.1. Jasmonic acid**

Jasmonic acid is the most important phytohormone linked to plant defense against herbivores and activates the expression of both direct and indirect defenses (Farmer and Ryan 1990; Steppuhn and Baldwin 2007; Shivaji et al. 2010; Scott et al. 2010). JA and its precursors and derivatives, collectively called jasmonates, represent a family of oxylipins that play an important role in a variety of plant processes including plant defense against insects and pathogens, abiotic stresses, growth and development, fertility and senescence (Kumari et al. 2006; Wasternack 2007; Shivaji et al. 2010; Scott et al. 2010; Kanno et al. 2011). Jasmonates are derived from linolenic acid through octadecanoid pathway (Wasternack 2007), and accumulates upon wounding and herbivory in plant tissues (Shivaji et al. 2010; Stout et al. 2009; Kanno et al. 2011). Jasmonic acid gets quickly accumulated in plant tissues surrounding the site of damage (Kanno et al. 2011), and induction of endogenous JA leads to the modulation of resistance related gene expression and the defensive metabolites involved in plant defense against herbivory (Korth and Thompson 2006; Bruinsma and Dicke 2008). A large number of genes involved in defense against herbivores are regulated by JA (Cipollini et al. 2004; Shivaji et al. 2010). Moreover, exogenous application of JA stimulates the changes in plants similar to those induced by insect herbivory (Farmer and Ryan 1990; Thaler et al. 1996; Kessler and Baldwin 2002; Browse and Howe 2008).

JA induced defenses play important role in mediating the physiological signals and plant interactions with a diverse array of ecological factors, including consumers, competitors and beneficial organisms. Kanno et al. (2011) found that white-backed planthopper, *Sogatella furcifera* (Horv.) infestation resulted in accumulation of JA in rice. Chewing of plant parts by insects causes the conversion of linolenic acid from cellular membranes into 12-oxophytodienoic acid (12-OPDA) by allene oxide synthase and allene oxide cyclase. OPDA is transferred to the peroxisome, where it is reduced by OPDA reductase 3 (OPR3), forming JA after decarboxylation (Walling 2000; Wasternack 2007). In addition to its role in the production of JA, OPDA signals the defense pathways individually. For example, OPDA signaling regulates the CORONATIN-INSENSITIVE 1 (COI1) -dependent and -independent transcription (Ribot et al. 2010), alters the intracellular calcium levels and cellular redox status (Walter et al. 2007). Jasmonates (most likely the JA-amino acid conjugate jasmonoyl–isoleucine) have been found to interact with the COI1 unit of an E3 ubiquitin ligase complex, termed SCFCOI1 (Skip/Cullin/F-box–COI1), which promotes binding of the COI1-unit to JAZ (jasmonate ZIM-domain) proteins, resulting in degradation of JAZ proteins, which otherwise suppresses JA-inducible gene expression (Sheard et al. 2010). A broad spectrum of defensive responses are induced by jasmonates that include antioxidative enzymes, PIs, VOCs (Parra-Lobato et al. 2009; Scott et al. 2010), alkaloid production, trichome formation, and secretion of EFN (Arimura et al. 2008; Wang et al. 2008). Concentration of indole glucosinolate, an important defensive compound, is induced by jasmonates (Cipollini et al. 2004). Furthermore, induction of arginase and Thr deaminase (TD2) by JA degrades the amino acids necessary for insect growth (Chen et al. 2005). JA has also been reported to affect calcium-dependent protein kinases (CDPK) transcript and activity in plants. CDPKs comprise of a large family of serine/threonine kinases in plants (34 members in *Arabidopsis*) and play an important role in plant defense

against a variety of biotic and abiotic stresses through signal transduction (Ludwig et al. 2004).

Effect of exogenous application of JA against insect herbivory has been well established. Exogenous application of JA in tomato induced plant defensive proteins and volatile compounds that attract the natural enemies of insect pests (Cipollini and Redman 1999; Thaler et al. 2002). The EFN produced by JA is used as an alternate food by natural enemies of insect pests (Kost and Heil 2005). JA induces various plant defensive enzymes involved in resistance against different stresses (Stout et al. 2009; Zhao et al. 2009; Shivaji et al. 2010). Cipollini and Redman (1999) reported that JA and/or MeJA at 1 mM concentration induced the activities of POD and PPO in tomato, and resulted in reduced larval weight of *M. sexta*. Wu et al. (2008) reported JA burst in the wild type *N. attenuata* plants treated with MeJA, which in turn increased the levels of phenolics, flavonoids, nicotine and trypsin proteinase inhibitors, and ultimately plant resistance against *Manduca sexta* (L.) larvae. Derivatives of JA such as MeJA induce antioxidant defense in sunflower seedlings (Parra-Lobato et al. 2009). Exogenous application of JA induced resistance in rice against rice water weevil, *Lissorhoptrus oryzophilus* Kuskel (Hamm et al. 2010) and *S. frugiperda* (Stout et al. 2009). Both the studies showed reduced larval infestation in JA treated plants. Infestation with *S. litura* and application of MeJA has been investigated to elevate the activity of PPO in radish, sweet pepper, tomato, and water spinach (Tan et al. 2011). Foliage application of MeJA led to the accumulation of phenolic compounds in maritime needles (Sampedro et al. 2011).

#### **2.3.6.2. Salicylic acid**

Salicylic acid, a benzoic acid derivative, is an important phytohormone involved in regulation of plant defense. It is an endogenous plant growth regulator that generates a wide

range of metabolic and physiological responses in plants involved in defense (Ananthkrishnan 1997; Zhao et al. 2009; Vicent and Plasencia 2011; Kanno et al. 2011). SA induces greater defense against piercing and sucking type of insect pests and also against the chewing pests (Ananthkrishnan 1997; Zhao et al. 2009; Peng et al. 2004; Thaler et al. 2010; Kanno et al. 2011). Its signaling is involved in local defense as well as induction of systemic resistance. Production of ROS by SA pathway has been proposed to induce resistance in plants against insect pests. SA mediates the production of H<sub>2</sub>O<sub>2</sub>, which in turn mediates plant defense against various insect pests, and H<sub>2</sub>O<sub>2</sub> actively damages the digestive system of insects leading to reduced growth and development (Maffei et al. 2007; Peng et al. 2004). Feeding by *M. persicae* induced the expression of genes associated with SA, and the genes involved in JA mediated pathways (Moran and Thompson 2001). Kanno et al. (2011) observed the accumulation of SA in rice infested with *S. furcifera*. Peng et al. (2004) recorded the induction of resistance by SA in tomato against *H. armigera*. Similar results were observed by Lamb and Dixon (1997). Ollerstam and Larson (2003) reported the induction of plant resistance in willow (*Salix viminalis* L.) against the gall midge, *Dasineura marginemtorquens* Bremi. Furthermore, SA signals the release of plant volatiles that attract the natural enemies of the insect pests, e.g., Lima bean and tomato plants infested by the spider mite attract the natural enemies of spider mite (De Boer et al. 2004).

Studies on marker genes associated with SA and JA/ET signaling have shown that aphids elicit all the three pathways mediated by JA, SA and ET signaling; however, the induction of SA signaling is more pronounced (Moran and Thompson 2001; Zhu-Salzman et al. 2004; Zhao et al. 2009). MeSA serves as a volatile signal that triggers induced defenses in plants, including HIPV emission, and a number of predaceous arthropods are attracted to MeSA under field conditions (De Boer et al. 2004; Maffei et al. 2007). SA application resulted in the reduced larval survival of the gall midge in willow (*Salix*

*viminalis* L.) and the SA-mediated gene expression was more in midge-resistant cultivars (Ollerstam and Larsson 2003). Moran and Thompson (2001) observed reduced fecundity of green peach aphid in SA treated *Arabidopsis*. The increase in SA and H<sub>2</sub>O<sub>2</sub> has also been demonstrated in cotton infested by the generalist, *H. zea* (Bi et al. 1997). SA induced resistance in maize against drought was pronounced through the increased activities of SOD, CAT, APX, glutathione reductase, monodehydroascorbate reductase, dehydroascorbate reductase and H<sub>2</sub>O<sub>2</sub> (Saruhan et al. 2012). In contrast Bi et al. (1997) did not observe the induction of resistance by SA in cotton against *H. zea*. SA application has been reported to induce greater levels of phenols in chickpea and groundnut (War et al. 2011a,b). Recently, it has been reported that SA when incorporated into artificial diet interferes with *H. armigera* respiratory complex leading to the inhibition of various enzymes (Akbar et al. 2011).

#### **2.4. Plant defense response and oviposition behavior of insect pests**

Insect oviposition is the first encounter between most of the insect pests and host plants and the oviposition preference or non preference is the most important step to determine plant resistance/or susceptibility to the insect pests. The successful oviposition will result in the successful emergence of the larvae and the greater infestation. So plants have evolved various defensive tactics to avoid oviposition by insect pests. It has been reported that plants can respond to insect oviposition and targets the attacker before plant harm is initiated. Plants respond to insect through various defensive strategies. Insect pests locate the host plants for oviposition based on the odor and visual stimuli. Female moths use various physical and chemical cues to select the suitable host for oviposition, and leaf surface chemicals play an important role in the selection of host plant for oviposition (Hilker et al. 2002; Chamarthi et al. 2011). Plants respond to insect oviposition through both direct and indirect defenses, which aim to get rid of the insect eggs and/or to kill them, thus

avoiding the damage by larvae that would hatch from them (Hilker et al. 2002; Hilker and Meiners 2006, 2010). Induced secondary metabolites, antinutritive compounds and toxins in plants in response to insect infestation and/or JA application result in the decreased oviposition and reduced insect growth and development (Thaler et al. 2002; Bruinsma and Dicke 2008). Oviposition also induces the release of volatiles in plants that attract natural enemies of the insect pests, thereby mediating tritrophic interaction, which is an important component of indirect defense (Hilker et al. 2002; Hilker and Meiners 2006, 2010), and the change in leaf surface chemicals also arrest egg parasitoids (Fatouros et al. 2007). The neoplasm formation is also an important and effective strategy developed by plants to avoid insect oviposition (Doss et al. 2000). It has been reported that in pea plants, eggs laid by pea weevil induces neoplasm formation, which dislodges the eggs by raising them above the surface (Doss et al. 2000). Hypersensitive response by plants is one more important plant defensive mechanism against insect oviposition (Shapiro and Devay 1987; Balbyshev and Lorenzen 1997; Desurmont and Weston 2011). Oviposition of *P. brassicae* and *P. napi* on *B. nigra* resulted in a hypersensitive response in the plant tissues within 24 hours of oviposition and caused egg killing in three days as the larvae rarely find way back to the host plant (Shapiro and Devay 1987; Balbyshev and Lorenzen 1997). Detachment of eggs through necrotic tissue formation has been reported in potato in response to Colorado potato beetle, *L. decemilineata* (Balbyshev and Lorenzen 1997). Oviposition also induces the production of ovicidal compounds that kill the eggs. For example, white backed plant hopper, *S. furcifera* oviposition induces the expression of a specific gene (*ovc*) that is involved in the production of an ovicidal compound, benzyl benzoate (Seino et al. 1996; Yamasaki et al. 2003). Oviposition non preference is a major component of host plant resistance to insect pests (Sharma and Nwanze 1997; Kessler and Baldwin 2002). The *P. brassicae* and *P. rapae* females have been found to prefer laying eggs on the plants without



eggs (Kirby and Spence 1863; Rothschild and Schoonhoven 1977; Bruinsma et al. 2009). The avoidance of oviposition has been associated with the oviposition induced plant defense and not to the chemicals released by the insect (Blaakmeer et al. 1994). Rothschild and Schoonhoven (1977) reported the avoidance of oviposition by *P. brassicae* females on the plants infested by insects and suggested that the avoidance was due to the herbivore induced plant defense that could affect the larval performance (Thompson and Pellmyr 1991; Shiojiri et al. 2002). JA is considered as an important elicitor of oviposition induced resistance. JA has been reported in the eggs of various Lepidopteran insects in higher concentration than in plant tissues or larval diet (Stanjek et al. 1997; Kessler and Baldwin 2002; Hilker and Meiners 2006). Furthermore, JA treated plants received less number of eggs from *P. rapae* and *P. brassicae* as compared to the untreated control plants (Bruinsma et al. 2009).

Inhibition of JA mediated response increased the oviposition and performance of insect herbivores (Stotz et al. 2002; Van Poecke and Dicke 2002; Reymond et al. 2004). The *S. littoralis* infested plants have been found less preferred by moths for oviposition (Anderson and Alborn 1999). MeSA released during infestation inhibits the oviposition of cabbage moth *M. brassicae* (Ulland et al. 2008). The tissue wounding by *Viburnum* spp. in response to *Pyrrhalta viburni* (Paykull) oviposition is a strong defensive response that causes egg destruction and/or expulsion of eggs (Desurmont and Weston 2011). Reduction of oviposition by plants can form an important and advantageous aspect of plant defense, since the insects can be restricted even at egg stage. The *Physalis pubescens* L. and *Physalis angulata* L. respond to *Heliothis subflexa* Guenee oviposition through necrosis, neoplasm and the combination of both (Petzold-Maxwell et al. 2011). Wireworm infestation in roots of cotton plants, *Gossypium hirsutum* L. led to the reduced oviposition by aboveground herbivore, *S. littoralis* as compared to the undamaged plants (Anderson et al. 2011). The

insect damaged tobacco plants deter the *H. virescens* females from oviposition and this deterrence has been attributed to the volatiles emitted by the plants after insect infestation (De Moraes et al. 2001). Furthermore, *Manduca quinquemaculata* (Haw.) females lay less number of eggs on the insect damaged and JA treated plants (Kessler and Baldwin 2002).

## **2.5. Effect of plant induced resistance on insects**

**2.5.1. Insect digestive enzymes:** The ability of insect pests to obtain required essential amino acids from dietary protein is very important for optimal growth and development. Any alteration in protein quality and quantity will pose a major challenge to insect growth as proteins are very important and commonly limiting nutrients for insect growth (Karowe 1989; Berenbaum 1995). A number of toxic secondary metabolites and defensive proteins are induced in plants in response to insect herbivory, which are either directly toxic to insect pests and/or reduce the nutrient quality of plant tissues, thereby, depriving off the insect of the essential nutrients (Green and Ryan 1972; Farmer and Ryan 1990; Chen et al. 2005; Scott et al. 2010). Plant proteinase inhibitors play a central role in plant defense against insect pests. PIs affect the insect midgut enzymes and inhibit their activity, thereby, reducing the insect growth and development (Green and Ryan 1972; Lawrence and Koundal 2002; Parde et al. 2010, 2012). An important PI in plants is the proteinases inhibitor II (PIN2), a serine proteinase inhibitor with trypsin and chymotrypsin inhibitory activities (Lawrence and Koundal 2002), and occurs in many plants (Pearce et al. 1991; Luo et al. 2009). The target enzymes of PIs are insect proteases. Proteases are the important enzymes involved in post-translational modification of proteins by mediating proteolysis at specific sites. In addition, lectins (carbohydrate binding proteins) are also important plant defensive proteins. They act as antinutritive and/or toxic substances by binding to membrane glycosyl groups lining the digestive tract, leading to an array of harmful systemic reactions (Chakraborti et al. 2009; Vandenborre et al. 2011), and thereby, interfere with the nutrient

digestion and absorption (Chakraborti et al. 2009). Disruption of lipids, carbohydrates, and proteins causes enlargement and/or atrophy of key tissues, which in turn alters the hormonal and immunological status, threatening the growth and development of insects (Saha et al. 2006; Chakraborti et al. 2009; Vandenborre et al. 2011).

During the last three decades, much effort has been put into the study of insect digestive enzymes, mainly of serine proteinases, which are the potent insect metabolic enzymes and are very important for insect growth and development (Pearce et al. 1991; Luo et al. 2009). Serine proteases are the major digestive proteinases in midgut of Lepidopteran larvae and protein components in peritrophic membranes are regarded highly resistant to digestive serine proteinase degradation (Wang and Granados 2001; Pechan et al. 2002; Li et al. 2009). They are involved in insect resistance to plant defensive compounds such as serine protease inhibitors (Jongsma et al. 1996; Mazumdar-Leighton and Broadway 2001; Haq et al. 2004). These enzymes are the main targets of the toxic plant secondary metabolites. Due to the alkaline pH (8-11) of insect gut, Lepidopteran, Dipteran, Orthopteran and Hymenopteran insects digest plant foods by serine proteases and metalloproteases (Ryan 1990). They are also the most abundant proteins in insect gut and play an important role in insect-plant interactions (Liao et al. 2007). They are utilized as digestive endopeptidase by the Lepidopteran larvae and their role in plant defense against plant serine protease inhibitors in the insect-plant interaction has been well established (Jongsma et al. 1995, 1996; Mazumdar-Leighton and Broadway 2001). Serine proteinase inhibition has been found to have marked effects on insects, since they are the main constituents of protein digestion (Azzouz et al. 2005; Habib and Fazili 2007; Hartl et al. 2010). Trypsin is involved in peptide bond hydrolyzation of the proteins, where the carboxyl groups are contributed by the lysine and arginine residues. Due to the ability of

protease inhibitors to inhibit insect midgut proteases, PIs has received attention as a target for biocontrol of insect pests.

### **2.5.2. Insect detoxifying enzymes:**

Enzymatic detoxification of toxic chemicals mediates the adaptation of insects to plants allelochemicals and plays an important role in chemical based insect-plant interaction (Lindroth 1989; Loayza-Muro et al. 2000; Francis et al. 2005). The mechanisms of detoxification that operate in insects depends on the host plant chemistry (Nitao 1989), and its levels are generally influenced by concentration of the allelochemicals in the plant (Wadleigh and Yu 1988; Leszczynski and Dixon 1992). The role of insect detoxification enzymes in the metabolism of insecticides, allelochemicals, and other xenobiotics has been studied in considerable detail (Conyers et al. 1998; Ortego et al. 1999; Francis et al. 2005; Chrzanowski et al. 2012). Monooxygenases, glutathione-S-transferase (GST) and esterase (EST) are the important detoxifying enzymes involved in metabolism of a broad range of foreign and endogenous compounds in insects, and play a potent role in insect defense against toxic compounds (Yu 1995; Conyers et al.1998; Francis et al. 2005; War et al. 2011c). GST defends insects through detoxification of toxic compounds including allelochemicals from plants, and is involved in insect resistance to host plant defense (Wadleigh and Yu 1988; Yu 1996; Francis et al. 2005; Scott et al. 2010). This family of enzymes has been implicated in neutralizing the toxic effects of insecticides (Huang et al. 1998; Ranson et al. 2001). It has been proposed that GST contributes to isothiocyanate detoxification in glucosinolate-feeding species (Wadleigh and Yu 1988). There are a number of reports showing the influence of glucosinolate-containing plants or other plant secondary metabolites incorporated in artificial diet on GST activity in insect herbivores including *S. frugiperda*, *S. litura*, *T. ni*, *M. persicae*, *Aulacorthum solani* (Kalt.) and *Acyrtosiphon pisum* Harris (Francis et al. 2005; Sintim et al. 2009). Glutathione-S-

transferase also detoxifies the plant xenobiotics and the insects are adapted to a broader range of plant chemicals (Leszczynski and Dixon 1992; Francis et al. 2005; Sintim et al. 2009). The conjugation of glutathione with electrophilic molecules and their subsequent elimination by increasing the solubility is catalyzed by GST (Enayati et al. 2005). The GST mediated metabolism has been reported to be induced by allelochemicals. The overproduction of GST in *M. persicae* has been attributed to insect adaptation to glucosinolates and isothiocyanates in members of *Brassicaceae* (Francis et al. 2005). Insects react strongly to the toxic allelochemicals, when provided with the natural host plant diet or incorporated in the artificial diet, by increasing the metabolic mechanisms that result in the production of detoxifying enzymes, such as monooxygenases, GST and esterase (Isman and Duffey 1982; Wadleigh and Yu 1988; Lee 1991; Vanhaelen et al. 2003; Krishnan and Kodrik 2006). Alteration in insect mid-gut enzymes by plant allelochemicals have been reported in *S. litura* (Mukherjee et al. 2003; Sintim et al. 2009). War et al. (2011a) observed the alteration in GST and esterase activity of *S. litura* treated with botanical pesticides and suggested that the presence of plant secondary metabolites could have regulated the GST activity.

Esterases are important detoxifying enzymes involved in insect resistance against insecticides and plant secondary metabolites (Lindroth 1989; Loayza-Muro et al. 2000). Esterase is one of the most important targets of the synthetic insecticides; however, insects have developed resistance to insecticides by producing large quantities of detoxifying enzymes such as, GST and esterase. Increased esterase activities are associated with insect resistance, and inhibition in esterase activity will have drastic effects on insect growth and development (Ahmad and Pardini 1990). Furthermore, enhanced levels of esterase in early days of development have been correlated with the higher food consumption and growth and maturation of adult females in *L. dispar* (Kapin and Ahmad 1980). Allelochemicals

could either reduce or inhibit detoxification mechanisms and possibly increase the susceptibility of insects to insecticides (Yu and Abo-Elghar 2000; Scott et al. 2010). Plant secondary metabolites have also been found to inhibit the esterase activity in insect pests (Smirle et al. 1996; Mukanganyama et al. 2003; Senthil Nathan et al. 2008; Caballero et al. 2008). The inhibition of esterase activity by plant secondary metabolites could result in increased susceptibility of insect pests, thereby could be an important indicator of plant resistance against insect pests.

## **2.6. Host plant resistance in groundnut**

Host plant resistance plays an important role in groundnut defense against a variety of insect pests. Trichomes have been associated with resistance in groundnut against jassids and thrips (Campbell et al. 1976; Dwivedi et al. 1986). Phenols and tannins induced by organic manures contribute to groundnut resistance against *S. litura* and *H. armigera* (Stevenson et al. 1993; Senguttuvan and Sujatha 2000; Rao 2003). Resistance against *A. craccivora* has been attributed to high levels of procyanidin polymers (condensed tannins) (Grayer et al. 1992; Rao 2003). Moreover, procyanidin at 0.005% in artificial diet reduced the honey dew production by *A. craccivora* (Grayer et al. 1992). Flavonoids such as quercetin 3-arabinosylgalactoside and quercetin 3-galactoside, kaempferol etc., are the important plant defensive components against many insect pests (Wightman and Ranga Rao 1994). The resistance to leaf miner (*A. modicella*) in groundnut has been attributed to the higher concentration of nitrogen, soluble sugars and polyphenols (Rao et al. 1998). Stevenson et al. (1993) observed the biochemical mechanism of resistance in groundnut genotypes against *S. litura* and concluded that quercetin, caffeoylquinic acids and diglycosides contribute to the resistance. They also showed that rutin and chlorogenic acid were highly toxic to *S. litura*.

Like other crops, groundnut also responds to various biotic and abiotic stresses. Induced resistance in groundnut against salinity showed the induction of various antioxidative enzymes such as POD, APX, CAT and secondary metabolites (Sankar et al. 2007). Water stress induced various antioxidative enzymes in groundnut. These include CAT, APX and POD (Neto et al. 2010). Groundnut plants infested with *Cercosporidium personatum* (Berk. and Curt.) showed induced activities of POD, PAL and LOX (Usha Rani and Jyothsna 2009). Furthermore, JA induced the activities of POD, SOD, CAT, and secondary metabolites in groundnut seedlings (Kumari et al. 2006). Induced resistance in groundnut by chitosan against leaf rust caused by *Puccinia arachidis* Speg has been studied by Sathiyabama and Balasubramanian (1998). Chitosan induced the endogenous concentration of SA and the activities of chitinase and  $\beta$ -glucanase, which in turn inhibited the growth of the pathogen. Cardoza et al. (2003) studied the induction of resistance in groundnut by fungus, *Sclerotium rolfsii* Saccodes against *S. exigua*. Foliar application of SA induced resistance in groundnut against leaf blight caused by *A. alternata* through the elevation of the activities of POD, PAL, chitinase,  $\beta$ -1,3 glucanase and the amounts of phenols after application of SA and inoculation with *A. alternata* (Chitra et al. 2008). Naz (2006) observed the increased phenolic content in groundnut treated with SA (500 ppm) + mepiquat chloride (1 ml L<sup>-1</sup>) and tannins (500 ppm) + mepiquat chloride (1 ml L<sup>-1</sup>) at 60 days after sowing. Induction of POD activity in groundnut by *Pseudomonas fluorescens* Migula contributes to induced resistance against nematode *Meloidogyne arenaria* Chitwood (Kalaiarasan et al. 2010). Furthermore, APX and CAT activity has been found to defend groundnut plants under drought stress (Akçay et al. 2010). The levels of SOD, CAT, POD, and of lipid peroxidation increased in groundnut seedlings on JA application (Kumari et al. 2006). Moreover, SA induces the resistance in groundnut against *Peanut mottle virus* through the elevation of activities of POD, APX, CAT, SOD and PAL (Kobeasy et al.

2011). War et al. (2011a,b) observed the induction of plant defensive enzymes and secondary metabolites in groundnut on application with SA.

Our understanding of plant defensive mechanisms against insect pests in groundnut, an important oilseed crop in India, is still limited. The present studies, were therefore, focused on plant defensive responses of groundnut genotypes against *S. litura* and *H. armigera* - the two important leaf defoliators, and the sap sucking insect, *A. craccivora* to gain an understanding of the possible use of induced plant responses. Furthermore, induction of resistance against insect pests by jasmonic and salicylic acids was also studied.



*Chapter 3*

*Materials and*

*Methods*

## MATERIALS AND METHODS

The following materials and methods were used to carry out the present work.

### 3.1. Chemicals

The chemicals used in this study were of analytical grade. Ethylene diamine tetra acetic acid (EDTA), bovine serum albumin (BSA), guaiacol, polyvinylpyrrolidone (PVP), jasmonic acid, salicylic acid, tannic acid, vanillin, linoleic acid, dithiothreitol (DTT), disodium hydrogen phosphate, sodium dihydrogen phosphate, nitro-blue tetrazolium salt (NBT), methionine, L-phenylalanine, 4-chloronaphthol, glucose, potassium iodide (KI), trypsin inhibitor, sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), and vanillin were obtained from Sigma Aldrich, USA. Catechol was obtained from Glaxo Laboratories, Mumbai, India. Coomassie brilliant blue-G250, tris-HCl, N,N,N',N'-tetramethy ethylene diammine (TEMED), ammonium persulphate (APS), acrylamide, N,N-methylene bisacrylamide, glycine, and trichloroacetic acid (TCA) were obtained from Sisco Research Lab., Mumbai, India. 2-mercaptoethanol, gallic acid and Folin-Ciocalteau reagent were obtained from Merck, Mumbai, India. Thiobarbituric acid (TBA), sucrose, DL-1,3-dihydroxyphenyl alanine (DOPA) and linoleic acid were obtained from HiMedia Pvt. Ltd., Mumbai, India. Ammonium sulphate was obtained from Qualigens Fine Chemicals, Mumbai.

The spectrophotometer used for the estimation of biochemical parameters was Hitachi UV – 2900 (Hitachi, Japan).

## 3.2. Insects

### 3.2.1. *Helicoverpa armigera*

*Helicoverpa armigera* larvae were collected from the field at ICRISAT, Patancheru, Andhra Pradesh, India, and reared on the natural host for one generation under laboratory conditions before introgression into the laboratory culture to avoid any viral, bacterial, or fungal infection. The larvae were reared on chickpea based artificial diet (Armes et al. 1992) at  $27 \pm 1$  °C. The pupae were washed with 2% sodium hypochlorite solution and transferred to plastic jars containing Vermiculite. Adults were transferred to wooden oviposition cages (30 x 30 x 30 cm), and provided with 10% honey or sucrose solution in a cotton swab as a food. Diaper liners (5 x 15 cm) and thin cotton wool sheets, which have a rough surface, were hung inside the cage as an oviposition substrate. The liners were removed daily and the eggs sterilized in 2% sodium hypochlorite solution. The liners were dried under a fan, and then placed inside the plastic cups for egg hatching. The *H. armigera* neonates were reared in groups of 200 to 250 in 200 ml plastic cups (having 2 to 3 mm layer of artificial diet on the bottom and sides) for five days. After five days, the larvae were individually reared in six cell well plates with each cell well 3.5 cm in diameter and 1.5 cm in depth, to avoid cannibalism. Each cell well was filled with sufficient amount of diet (7 ml) to support larval development until pupation. The laboratory culture was supplemented with field-collected population every six months to maintain the heterogeneity of the laboratory culture. Neonate larvae were used for the experiments.

**Table 3.1. Composition of semi-synthetic diet for *H. armigera***

<b>Diet parts</b>	<b>Ingredients</b>	<b>Quantity (g) per 1,000 ml diet</b>
Part A	Chickpea flour	300
	Sorbic acid	3.0
	Methyl- <i>p</i> -hydroxybenzoate	5.0
	Ascorbic acid	4.7
	Yeast	48
	Auromycin powder	11.5
	Cholesterol	1.5
	Formaldehyde (1%)	20 ml
	Multivitamin solution (A,B,D,E,C) drops	10 $\mu$ l
	Water	450 ml
Part B	Agar-agar	17.3
	Water	800

**3.2.1.1. Diet preparation:** The diet was prepared as follows:

1. Measured quantities of part A were mixed.
2. Agar-agar was added to water in a separate container and boiled for 5 min (Part B).
3. Part A and Part B were mixed thoroughly in a blender to get an even consistency.
4. The diet was poured into small plastic cups and allowed to cool under a laminar flow for 1 to 2 h.

**3.2.2. *Spodoptera litura***

Egg masses of *S. litura* were collected from the groundnut fields, and the larvae were reared on groundnut leaves for one generation under laboratory conditions ( $26 \pm 1$  °C;  $11 \pm 0.5$  h photoperiod, and  $75 \pm 5\%$  relative humidity). In the subsequent generations, the larvae were reared on the same semi-synthetic diet used for rearing *H. armigera*. The pupae were washed in 0.2% sodium hypochlorite solution and placed in moistened Vermiculite bed in a plastic jar for adult emergence. After emergence, the adults were transferred to oviposition cages (30 x 30 x 30 cm), and butter paper sheets (15 x 10 cm) were provided for oviposition. The adults were provided with 10% sucrose solution as food. The egg masses laid on butter papers were washed with 0.1% sodium hypochlorite solution for 2 to 3 minutes to surface sterilize the eggs. Newly emerged larvae were used for bioassays.

**3.2.3. *Aphis craccivora***

*Aphis craccivora* adults were collected from the groundnut fields at ICRISAT, Patancheru, Andhra Pradesh, India, and released on potted groundnut plants enclosed in cages under greenhouse conditions. The groundnut plants were raised in pots (30 cm

diameter) containing soil, sand and farm yard manure (2: 2: 1) inside the greenhouse at  $27 \pm 3$  °C. To avoid overcrowding, the aphids were transferred to new plants every 15 days.

### **3.3. Evaluation of groundnut (*Arachis hypogaea* L.) genotypes for resistance to insects under field conditions**

Five genotypes of groundnut were evaluated for resistance to insects under field conditions, including four genotypes earlier known to be resistant to insects [(ICGV 86699, ICGV 86031, ICG 2271 (NCAc 343), ICG 1697 (NCAc 17090)], and a susceptible check, JL 24 (Sharma et al. 2003). The crop was grown during the 2010/11 rainy seasons. There were three replications in a randomized complete block design. Each plot had two rows, 2 m long, and the material was planted on ridges 75 cm apart. For breaking dormancy, the seeds were treated with Ethrel (Imperial Chemical Industries, Berks, UK) before sowing. The seeds were sown 5 - 7 cm below the soil surface by hand with a spacing of 15 cm between the plants. The experimental plots were not sprayed with any insecticide. Weeds were removed as needed, and the field was maintained under rainfed conditions. Resistance/susceptibility of groundnut genotypes to *H. armigera*, *S. litura* and leafhoppers was measured in terms of plant damage on a 1 - 9 visual damage rating scale (1 =  $\leq$  10% damage and 9 =  $\geq$  80% damage) (Fig. 3.1).

#### **3.3.1. Biochemical profile of groundnut genotypes raised in the field**

Leaves were randomly collected from the groundnut plants at 20 days after germination to study the activities of various defensive enzymes such as peroxidase (POD), polyphenol oxidase (PPO), superoxide dismutase (SOD), ascorbate peroxidase (APX), lipoxygenase (LOX), catalase (CAT), phenylalanine ammonia lyase (PAL), trypsin proteinase inhibitor (PI), and total amounts of phenols, condensed tannins, flavonoids, carbohydrates, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and malondialdehyde (MDA).

### **3.3.1.1 Enzyme extraction**

Fresh leaves (0.5 g) were ground in 3 ml of ice cold 0.1 M Tris-HCl buffer (pH 7.5) containing 5 mM 2-mercaptoethanol, 1% polyvinylpyrrolidone (PVP), 1 mM DTT, and 0.5 mM EDTA. The homogenate was centrifuged at 14,000 rpm for 20 min and the supernatant was collected. The supernatant was subjected to protein precipitation and dialysis.

### **3.3.1.2. Precipitation of proteins**

Proteins were precipitated by salt method using ammonium sulphate ( $\text{NH}_4\text{SO}_2$ ). Ammonium sulphate (1.2 g) was added to 5 ml of the protein extract to obtain 40% saturation. The solution was kept overnight at 4 °C and then centrifuged at 14,000× g for 30 min. The pellet was collected and the supernatant was used for further precipitation. For 80% saturation, ammonium sulphate was added at the rate of 0.28 g ml<sup>-1</sup>. The solution was kept overnight at 4 °C and the salt precipitated proteins were collected after centrifugation at 14,000× g for 30 min. The pellets were pooled together and dissolved in buffer (0.1 M Tris-HCl buffer, pH 7.5, containing 0.5 mM EDTA and 1 mM DTT). The protein solution was dialyzed using dialysis bag (Sigma-Aldrich, USA).

### **3.3.1.3. Activation of the dialysis bag and dialysis**

Activation solution (100 mM sodium bicarbonate, pH 7.0, containing 10 mM EDTA) was taken in a beaker with the dialysis bag immersed in it. The solution was agitated at 60 °C for 2 h and changed thrice with the fresh one. The solution was then replaced with distilled water and washed for 1 h. This step was repeated several times to

ensure the solution became clear. The activated dialysis bag was stored in 10% ethanol at 4 °C until use.

For dialysis, the bag was washed with distilled water, sealed with a plastic clip on one end and again washed with the distilled water. The bag was filled with the protein sample and sealed on the other end with a plastic clip. The dialysis was carried out for 18 h in the preceding buffer at 4 °C. The buffer was changed after every 3 h, and the dialyzed sample was used as the enzyme source.

#### **3.3.1.4. Enzyme assays**

##### **3.3.1.4.1. Peroxidase (POD) assay**

Peroxidase activity was estimated as per the method of Shannon et al. (1966) with slight modification. The reaction mixture (2.9 ml) containing 0.1 M sodium phosphate buffer (pH 6.5), 0.8 mM H<sub>2</sub>O<sub>2</sub> and 5 mM guaiacol was taken in a test tube. To the reaction mixture, 0.1 ml of enzyme source was added and the absorbance was read at 470 nm for 2 min at 15 sec intervals. The enzyme activity was expressed as IU g<sup>-1</sup> FW. One unit of POD activity was defined as the change in absorbance by 0.1 unit per minute under conditions of assay.

##### **3.3.1.4.2. Polyphenol oxidase (PPO) assay**

Polyphenol oxidase activity was estimated as per the method of Mayer and Harel (1979) with some modifications. To 2.9 ml of 0.1 M sodium phosphate buffer (pH 6.8), 0.1 ml of enzyme source and 0.1 ml of substrate (0.05 M catechol) were added. Absorbance was read at 420 nm for 3 min at 30 sec interval. Enzyme activity was expressed as IU g<sup>-1</sup> FW. One unit of PPO was defined as the change in absorbance by 0.1 unit per minute under conditions of the assay.



#### **3.3.1.4.3. Phenylalanine ammonia lyase (PAL) assay**

Phenylalanine ammonia lyase was estimated as described by Campos-Vergas and Saltveit (2002) with slight modifications. To 0.4 ml of 50 mM L-phenylalanine (dissolved in 20 mM potassium phosphate buffer, pH 8.8), 0.2 ml of supernatant and 0.4 ml of 50 mM potassium phosphate buffer (pH 8.8) were added. The reaction mixture was incubated at 40 °C for 30 min. Change in absorbance was measured at 290 nm and PAL activity was expressed as  $\mu\text{mol cinnamic acid min}^{-1} \text{mg}^{-1}$  protein.

#### **3.3.1.4.4. Lipoxygenase (LOX) assay**

Lipoxygenase activity was measured by following the method of Hildebrand and Hymowitz (1981) with slight modifications. To 0.95 ml reaction mixture containing 1 mM linoleic acid dispersed in 0.1 M sodium phosphate buffer (pH 7.0), 0.05 ml of partially purified enzyme extract was added. Absorbance was read at 234 nm for 2-3 min. One unit of enzyme activity was defined as the increase in absorbance by 0.01 per min, and expressed as  $\text{IU g}^{-1}$  FW.

#### **3.3.1.4.5. Superoxide dismutase (SOD) assay**

The activity of SOD was assayed as described by Beauchamp and Fridovich (1971) with slight modifications. To 3 ml of 0.05 M sodium phosphate buffer with 0.1% NaCl (pH 7.8), 0.3 ml of 0.1 mM EDTA, 0.3 ml of 0.13 mM methionine, 0.1 ml of 0.02 mM KCN, 0.3 ml of 0.75 mM nitroblue tetrazolium salt (NBT), 0.3 ml of 0.02 mM riboflavin and 0.1 ml of enzyme extract were added. The reaction mixture was illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes for 1 hour. Identical solutions that were kept under dark served as blanks. Absorbance was read at 560 nm against the blank. SOD activity was expressed in  $\text{IU g}^{-1}$  FW.

#### **3.3.1.4.6. Catalase (CAT) assay**

Catalase activity was determined by using the method of Zhang et al. (2008). The reaction mixture consisted of 1 ml of Tris- HCl buffer (pH 7.0), 0.1 ml of partially purified enzyme extract and 0.2 ml of H<sub>2</sub>O<sub>2</sub>. Absorbance was read at 240 nm for 2 min and the enzyme activity was expressed as  $\mu\text{mol min}^{-1} \text{mg}^{-1}$  protein.

#### **3.3.1.4.7. Ascorbate peroxidase (APX) assay**

To determine the APX activity, method of Asada and Takahashi (1987) was followed with slight modifications. Leaf tissue (0.2 g) was homogenized in a pestle and mortar with 3 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 1% polyvinylpyrrolidone (PVP) and 1mM ascorbic acid. After filtering through a double-layered cheese cloth, the homogenate was centrifuged at 18,000× g for 20 min at 4 °C. The supernatant was collected and subjected to precipitation and dialysis as mentioned above. The partially purified sample was used as the enzyme source. The reaction mixture (1 ml) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H<sub>2</sub>O<sub>2</sub> and 0.2 ml of partially purified enzyme extract. Decrease in absorbance at 290 nm due to ascorbate oxidation was measured against the blank and the enzyme activity was expressed as IU g<sup>-1</sup> FW.

#### **3.3.1.4.8. Proteinase inhibitor (PI) activity**

Leaf sample (0.2 g) was homogenized in 4 ml of 50 mM Tris-HCl buffer (pH 7.8) containing 5% PVP, 0.016 M phenyl urea, 0.03 M KCl, 0.05 M EDTA and 0.4 mM ascorbic acid. The homogenate was filtered through three layers of cheese cloth and centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant was collected, precipitated by ammonium sulphate, dialyzed and used as the protein inhibitor source. All the steps were carried out on ice to ensure the lowest possible temperature. The PI activity was estimated by following the method of Kakade et al. (1969) with slight modifications using *N*- $\alpha$ -

benzoyl-D<sub>L</sub>-arginyl-*p*-nitroanilide (BApNA) as substrate and trypsin as a standard. The reaction mixture consisted of 0.3 ml of supernatant, 0.3 ml of trypsin (2 mg in 40 ml of 0.001 M HCl), and 2.1 ml of 1 mM BA<sub>p</sub>NA (15 mg dissolved in minimum volume of DMSO and adjusted its final volume to 50 ml with 0.05 M Tris – HCl, pH 8.2, containing 0.03 M CaCl<sub>2</sub>). The final concentration of BA<sub>p</sub>NA in reaction mixture was 0.54 mM with 180 units of trypsin. Commercially available trypsin inhibitor was used as a positive control. The reaction mixture was incubated at 37 °C for 15 min in a shaking water bath and the reaction stopped by adding 0.3 ml of 30% glacial acetic acid. Absorbance was read at 410 nm against the blank. The PI activity against trypsin was expressed as percentage inhibition.

### **3.3.1.5. Estimation of secondary metabolites**

#### **3.3.1.5.1. Phenolic content**

Fresh leaves (0.5 g) were homogenized in 3 ml of 80% methanol and agitated for 15 min at 70 °C. The solution was centrifuged at 10, 000 rpm for 10 min and the supernatant collected. The supernatant was used for the estimation of total phenols, condensed tannins and total flavonoids. The Phenolic content was estimated as per Zieslin and Ben-Zaken (1993) method with some modifications. To 2 ml of 2% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), 1 ml of methanol extract was added. The solution was incubated for 5 min at room temperature after which 0.1 ml of 1 N Folin-Ciocalteu reagent was added. The solution was incubated again for 10 min and absorbance of the blue color measured at 760 nm. Phenolic concentration was determined from standard curve prepared with gallic acid and was expressed as µg Gallic acid equivalents g<sup>-1</sup> FW (µg GAE g<sup>-1</sup> FW).

#### **3.3.1.5.2. Condensed tannins**

Condensed tannins content was estimated by using vanillin-hydrochloride method as described by Robert (1971), with some modifications. The 0.5 ml of supernatant was added to 2.5 ml of vanillin-HCl reagent [equal volumes of 8% HCl (in methanol) and 4% vanillin (in methanol) and the solutions mixed just before use]. The reaction mixture was incubated at room temperature for 20 min and the absorbance read at 500 nm against a blank containing the reagent alone. Catechin was used as the standard. The total amount of condensed tannins was expressed as  $\mu\text{g}$  catechin equivalents  $\text{g}^{-1}$  FW ( $\mu\text{g}$  CE  $\text{g}^{-1}$  FW).

#### **3.3.1.5.3. Total flavonoids**

Total flavonoid content was determined by the modified aluminum chloride method as described by Woisky and Salatino (1998). Leaf extract (0.2 ml) was added to 0.8 ml of distilled water in a test tube. To the above solution, 0.06 ml of 5%  $\text{NaNO}_3$  was added. The solution was allowed to stand for 5 min. To the solution, 0.06 ml of 10%  $\text{AlCl}_3$  and 0.4 ml of 1 M NaOH was added and mixed well. The absorbance was read at 510 nm. The total amount of flavonoids was expressed as  $\mu\text{g}$  catechin equivalents  $\text{g}^{-1}$  FW ( $\mu\text{g}$  CE  $\text{g}^{-1}$  FW).

#### **3.3.1.5.4. Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) content**

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) content was estimated by the method of Noreen and Ashraf (2009). Fresh leaf tissue (0.1 g) was homogenized in 2 ml of 0.1% (w/v) trichloroacetic acid (TCA) in a pre-chilled pestle and mortar, and the homogenate was centrifuged at 10,000 rpm for 15 min. To 0.5 ml of supernatant, 0.5 ml of phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide (KI) were added. The absorbance was read at 390 nm.  $\text{H}_2\text{O}_2$  concentration was determined by using an extinction coefficient of  $0.28 \mu\text{M}$   $\text{cm}^{-1}$  and expressed as  $\mu\text{mol}$   $\text{g}^{-1}$  FW.

#### **3.3.1.5.5. Malondialdehyde (MDA) content**

The level of lipid peroxidation was determined in terms of thiobarbituric acid-reactive substances (TBARS) as described by Carmak and Horst (1991) with minor modifications. Fresh leaf tissue (0.2 g) was homogenized in 3 ml 0.1% (w/v) trichloroacetic acid (TCA) at 4 °C. The homogenate was centrifuged at  $20,000 \times g$  for 15 min. 0.5 ml of supernatant was added to 3 ml 0.5% (v/v) thiobarbituric acid (TBA) in 20% TCA. The mixture was incubated at 95 °C in a shaking water bath for 50 min and the reaction stopped by cooling the tubes in an ice water bath. Then samples were centrifuged at 10,000 rpm for 10 min and the absorbance of the supernatant read at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The concentration of TBARS was calculated using the absorption coefficient  $155 \text{ mmol}^{-1} \text{ cm}^{-1}$  and expressed as  $\mu\text{mol g}^{-1} \text{ FW}$ .

#### **3.4. Consumption, digestion and utilization of food by *H. armigera* and *S. litura***

To study the consumption, digestion and utilization of food by *H. armigera* and *S. litura*, the detached leaf assay (Sharma et al. 2005) was followed (Fig. 3.2). Five groundnut genotypes ICGV 86699, ICGV 86031, ICG 2271 (NCAc 343), ICG 1697 (NCAc 17090) and JL 24 were grown under greenhouse conditions at ICRISAT, Patancheru, Andhra Pradesh, India (as mentioned earlier). First fully expanded leaves (tetrafoliates) were collected from 20 day old plants and brought to lab in ice box. One leaf was placed in each 100 ml plastic cup containing 3% agar-agar. The tetrafoliates were embedded into agar-agar to keep them fresh. Third-instar larvae with almost similar physiological conditions and size were used for bioassays. Larvae were starved for 4 h and weighed before each experiment so that they could feed on the leaves efficiently. One larva was released in each cup. The dry weight of the introduced food was determined by multiplying the fresh weight of the food remaining after larval consumption by a standard factor, determined as the percentage dry matter in each genotype. It was determined by maintaining an aliquot of the food under similar conditions in the absence of larvae, weighing it, then drying and reweighing it. Dry

and fresh weights of each aliquot were used to calculate the percentage dry matter. Uneaten food and frass were removed after the experiment, weighed and dried at 65 °C for 72 h in a hot-air oven. The difference between the dry weight of the uneaten food and the calculated dry weight of the offered food was the dry weight of the food consumed by the larvae. Larval weight gain was calculated as the difference between the weight of the larvae before and after the feeding period.

The nutritional indices proposed by Waldbauer (1968) and described by Sharma and Franzmann (2000) were used to compute the food consumption, digestion, and efficiency of conversion of the ingested food into body matter. The consumption index (CI) was calculated by Hopkins (1912) formula as the animals' rate of food intake in relation to its mean weight during the feeding period.

The consumption index (CI) was calculated as follows:

$$CI = \frac{\text{Weight of food ingested}}{\text{Duration of feeding period} \times \text{Mean weight of insect}} \times 100$$

The approximate digestibility (AD) of food was calculated as from following formula:

$$AD = \frac{\text{Weight of food ingested} - \text{weight of frass}}{\text{Weight of food ingested}} \times 100$$

Efficiency of conversion of ingested food into body matter (ECI) was calculated as follows:

$$ECI = \frac{\text{Weight gained by the larva}}{\text{Weight of food ingested}} \times 100$$

The efficiency with which larvae converted digested food into body matter (ECD) was calculated as below:

$$\text{ECD} = \frac{\text{Weight gained by the larva}}{\text{Weight of food ingested} - \text{weight of frass}} \times 100$$

### **3.5. Induced resistance in groundnut against chewing and sap sucking insects under greenhouse conditions**

Five groundnut genotypes ICGV 86699, ICGV 86031, ICG 2271 (NCAc 343), ICG 1697 (NCAc 17090) and JL 24 were grown under greenhouse conditions at ICRISAT, Patancheru, Andhra Pradesh, India, to study the induced responses against the chewing (*H. armigera* and *S. litura*), and a sap sucking (*A. craccivora*) insect (Fig. 3.3). Groundnut plants were grown in plastic pots (30 cm diameter and 40 cm deep) and were maintained as per normal agronomic practices. The pots were filled with a mixture of soil, sand, and farmyard manure (2:1:1). Five seeds were sown in each pot at 7 cm below the soil surface. The plants were watered as needed. Two seedlings with similar growth were retained in each pot at 10 days after seedling emergence. The greenhouse was cooled by desert coolers to maintain the temperature at  $26 \pm 5$  °C and  $65 \pm 5\%$  relative humidity. Twenty day old plants were infested with ten newly emerged *H. armigera* and *S. litura* larvae or 10 apteral adults of *A. craccivora*.

#### **3.5.1. Insect infestation**

One plant in each pot was covered with a plastic jar cage (11 cm diameter, 26 cm in height) with two wire-mesh screened windows (4 cm diameter) on the sides. The top of the plastic jar cage was covered with a lid fitted with wire-mesh screen. The plants were infested with neonates of *H. armigera* and *S. litura* or the adults of *A. craccivora*.

#### **3.5.2. Plant enzyme assays**

After six days of insect infestation, leaves were collected from insect infested and uninfested control plants and the activities of various defensive enzymes such as POD, PPO, SOD, APX, LOX, CAT, PAL, and PI, and total amounts of secondary metabolites such as phenols, condensed tannins, flavonoids, and of carbohydrates, H<sub>2</sub>O<sub>2</sub>, MDA, and glutathione were estimated as described above.

### **3.5.3. Effect of induced resistance insect biology**

Plant damage by *H. armigera* and *S. litura* was recorded on a 1 - 9 visual damage rating scale (1 = ≤ 10% damage, and 9 = ≥ 80% damage) and that of *A. craccivora* on 1-5 scale (1 = highly resistant and 5 = highly susceptible). The larvae of *H. armigera* and *A. craccivora* nymphs and adults were recovered from the infested plants, counted and weighed using a digital balance (Mettler Toledo, AB304-S) to record insect survival and larval.

### **3.5.4. HPLC fingerprinting of flavonoids**

The phenolic samples extracted from *H. armigera* and *A. craccivora* infested and uninfested plants were run through the HPLC to study the difference between the phenolic profiles of infested and uninfested control plants. HPLC system used for the analysis of phenolic compounds was of Waters Series consisting of a Separation module (2695) with Controller (600), and equipped with photodiode- array detector (2996). Methanolic extracts were filtered through a polyvinyl difluoride filter (PVDF; Millipore, Millex-GV, filter 0.22 µm dia.) membrane before HPLC analysis. Separation of the compounds was performed on an Atlantis C<sub>18</sub> column (4.6 × 250 mm; Atlantis, Ireland) at a flow-rate of 1 ml min<sup>-1</sup> for 40 min with 20 µl injected volume of the extract. The column was used at ambient temperature. The mobile phase was water (A)-acetonitrile (B) (v/v) containing 1%



orthophosphoric acid. The mobile phase was filtered through a 0.45 µm membrane filter and de-aerated using a sonicator (D-Compact, 443). The elution profile used was: 0 to 5 min, 65% A, 35% B (isocratic); 5 to 12 min, 35 to 40% B in A (linear gradient); 12 to 20 min, 40 to 45% B in A (linear gradient); 20 to 30 min, 55% A, 45% B (isocratic); 30 to 35 min, 45 to 35% B in A (linear gradient): 35 to 40 min, 65% A, 35%. The compounds were identified by comparing the sample chromatograms with the standard chromatograms.

### **3.5.5. Native polyacrylamide gel electrophoresis of peroxidase and polyphenol oxidase of *A. craccivora* and *H. armigera* infested plants**

Proteins extracted from *A. craccivora* and *H. armigera* infested plants were precipitated by ammonium sulphate and dialyzed. The dialyzed protein samples were subjected to native polyacrylamide gel electrophoresis (native PAGE) to see the banding pattern of POD and PPO. The PAGE apparatus used was obtained from Thermo Scientific (Model P10DS; Owl Separation Systems, Inc. Portsmouth, USA). No SDS was added to separating or stacking gel or the tank buffer. Gel electrophoresis was carried out in 10% separating and 4% stacking gel. The preparation and casting of the gel was performed by the method of Laemmli (1970).

#### **3.5.5.1. Staining of gels**

For POD activity, the native gels were soaked in 50 mM sodium acetate buffer (pH 5.0) for 5 min. To the soaking gel, 10 mg of 4-chloronaphthol (dissolved in 0.5 ml of methanol) and 20 µl of 30% hydrogen peroxide were added. After 15-30 min, zones of peroxidase activity occurred as black bands. The gels were fixed with 7% glacial acetic acid. For PPO activity, the gels were incubated in a staining mixture consisting of 100 ml of 0.1 M sodium acetate buffer (pH 5.0) containing 0.1 g of DL-1,3- dihydroxyphenyl alanine

and 0.1 g of catechol. The gels were incubated with the substrate for 30 min in dark until the bands appeared.

### **3.6. Effect of pre-infestation of groundnut plants by *H. armigera* and *S. litura* on growth and development of subsequently infesting *H. armigera* and *S. litura* larvae**

Twenty-day-old groundnut plants of five genotypes grown in the glasshouse were infested with *H. armigera* and *S. litura*. Ten plants in each genotype were infested with 10 neonates of the insects. The uninfested plants were maintained as control. After five days of infestation, the surviving larvae were collected and weighed. After two days, the pre-infested plants were then reinfested with 4 h starved third-instar larvae of *H. armigera* or *S. litura*. The larvae were weighed before infestation, and two larvae were released on each plant. The previously uninfested plants were also infested. The larvae were allowed to feed for 3 days, after which, the surviving larvae were collected, starved for 4 h and weighed to record data on larval survival and weight gain. Also, the activities of insect gut enzymes such as serine and trypsin (digestive) proteases, and esterase and glutathione-S-transferase (GST, detoxifying) were estimated (discussed in section 3.7.4).

### **3.7. Role of jasmonic acid and salicylic acid in induced resistance in groundnut against *H. armigera* and *S. litura***

#### **3.7.1. Treatments**

Plants were treated with JA and SA to study their role in induced resistance in groundnut against insect pests. To prepare the 1 mM JA solution, 21 mg of JA was dissolved in 1 ml of ethanol, and the JA/ethanol solution was dispersed in 100 ml of water to make the desired concentration (Hamm et al. 2010). The 1 mM SA was prepared by dissolving 0.069 g of SA in 5 ml of ethanol and then dissolved in water to form the appropriate concentration.

### **3.7.2. Induced resistance by JA and SA against *H. armigera***

Plants in each genotype were grouped into six sets.

Group I : Plants were pre-treated with JA (1 mM) for 24 h and then infested with *H. armigera* (PJA + HIN)

Group II : Plants were pretreated with SA for 24 h and then infested with *H. armigera* (PSA + HIN)

Group III : Plants were sprayed with JA (1 mM) and simultaneously infested with *H. armigera* (JA + HIN)

Group IV : Plants were sprayed with SA (1 mM) and simultaneously infested with *H. armigera* (SA + HIN)

Group V : Plants were infested with *H. armigera* (HIN)

Group VI : Plants were maintained as control (sprayed with ethanol dissolved in water).

After 6 days of treatment, the leaves were collected from the plants for evaluation of biochemical attributes of induced resistance, and the insects collected were used for estimation of activities of the gut enzyme.

### **3.7.3. Induced resistance by JA and SA against *S. litura***

Plants in each genotype were grouped into six sets;

Group I : Plants pre-treated with JA (1 mM) for 24 h and then infested with *S. litura* (PJA + SIN)

Group II : Plants pretreated with SA for 24 h and then infested with *S. litura* (PSA + SIN)

Group III : Plants sprayed with JA (1 mM) and simultaneously infested with *S. litura* (JA + SIN)

Group IV : Plants sprayed with SA (1 mM) and simultaneously infested with *S. litura* (SA + SIN)

Group V : Plants sprayed with SA (1 mM)

Group VI : Plants maintained as control (sprayed with ethanol dissolved in water).

After 6 days of treatment, leaves were collected from the plants for evaluation of the biochemical attributes of the induced resistance to *S. litura*.

#### **3.7.4. Effect of JA and SA induced resistance of plants on insect physiology**

To understand the effect of JA and SA induced resistance on insects, the *H. armigera* and *S. litura* larvae fed on JA and SA treated and untreated plants were collected after 6 days of treatment. The larvae were counted and weighed to record the data on larval survival and weight. The surviving larvae were dissected, and midgut was isolated to study the activities of important midgut enzymes such as serine proteases, trypsin, esterase and GST.

##### **3.7.4.1. Total serine protease assay**

The larvae were dissected and midgut was extracted in 0.2 M sodium phosphate buffer (pH 7.5). The midguts were removed and homogenized in 0.1 M glycine-NaOH buffer, pH 10, containing 1 mM EDTA. The homogenate was filtered through three layered cheese cloth and centrifuged at 10, 000 rpm for 20 min at 4 °C. The supernatant was collected and used as enzyme for serine protease and trypsin activity. Serine protease activity of insect midgut was estimated by following the method of Hegedus et al. (2003) using azocasein as a substrate. To 0.04 ml of midgut supernatant, 0.3 ml of 1% azocasein solution (prepared in 0.05 M glycine-NaOH buffer, pH 10) was added. The reaction mixture was incubated at 28 °C for 15 min, and then 0.34 ml of 10% TCA was added to it. The

reaction mixture was incubated again for 1 h at room temperature and centrifuged at 14,000× g for 10 min. The supernatant was collected in a separate tube and 0.68 ml of 1 M NaOH added to it. Absorbance was read at 495 nm.

Total midgut serine protease activity (SP) was calculated by subtracting the azocasein blank absorbance from sample absorbance divided by incubation time in min multiplied by 1000.

$$SP = \frac{\text{Abs}_{(\text{sample})} - \text{Abs}_{(\text{blank})}}{\text{Incubation time (min)}} \times 1000$$

Units are tryptic activity (mu) per min of incubation per mg insect body weight ( $\text{mu min}^{-1} \text{mg}^{-1}$  protein).

#### **3.7.4.2. Trypsin assay**

Trypsin activity of the insect midgut was determined as per the method described by Perlmann and Lorand (1970). Larval midgut extract (0.15 ml) was added to 1 ml of 1 mM BApNA (in 0.2 M glycine - NaOH buffer, pH 10). The reaction mixture was incubated at 37 °C for 10 min. The reaction was terminated by adding 0.2 ml of 30% acetic acid. Absorbance was read at 410 nm and the enzyme activity was expressed as ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$  protein).

#### **3.7.4.3. Esterase assay**

The larvae were dissected in 0.1 M sodium phosphate buffer (pH 7.5), midguts removed and homogenized in 0.1 M sodium phosphate buffer (pH 7.5) containing 1mM EDTA. The homogenate was filtered through three layered cheese cloth and centrifuged at 15,000× g for 15 min at 4 °C. The supernatant was collected and used as a enzyme source

for esterase and GST. The esterase activity was determined according to the method of Van Aspreen (1962) with slight modifications. To 2 ml of 1.5 mM 1-naphthyl acetate solution, 0.1 ml of diluted enzyme sample (10 times with 0.1 M sodium phosphate buffer) was added. This mixture was incubated at 25 °C for 30 min. The reaction was stopped by addition of Fast Blue B (in 5% SDS) staining solution. The reaction mixture was incubated for 15 min and absorbance recorded at 490 nm. The concentration of hydrolyzed substrate was determined from standard curve of 1-naphthol. Specific activity was expressed as  $\mu\text{mol}$  of 1-naphthol formed  $\text{min}^{-1} \text{mg}^{-1}$  protein.

#### **3.7.4.4. Glutathione -S- transferase assay**

Glutathione -S- transferase activity was determined using 1-chloro-2, 4-dinitrobenzene (CDNB) and reduced GSH as substrates according to Habig et al. (1974) with slight modifications. To 1 ml of phosphate buffer (pH 7.5), 0.1 ml of CDNB (25 mM) and 1.6 ml of distilled water were added. The reaction was started by adding 0.1 ml of diluted enzyme solution (the stock solution was diluted 10 fold with 0.1 M sodium phosphate buffer, pH 7.5). The reaction mixture was incubated at 37 °C for 5 min and 0.1 ml of 20 mM GSH added. Optical density at 340 nm was recorded at 30 s intervals for 3 min. The enzyme activity was calculated with an extinction coefficient of  $9.6 \text{ mM cm}^{-1}$  for CDNB. Specific activity was expressed as  $\text{nmol}$  of CDNB conjugate formed  $\text{min}^{-1} \text{mg}^{-1}$  protein.

### **3.8. Orientation behavior of natural enemies in response to chemical cues from host plants**

#### **3.8.1. *Campoletis chloridae***

*Campoletis chloridae* Uchida (Hymenoptera: Ichneumonidae) is a larval parasitoid of *H. armigera* and an important biological control agent (Fig. 3.4). The *C. chloridae*

cocoons were collected from the groundnut and chickpea fields at ICRISAT, Patancheru, Andhra Pradesh, India, and reared at  $27 \pm 2$  °C, and 65–75% relative humidity under laboratory conditions on second instars of *H. armigera*. The cocoons were placed individually in glass vials for adult emergence. After emergence, the adults were transferred to the rearing cages for mating and provided with 10% sucrose solution as a food. The sucrose solution was changed on alternate days. After mating, the females were used for the parasitization of 2<sup>nd</sup> instar *H. armigera* larvae. For parasitization, the *H. armigera* larvae were placed in a transparent plastic vial (15 ml) kept in an inverted position, and one mated female was released inside each vial by an aspirator. After parasitization, the larvae were transferred to the rearing tubes containing artificial diet for further development. After 8 - 9 days of parasitization, the cocoon formation occurs in the successfully parasitized larvae, from which adults emerge in 5-7 days. The newly emerged females were used to study the behavioral response to different groundnut genotypes by using a Y-tube olfactometer.

### **3.8.2. *Trichogramma chilonis***

*Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) parasitized *Corcyra cephalonica* (Stai.) egg cards were obtained from Acharya N. G. Ranga Agricultural University (ANGRAU), Hyderabad, Andhra Pradesh, India. The egg cards were transferred to glass vials for adult emergence. On emergence, the adults were fed on 10% honey solution. The females were separated under microscope and used to study the behavioral response towards different groundnut genotypes through Y-tube olfactometer.

### **3.8.3. Olfactometer setup**

The Y-tube olfactometer setup consisted of a base tube (10 cm long, 4 cm diameter) and two lateral arms (20 cm long, 4 cm diameter). Each arm was fitted with 10 cm long terminal segment (Fig. 3.5). The terminal segment was connected to Teflon tubing. The

incoming air from the air pump was passed through activated charcoal and humidified with distilled water before being passed into the olfactometer. The filtered air passed through two arms of the olfactometer, and then to base tube at 260 ml min<sup>-1</sup>. The air flow was controlled by an air flow meter (Fischer Porter 37070, Gottingen, Germany: D10A 6142N 9407 N 1723). Two fluorescent lamps (40 Watts; Philips GE, F20T12-PL/AQ) were positioned above the arms of the Y-tube to ensure uniform light intensity on both the arms. The orientation of the *C. chloridae* and *T. chilonis* to the odors from different groundnut genotypes was recorded in the following sets with 20 replicates in each set.

First the number of selections and the time taken to respond to a groundnut genotype in comparison to blank air was recorded. In one arm of the Y-tube, groundnut leaves were placed, while the other arm served as a control. The parasitoids were released at one end of the tube and the number of selections was recorded. The time to reach the chosen target was recorded using a stopwatch.

In the second setup, the choice of the parasitoids between the resistant and the susceptible genotypes was studied. In one arm of the Y-tube, susceptible genotype, JL 24 was placed, while in the other arm, the resistant test genotypes were placed individually. The number of selections and time taken to reach the selected genotype were recorded.

In the third setup, the healthy uninfested leaves were placed in one arm of the Y-tube; while and infested leaves of the same genotype with third instar larvae of *H. armigera* were placed in the other arm. The parasitoids were released after the insect started feeding on the leaves, and the number of selections and the time taken to reach the selected sample were recorded. The olfactometer data were analyzed using Chi-Square test to judge the significance of differences in dual-choice assays in the olfactometer.

### **3.9. Effect of JA, SA and insect infestation on trichome density of groundnut plants**



Five groundnut genotypes were grown under greenhouse conditions at ICRISAT, Patancheru, Andhra Pradesh, India, to study the effect of JA, SA and insect infestation on trichome density as an induced response to insect damage. The plants were raised as described above. Twenty days after seedling emergence, the plants in each genotype were treated with JA, SA, and infested with neonates of *H. armigera*. After 5 and 10 days of treatment, newly expanded tetrafoliates were collected from each plant and used to record the trichome density. The tetrafoliates from the treated and untreated plants were immersed in water and incubated at 70 °C for 2-4 min. The samples were cleared in 90% ethanol for one day and transferred to ethanol: acetic acid (2:3 ratio) for 24 h. The leaf samples were stored in 90% lactic acid solution. To record the trichome density, the leaves were examined at a magnification of 100x under a stereomicroscope (Olympus 598472, Japan). Trichome density was expressed as numbers of trichomes per mm<sup>2</sup>.

### **3.10. Effect of JA, SA and insect infestation on the ovipositional behavior of *H. armigera***

Five groundnut genotypes were grown under greenhouse conditions at ICRISAT, Patancheru, Andhra Pradesh, India to study the ovipositional behavior of *H. armigera* females under no-choice conditions on plants treated with JA, SA, insect-infested, and uninfested control plants. Two plants were retained in each pot after ten days of emergence, and 20 day old plants were used for the experiment.

One plant in each pot was covered by a plastic cage. Newly emerged *H. armigera* adults were used for oviposition. Plants in each genotype were divided into six groups.

Group I : Plants pretreated with 1 mM JA for one day and one pair (one male and one female) of *H. armigera* was released inside the cage (PJA + HA).

Group II : Plants pretreated with 1 mM SA for one day prior to the release of one pair of *H. armigera* adults (PSA + HA).

Group III : Plants preinfested for one day with five third-instar larvae of *H. armigera* and one pair of *H. armigera* adults was released inside the cage (PHI + HA).

Group IV : Plants sprayed with 1 mM JA and one pair of *H. armigera* adults was released at the same time (JA + HA).

Group V : Plants sprayed with 1 mM SA and one pair of *H. armigera* adults was released simultaneously (SA + HA).

Group VI : Only a pair of *H. armigera* adults was released on un-treated plants (HA).

The adults were provided with 10% sucrose solution and kept inside the cage for 6 days. After 6 days, the adults were removed from the plants and eggs laid on the plants were recorded. Eggs on walls and lid of the jar were not taken into consideration. The neonates on some plants were also counted as eggs.

### **3.11. Effect of flavonoids, lectins and phenyl $\beta$ -D-glucoside on growth and development, and midgut enzymes of *H. armigera* and *S. litura***

Effect of flavonoids on insect growth and development was studied by feeding the larvae on flavonoids incorporated into the artificial diet. Ten flavonoids: quercetin, cinnamic acid, caffeic acid, chlorogenic acid, catechin, trihydroxyflavone, gentic acid, ferulic acid, protocatechuic acid and umbelliferone were bioassayed using diet incorporation assay (Narayanamma et al. 2007). Neonates of *H. armigera* and *S. litura* neonates were released on the diet containing three concentration of each flavonoid (100, 500, and 1000 ppm). One larva was released in each cell well in a 20 well plastic plate. Four replications were maintained for each treatment with 10 larvae in each replication. Larvae fed on untreated diet were maintained as a control. After 5 and 10 days of treatment, larval survival and weights were recorded. The larvae after 10 days of treatment were used to study the effect of flavonoids on gut enzyme activities such as serine protease, trypsin, esterase and GST.

Groundnut leaf lectin (*A. hypogaea*) and ConA (*Canavalia ensiformis* (L.) and phenyl phenyl  $\beta$ -D-glucoside were also incorporated into the artificial diet to study their affects on biology of *H. armigera* and *S. litura*. The concentrations used were; 1.25, 2.5 and 5  $\mu\text{g ml}^{-1}$  of diet. Four replications were maintained for each treatment and 10 neonate larvae individually were used in each replication. The larvae fed on untreated diet were maintained as a control. After 5 and 10 days after treatment, larval survival and larval weights were recorded. At 10 DAT larvae were used to study insect gut enzymes (esterase, GST, trypsin, and serine protease) after 10 days of feeding.

### **3.12. Statistical analysis**

All the experiments were carried out in a completely randomized design. The experiments were replicated three times with many replicates based on the experiment. Data were analyzed statistically using repeated analysis of variance (ANOVA), individual means were compared with Tukey's honestly significantly different (HSD) means separation test. Dunnett's  $t'$  test, Chi-square test and, Tukey's test were performed by SPSS (ver. 11.5, Chicago, USA) and SAS (ver. 9.2).



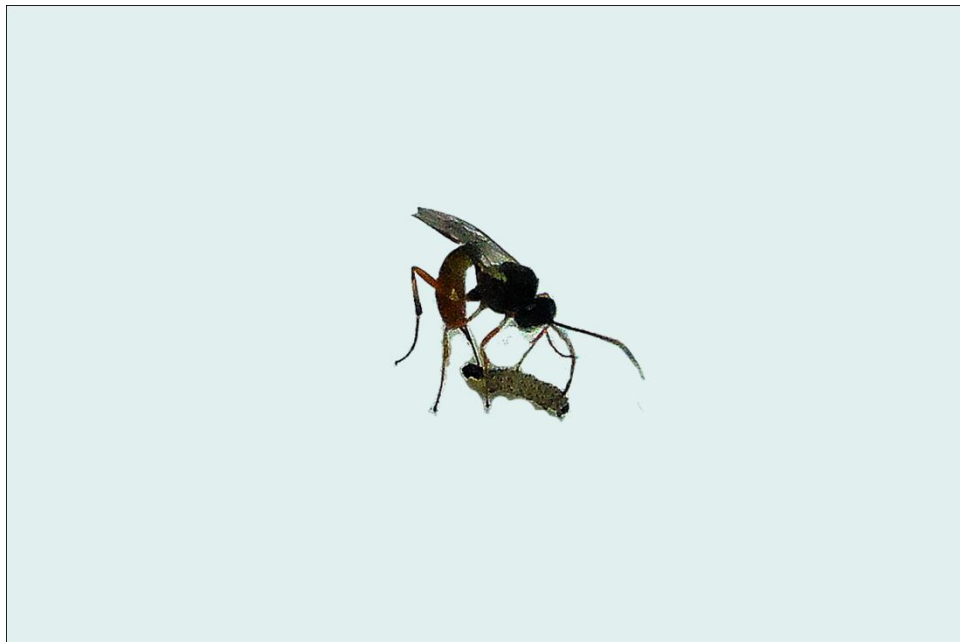
**Fig. 3.1: Groundnut plants in field. Susceptible JL 24 (left) and resistant ICGV 86699 (right)**



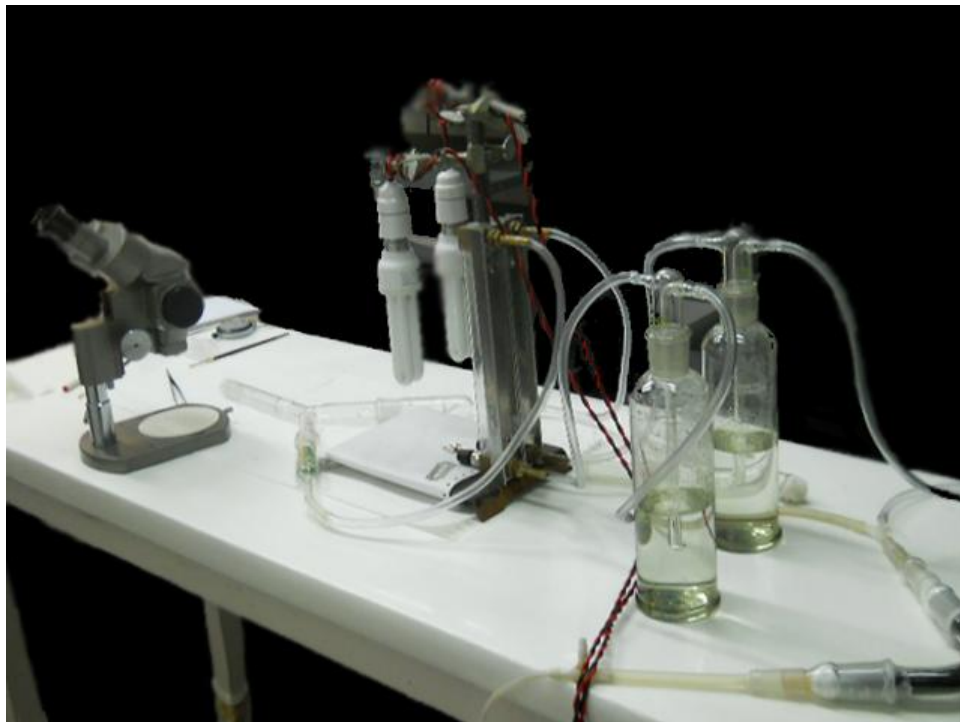
**Fig 3.2: Detached leaf assay for evaluation of host plant resistance against *Helicoverpa armigera***



**Fig. 3.3: Glasshouse experiment for induced resistance**



**Fig. 3.4: *Campoletis chloridae* female parasitizing *Helicoverpa armigera* larva**



**Fig. 3.5: Olfactometer experimental set up**

# *Chapter 4*

## *Results*

## RESULTS

The following pages summarize the results of the various experiments undertaken in the present study. The level of insect resistance by different groundnut genotypes due to their morphological traits and due to the external application of salicylic acid and jasmonic acid was studied by analyzing different physiological and biochemical changes in host plants and insect pests. Moreover the effect of pest infestation in host plants on parasitoids' orientation behavior was also studied. All the parameters were statistically analyzed.

### **4.1. Evaluation of groundnut genotypes for insect resistance under open field conditions**

#### **4.1.1. Leaf damage**

The leaf damage due to foliage feeders, especially *H. armigera* and *S. litura* was significantly lower in ICGV 86699 (2.6), ICGV 86031 (3.1), ICG 2271 (2.9) and ICG 1697 (3.2) ( $F_{(4,14)} = 54.4$ ,  $P \leq 0.001$ ) than in JL 24 (7.0) (Table 4.1.1). Similar trend was observed for leafhopper damage (2.0 - 6.0) ( $F_{(4,14)} = 36.2$ ,  $P \leq 0.001$ ).

#### **4.1.2. Biochemical profile of the groundnut plants grown under field conditions**

The biochemical constituents viz., POD, PPO, PAL, LOX, CAT, SOD and APX, and the secondary metabolites such as total phenols, condensed tannins, flavonoids and  $H_2O_2$ , MDA and total proteins of groundnut genotypes showed a considerable variability (Table 4.1.2a,b). Amongst the genotypes tested, greater POD, PPO and PAL activities were observed in ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697 ( $F_{(4,14)} = 24.2$ , 46.8 and 32.4, respectively,  $P < 0.01$ ) than in JL 24. The LOX and CAT activities were considerably greater in ICGV 86699 and ICGV 86031 ( $F_{(4,14)} = 98.3$ , 49.7 and 32.6 for PAL LOX and CAT, respectively,  $P < 0.01$ ) than ICG 2271, ICG 1697, and JL 24. The SOD and APX



activities were significantly greater in ICGV 86699 and ICGV 86031 ( $F_{(4,14)} = 34.6, 23.4$  for SOD and APX, respectively,  $P < 0.01$ ) than in ICG 2271, ICG 1697 and JL 24. There were no significant differences in total phenolic and tannin contents between ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697, but was significantly greater ( $F_{(4,14)} = 34.9$  and  $25.8$  for phenolic and tannins, respectively,  $P < 0.001$ ) than that of JL 24. The  $H_2O_2$  content was significantly higher in ICGV 86699 ( $F_{(4,14)} = 76.1$ ,  $P < 0.01$ ). Protein content was significantly higher in ICGV 86699, ICGV 86031 and ICG 2271 ( $F_{(4,14)} = 34.6$ ,  $P < 0.05$ ) than in ICG 2271, ICG 1697 and JL 24. The ICG 2271, ICG 1697 and JL 24 had greater MDA content ( $F_{(4,14)} = 65.3$ ,  $P = 0.05$ ) than that of ICGV 86031 and ICGV 86699. A strong negative correlation was observed between various enzyme activities and the severity of damage by *H. armigera*, *S. litura* and leaf hopper. Condensed tannins, phenols,  $H_2O_2$  and total proteins were negatively correlated with severity of damage by *H. armigera*, *S. litura* and leaf hopper, while no significant correlation was observed between MDA content and pest damage.

## **4.2. Food consumption and utilization by *Helicoverpa armigera* and *Spodoptera litura***

### **4.2.1. *Helicoverpa armigera***

The consumption index (CI) for *H. armigera* per unit body weight was significantly lower in the larvae fed on ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 as compared to those fed on the susceptible check, JL 24 (Table 4.2.1). The approximate digestibility (AD) was lower on the insect-resistant genotypes, ICGV 86699, ICGV 86031, ICG 1697 and ICG 2271 (36.5 – 45.4%) as compared to the larvae fed on JL 24 (67.5%). In addition, efficiency of conversion of ingested food (ECI) was significantly lower in larvae fed on the insect-resistant genotypes, ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 (21.3 – 28.2%) as compared to the larvae fed on the susceptible check, JL 24 (54.1%).

Efficiency of conversion of digested food (ECD) varied from 23.6 - 30.2% on the insect-resistant genotypes, while larvae fed on JL 24 showed ECD value of 45.7%.

#### **4.2.2. *Spodoptera litura***

Food consumption and conversion of ingested and digested food into body matter by *S. litura* larvae showed a considerable variation across the groundnut genotypes (Table 4.2.2). The *S. litura* larvae fed on the insect-resistant genotypes ICGV 86699 and ICG 2271 showed significantly less CI than those fed on ICGV 86031, ICG 1697 and JL 24. Larvae fed on insect-resistant genotypes viz., ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 did not show any significant differences in AD, but the values were significantly different than the larvae fed on JL 24. The larvae fed on ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 exhibited reduced ECI (24.6 – 29.1%) and ECD (25.8 – 37.8%) as compared to the larvae fed on JL 24 (ECI 54.1% and ECD 45.7%).

### **4.3. Induced resistance in groundnut against *H. armigera*, *S. litura* and *A. craccivora* under greenhouse conditions**

#### **4.3.1. Biochemical profile**

**4.3.1.1. POD activity:** Plants infested with *H. armigera*, *S. litura* and *A. craccivora* showed significantly greater POD activity [ICGV 86699 ( $F_{(3,11)} = 34.3$ ,  $P < 0.001$ ), ICGV 86031 ( $F_{(3,11)} = 25.4$ ,  $P < 0.01$ ), ICG 2271 ( $F_{(3,11)} = 28.2$ ,  $P < 0.05$ ), ICG 1697 ( $F_{(3,11)} = 19.3$ ,  $P < 0.01$ ), and JL 24 ( $F_{(3,11)} = 25.9$ ,  $P < 0.05$ )] as compared to the uninfested control plants (Fig. 4.3.1a). Across the genotypes, ICGV 86699 showed a strong induction of POD activity in all the treatments infested with insects [*H. armigera* ( $F_{(4,14)} = 45.4$ ,  $P < 0.05$ ); *S. litura* ( $F_{(4,14)} = 33.5$ ,  $P < 0.01$ ), *A. craccivora* ( $F_{(4,14)} = 23.5$ ,  $P < 0.05$ )]. The constitutive levels of POD were significantly high in insect-resistant genotypes ( $F_{(4,14)} = 12.3$ ,  $P < 0.05$ ) than that of JL

24. The JL 24 also exhibited increased POD activity following insect infestation, but the activity was not at par with that of the insect-resistant genotypes.

**4.3.1.2. PPO activity:** Greater induction in PPO activity was observed in *H. armigera* and *S. litura* infested plants in ICGV 86699 ( $F_{(3,11)} = 35.3$ ,  $P < 0.001$ ) and ICGV 86031 ( $F_{(3,11)} = 89.4$ ,  $P < 0.001$ ), ICG 2271 ( $F_{(3,11)} = 32.3$ ,  $P < 0.05$ ) and ICG 1697 ( $F_{(3,11)} = 19.5$ ,  $P < 0.01$ ) than their respective *A. craccivora* infested and control plants (Fig. 4.3.1b). No significant differences were recorded in PPO activity between *H. armigera*, *S. litura* and *A. craccivora* infested plants of JL 24 ( $F_{(3,11)} = 15.9$ ,  $P < 0.05$ ). Across the genotypes, ICGV 86699 and ICGV 86031 plants infested with *H. armigera* and *S. litura* showed significantly greater PPO activity ( $F_{(4,14)} = 78.4$  and  $67.2$ , respectively,  $P < 0.001$ ), than that of ICG 2271, ICG 1697 and JL 24. *A. craccivora* infested plants of ICGV 86699, ICGV 86031 and ICG 1697 had significantly greater PPO activity ( $F_{(4,14)} = 23.8$ ,  $P < 0.05$ ) than those of ICG 2271 and JL 24. Constitutive levels of PPO activity were higher in ICGV 86031; however, the difference was not significant across the tested genotypes.

**4.3.1.3. PAL activity:** A strong induction of PAL activity was observed in groundnut plants after insect infestation (Fig. 4.3.1c). The *H. armigera*, *S. litura* and *A. craccivora* infested plants had greater PAL activity in ICGV 86699 ( $F_{(3,11)} = 34.5$ ,  $P < 0.001$ ), ICG 2271 ( $F_{(3,11)} = 12.67$ ,  $P < 0.01$ ), ICG 1697 ( $F_{(3,11)} = 18.9$ ,  $P < 0.05$ ), and JL 24 ( $F_{(3,11)} = 11.5$ ,  $P < 0.05$ ) than the uninfested control plants. However, in ICGV 86031, *H. armigera* and *S. litura* infestation elicited significantly greater PAL activity ( $F_{(3,11)} = 33.3$ ,  $P < 0.01$ ) than *A. craccivora* infested plants. Across the genotypes tested, ICGV 86699 and ICGV 86031 plants infested with *H. armigera* and *S. litura* exhibited greater PAL activity ( $F_{(4,14)} = 23.2$ ,  $P < 0.05$ ) than those of ICG 2271, ICG 1697 and JL 24. The PAL activity in *A. craccivora* infested plants of ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 was significantly higher ( $F_{(4,14)} = 18.6$ ,  $P < 0.05$ ) than those of JL 24. The constitutive levels of PAL in insect-

resistant genotypes were significantly greater than in the susceptible genotype, JL 24 ( $P < 0.05$ ).

**4.3.1.4. LOX activity:** Insect infestation resulted in increased levels of LOX in all the genotypes tested (Fig. 4.3.1d). The induction was significantly greater in plants infested with *H. armigera*, *S. litura* and *A. craccivora* in ICGV 86699 ( $F_{(3,11)} = 16.3$ ,  $P < 0.01$ ), ICGV 86031 ( $F_{(3,11)} = 8.9$ ,  $P < 0.05$ ), and ICG 1697 ( $F_{(3,11)} = 11.6$ ,  $P < 0.05$ ) and JL 24 ( $F_{(3,11)} = 6.8$ ,  $P < 0.05$ ) than the uninfested control plants. In ICGV 2271, LOX activity in *H. armigera* and *S. litura* infested plants were significantly higher ( $F_{(3,11)} = 18.5$ ,  $P < 0.01$ ) than those infested with *A. craccivora*, and the uninfested control plants. Insect-resistant genotypes showed greater increase in LOX activity in plants infested with *H. armigera* ( $F_{(4,14)} = 9.1$ ,  $P < 0.05$ ), *S. litura* ( $F_{(4,14)} = 13.1$ ,  $P < 0.05$ ), and *A. craccivora* ( $F_{(4,14)} = 5.2$ ,  $P < 0.05$ ) than the corresponding treatments of JL 24. Even the constitutive levels of LOX activity were significantly greater in insect-resistant genotypes than in JL 24.

**4.3.1.5. SOD activity:** The *H. armigera*, *S. litura* and *A. craccivora* infestation increased the SOD activity in all the groundnut genotypes (Fig. 4.3.1e). The induction was significantly higher in *H. armigera* and *S. litura* infested plants of ICGV 86699 ( $F_{(3,11)} = 68.7$ ,  $P < 0.01$ ) and ICG 2271 ( $F_{(3,11)} = 23.5$ ,  $P < 0.05$ ) than *A. craccivora* infested and uninfested control plants. However, there were no significant differences in SOD activity between *H. armigera*, *S. litura* and *A. craccivora* infested plants of ICGV 86031, ICG 1697, and JL 24. Across the tested genotypes, ICGV 86699 and ICG 1697 had greater SOD activity in *H. armigera* ( $F_{(4,14)} = 98.1$ ,  $P < 0.001$ ) and *S. litura* infested plants ( $F_{(4,14)} = 45.6$ ,  $P < 0.01$ ) than those of ICGV 86031, ICG 2271 and JL 24, whereas *A. craccivora* infested plants of ICG 1697 exhibited greater SOD activity ( $F_{(4,14)} = 34.7$ ,  $P < 0.05$ ) than that of ICGV 86699, ICGV 86031, ICG 2271 and JL 24. Constitutive levels of SOD activity were almost similar across the genotypes tested.

**4.3.1.6. APX activity:** The APX activity was significantly greater in *H. armigera* and *S. litura* infested plants of ICGV 86699 ( $F_{(3,11)} = 43.8$ ,  $P < 0.01$ ), ICGV 86031 ( $F_{(3,11)} = 27.8$ ,  $P < 0.01$ ), and ICG 1697 ( $F_{(3,11)} = 12.3$ ,  $P < 0.05$ ) than those infested with *A. craccivora*, and the uninfested control plants (Fig. 4.3.1f). The *H. armigera*, *S. litura* and *A. craccivora* infested plants of ICG 2271 and JL 24 had greater APX activity than the uninfested control plants ( $P < 0.05$ ). Across the genotypes, *H. armigera* and *S. litura* infested plants of ICGV 86699, ICGV 86031 and ICG 1697 showed significantly greater APX activity ( $F_{(4,14)} = 32.4$ ,  $P < 0.01$ ) than those of ICG 2271 and JL 24. The *A. craccivora* infested plants of insect-resistant genotypes had significantly higher APX activity ( $F_{(4,14)} = 19.1$ ,  $P < 0.01$ ) than the *A. craccivora* infested plants of JL 24. Constitutive levels of APX did not differ significantly among the insect-resistant genotypes, but they were significantly higher than that of JL 24.

**4.3.1.7. CAT activity:** Insect infestation resulted in increased activity of CAT (Fig. 4.3.1g). Plants infested with *H. armigera* and *S. litura* had significantly greater CAT activities ( $F_{(3,11)} = 12.2$ , 18.9, 17.7, 8.6, and 9.5, respectively for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24,  $P < 0.05$ ) than the *A. craccivora* infested and uninfested control plants. Among the genotypes, ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 had significantly greater CAT activity in plants infested with *H. armigera* ( $F_{(3,14)} = 23.6$ ,  $P < 0.05$ ) and *S. litura* ( $F_{(4,14)} = 17.2$ ,  $P < 0.05$ ). The *A. craccivora* infested plants of ICG 1697 exhibited significantly greater CAT activity ( $F_{(3,14)} = 14.2$ ,  $P < 0.05$ ) than those of ICGV 86699, ICGV 86031, ICG 2271 and JL 24. Constitutive levels of CAT activity were higher in insect-resistant genotypes than in the susceptible check, JL 24 ( $P < 0.05$ ).

**4.3.1.8. Total phenols:** Insect damage resulted in a tremendous increase in the amounts of phenolic compounds as compared to the uninfested control plants (Fig. 4.3.1h). The *H. armigera* and *S. litura* infested plants showed significantly greater phenolic content ( $F_{(3,11)} =$

39.4, 16.8, 28.1, and 13.6, respectively for ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697,  $P < 0.01$ ) than *A. craccivora* infested and the uninfested control plants. No significant differences were observed between *H. armigera*, *S. litura* and *A. craccivora* infested plants of the susceptible check, JL 24, but were significantly greater than the uninfested control plants. Across the genotypes, the phenolic content of insect-infested plants of the insect-resistant genotypes was significantly higher [*H. armigera* ( $F_{(4,14)} = 16.2$ ,  $P < 0.01$ ), *S. litura* ( $F_{(4,14)} = 10.8$ ,  $P < 0.01$ ) and *A. craccivora* ( $F_{(4,14)} = 14.3$ ,  $P < 0.01$ )] than that of JL 24. Constitutive levels of phenols were not statistically different among the insect-resistant genotypes, but significantly higher ( $F_{(4,14)} = 9.3$ ,  $P < 0.05$ ) than the susceptible genotype, JL 24.

**4.3.1.9. Condensed tannins:** The *H. armigera*, *S. litura* and *A. craccivora* infested plants had greater amounts of condensed tannins in all the tested genotypes ( $F_{(3,11)} = 13.7$ , 21.1, 7.4, 18.1 and 11.6, respectively for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24,  $P < 0.01$ ) than the uninfested control plants (Fig. 4.3.1i). Across the genotypes, ICGV 86699 had significantly higher tannin content in all the treatments [*H. armigera* infested ( $F_{(4,14)} = 11.4$ ,  $P < 0.01$ ), *S. litura* infested ( $F_{(4,14)} = 18.1$ ,  $P < 0.01$ ), *A. craccivora* infested ( $F_{(4,14)} = 9.2$ ,  $P < 0.05$ ) than that of ICGV 86031, ICG 2271, ICG 1697 and JL 24. Constitutive levels of condensed tannins were significantly higher in insect-resistant genotypes ( $F_{(4,14)} = 21.4$ ,  $P < 0.05$ ) than that of the susceptible check, JL 24.

**4.3.1.10. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content:** Insect infestation resulted in a substantial increase in H<sub>2</sub>O<sub>2</sub> in all the genotypes (Fig. 4.3.1j). H<sub>2</sub>O<sub>2</sub> content was significantly greater in *H. armigera* and *S. litura* infested plants of ICGV 86699, ICG 1697 and JL 24 ( $F_{(3,11)} = 11.2$ , 14.4 and 23.1, respectively, all  $P < 0.05$ ) than the *A. craccivora* infested and uninfested control plants. ICGV 86031 and ICG 2271 had higher H<sub>2</sub>O<sub>2</sub> content in *H. armigera*, *S. litura* and *A. craccivora* infested plants than the uninfested controls ( $F_{(3,11)} =$

17.5 and 9.6, respectively for ICGV 86699 and ICG 2271,  $P < 0.05$ ). Across the genotypes, no significant differences were observed in  $H_2O_2$  among the resistant genotypes in all the treatments; however, the differences were significant in comparison to the susceptible check, JL 24.

**4.3.1.11. MDA content:** A significant increase in MDA content was observed in insect-infested plants as compared to the uninfested controls (Fig. 4.3.1k). Greater MDA content was observed in *H. armigera* and *S. litura* infested plants in ICGV 86699 ( $F_{(4,14)} = 23.4$ ,  $P = 0.05$ ) as compared to *A. craccivora* infested, and the uninfested control plants. The *H. armigera*, *S. litura* and *A. craccivora* infested plants of ICGV 86031, ICG 2271, ICG 1697 and JL 24 had greater MDA content ( $F_{(3,11)} = 12.5, 17.3, 20.5$  and  $45.5$ , respectively,  $P < 0.01$ ) than that of the uninfested control plants. JL 24 exhibited greater amounts of MDA in *H. armigera*, *S. litura* and *A. craccivora* infested plants ( $F_{(4,14)} = 34.2, 29.8$  and  $18.3$ , respectively,  $P < 0.05$ ) than those of ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697.

**4.3.1.12. Protein content:** Plants infested with *H. armigera*, *S. litura* and *A. craccivora* had greater protein content than the respective uninfested control plants in all the tested genotypes ( $F_{(3,11)} = 15.4, 21.8, 20.3, 17.7$  and  $19.8$  for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24,  $P < 0.01$ ) (Fig. 4.3.1l). Insect-resistant genotypes had greater protein content in the insect-infested plants in all the treatments [*H. armigera* ( $F_{(4,14)} = 24.3$ ,  $P < 0.01$ ), *S. litura* ( $F_{(4,14)} = 32.4$ ,  $P < 0.01$ ) and *A. craccivora* ( $F_{(4,14)} = 19.4$ ,  $P < 0.05$ )] than in the susceptible check, JL 24. The constitutive protein content of insect-resistant genotypes was higher ( $F_{(4,14)} = 15.4$ ,  $P < 0.05$ ) than that of JL 24.

**4.3.1.13. Proteinase inhibitor (PI) activity:** The *in vitro* percent PI activity of ICGV 86699 and ICGV 86031 was significantly higher in plants infested with *H. armigera* (33.5 and 30.9) and *S. litura* (30.6 and 28.2) as compared to the *A. craccivora* infested (23.5 and

20.6), and uninfested control plants (21.6 and 19.4) (Table 4.3.1). Across genotypes, ICGV 86699 and ICGV 86031 infested plants showed strong PI activity in *H. armigera*, *S. litura* and *A. craccivora* infested plants than the corresponding treatments in ICG 2271, ICG 1697 and JL 24. However, the constitutive levels of PI activity were almost similar in the insect-resistant genotypes, but significantly higher than those of JL 24.

#### **4.3.2. Relative susceptibility of groundnut genotypes to insect pests**

JL 24 suffered greater damage by *S. litura*, *H. armigera* and *A. craccivora* (7.9, 7.5 and 4.2, respectively) than ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 (Table 4.3.2). Survival of *S. litura* and *H. armigera* larvae was significantly lower in the resistant genotypes; ICGV 86699 (41.2 and 33.5%), ICGV 86031 (48.7 and 39.4%), ICG 2271 (52.3 and 45.6%) and ICG 1697 (50.6 and 48.3%) than on the susceptible check, JL 24 (80.3 and 77.5%). The genotypes exhibiting low susceptibility to *S. litura* and *H. armigera* were also less susceptible to the aphid, *A. craccivora*, and least aphid damage was recorded in ICGV 1697 (DR 2.0) and the highest (DR 4.2) in JL 24. Similar trend was observed in terms of numbers of aphids. ICG 1697 had the least numbers of aphids (19 per plant), while JL 24 had the highest (56.5 aphids per plant). Weights of *H. armigera* and *S. litura* larvae were significantly lower (55.5-68.9 and 65.4-79.2 mg/5 larvae, respectively) on ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697 than those fed on the susceptible check, JL 24 (120.3 and 95.5 mg/5 larvae, respectively).

#### **4.3.3. HPLC fingerprinting of flavonoids of *H. armigera* and *A. craccivora* infested plants**

Plants infested with insects showed more number of peaks as compared to the uninfested control plants. The *H. armigera* and *A. craccivora* infested plants showed considerable differences in peaks in all the genotypes. The *H. armigera* infested plants of



ICGV 86699 had more number of peaks (16) as compared to *A. craccivora* infested (9) and uninfested control plants (8) (Fig.4.3.3.1). *H. armigera* infested and *A. craccivora* infested plants of ICGV 86031 showed equal number of peaks (8 each). The uninfested control plants had six peaks (Fig.4.3.3.2). In ICG 2271, more of peaks were observed in *A. craccivora* infested plants (15) as compared to the *H. armigera* infested (6) and uninfested control plants (6) (Fig.4.3.3.3). The number of peaks observed in HPLC chromatogram of ICG 1697 was seven in *H. armigera* infested, eight in *A. craccivora* infested, and eight in uninfested control plants (Fig.4.3.3.4). The chromatogram of JL 24 had seven, six and five peaks respectively, for *H. armigera*, *A. craccivora* and uninfested control plants (Fig.4.3.3.5). Chlorogenic and syringic acids were the main compounds found in all the genotypes. The *H. armigera* infested plants of ICGV 86699 and *A. craccivora* infested plants of ICG 2271 had chlorogenic acid, caffeic acid, syringic acid, catechin, ginsitin, ferulic acid, vanillic acid, umbelliferone and quercetin as the main identified compounds in the former, and syringic acid, ginsitin, ferulic acid and cinnamic acid in the latter. Moreover, chlorogenic and syringic acids were found in almost all the chromatograms.

#### **4.3.4. Native PAGE profile of POD and PPO of *H. armigera* and *A. craccivora***

Native PAGE revealed four isozymes for both POD and PPO in the tested groundnut genotypes infested with *H. armigera* and *A. craccivora* (Fig. 4.3.4). The expression of POD3 was more prominent in all the genotypes; however, *H. armigera* infested plants showed more intense bands than the *A. craccivora* infested plants. Isozyme POD1 was more prominent in ICGV 86699 and ICGV 86031 than in ICG 2271, ICG 1697 and JL 24. The isozyme POD2 was highly distinct in *H. armigera* infested plants than the *A. craccivora* infested plants, and more prominent in ICGV 86031, followed by ICGV 86699, ICG 2271 and ICG 1697.

The PPO showed four isozymes in groundnut genotypes (Fig. 4.3.4). Isozyme PPO1 was highly prominent in JL 24 *A. craccivora* infested plants than ICCGV 86699, ICGV 86031, ICG 2271 and ICG 1697. Isozyme PPO2 was highly prominent in all the genotypes with intense bands in *H. armigera* infested plants as compared to the *A. craccivora* infested plants. ICGV 86031, ICG 2271 and ICG 1697 showed light bands of PPO1. PPO4 showed highly dense band in *H. armigera* infested plants of ICGV 86699.

#### **4.4. Jasmonic acid and salicylic acid mediated induced resistance in groundnut against *H. armigera* and *S. litura***

Treatment of plants with JA and SA showed considerable effect on the activity of plant defensive enzymes and secondary metabolites. Further, a substantial effect was observed on insects fed on JA and SA treated plants, which are discussed below.

##### **4.4.1. JA and SA induced resistance in groundnut against *H. armigera***

###### **4.4.1.1. Plant defensive traits**

**(a). POD activity:** Increase in POD activity was observed in groundnut genotypes subjected to various treatments (Fig. 4.4.1.1a). The PJA + HIN treated plants showed significantly greater POD activity in ICGV 86699 and ICG 2271 ( $F_{(5,17)} = 23.4$  and  $48.1$ , respectively,  $P < 0.01$ ) as compared to PSA + HIN, JA + HIN, SA + HIN, HIN and the untreated control plants. In ICGV 86031, PJA + HIN and JA + HIN treated plants showed significantly greater POD activity ( $F_{(5,17)} = 12.6$ ,  $P < 0.01$ ) than PSA + HIN, SA + HIN, HIN treated and the untreated control plants. The PJA + HIN, PSA + HIN and JA + HIN treated plants of ICG 1697 had significantly greater POD activity ( $F_{(5,17)} = 11.5$ ,  $P < 0.05$ ) as compared to SA + HIN, HIN treated and untreated control plants. In JL 24, significantly greater POD activity was observed in PJA + HIN, PSA + HIN and JA + HIN treated plants ( $P < 0.05$ ) than that of SA + HIN, HIN and the untreated control plants. Across the genotypes, ICGV

86699, ICGV 86031, ICG 2271 and ICG 1697 exhibited significantly greater POD activity in all the treatments ( $F_{(4,14)} = 12.7, 21.3, 25.1, 8.6$  and  $15.7$ , respectively, for PJA + HIN, PSA + HIN, JA + HIN, SA + HIN and HIN,  $P < 0.05$ ) than in JL 24. Constitutive levels of POD activity were also significantly greater in the insect-resistant genotypes ( $P < 0.01$ ) than the susceptible check, JL 24.

**(b). PPO activity:** Treatment with JA and SA, and insect infestation resulted in increased levels of PPO activity (Fig. 4.4.1.1b). Among the treatments, PJA + HIN induced significantly greater PPO activity than PSA + HIN, JA + HIN, SA + HIN treated, HIN infested, and the uninfested control plants in ICGV 86699 ( $F_{(5,17)} = 25.7, P < 0.01$ ), ICGV 86031 ( $F_{(5,17)} = 23.4, P < 0.01$ ) and ICG 1697 ( $F_{(5,17)} = 11.9, P < 0.05$ ). The PJA + HIN and JA + HIN treated plants of ICG 2271 and JL 24 showed significantly greater PPO activity ( $F_{(5,17)} = 20.1$  and  $18.7$  respectively,  $P < 0.05$ ) as compared to the SA + HIN, HIN and the untreated control plants. Across the genotypes, ICGV 86699, ICGV 86031 and ICG 1697 had significantly higher PPO activity in PJA + HIN treated plants ( $F_{(4,14)} = 16.7, P < 0.05$ ) than those of ICG 2271 and JL 24. The PSA + HIN treated plants of ICGV 86699 exhibited greater PPO activity ( $F_{(4,14)} = 10.3, P < 0.05$ ) than ICGV 86031, ICG 2271 and ICG 1697. Significantly greater PPO activity was observed in JA + HIN treated plants of ICGV 86699, ICGV 86031 and ICG 2271 ( $F_{(4,14)} = 22.5, P < 0.05$ ) as compared to those of ICG 1697 and JL 24. Constitutive levels of PPO activity were significantly higher in insect-resistant genotypes ( $F_{(4,14)} = 8.9, P = 0.05$ ) than JL 24.

**(c). PAL activity:** The PJA + HIN, PSA + HIN and JA + HIN treated plants showed significantly greater PAL activity ( $F_{(5,17)} = 45.7, 22.9, 34.6, 16.9$  and  $11.6$  for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24, respectively,  $P < 0.05$ ) than the SA + HIN, HIN and the untreated control plants (Fig. 4.4.1.1c). Among the genotypes, ICGV 86699, ICGV 86031 and ICG 2271 and ICG 1697 exhibited significantly greater PAL activity in

PJA + HIN, PSA + HIN and JA + HIN treated plants ( $F_{(4,14)} = 21.8, 11.9$  and  $16.4$ , respectively,  $P < 0.01$ ) as compared to JL 24. Constitutive levels of PAL activity in ICGV 86699 were significantly higher ( $P < 0.05$ ) than rest of the genotypes. .

**(d). LOX activity:** Among the treatments within a genotype, PJA + HIN and JA + HIN treated plants showed significantly greater LOX activity in all the tested genotypes ( $F_{(5,17)} = 32.5, 21.3, 23.9, 21.9$  and  $13.2$  for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL24, respectively,  $P < 0.05$ ) than the plants treated with SA + HIN and the uninfested control plants (Fig. 4.4.1.1d). Across the genotypes, ICGV 86699, ICGV 86031 and ICG 2271 plants treated with PJA + HIN and JA + HIN showed significantly greater LOX activity ( $F_{(4,14)} = 32.1$  and  $24.6$ , respectively,  $P < 0.01$ ) than the respective treatments of ICG 1697 and JL 24. No significant differences were observed in LOX activity of plants treated with PSA + HIN, SA + HIN, HIN and the untreated plants.

**(e). SOD activity:** The PJA + HIN treated plants had significantly greater SOD activity in ICGV 86699 and ICG 1697 ( $F_{(5,17)} = 11.3$  and  $15.2$ , respectively,  $P < 0.05$ ) than PSA + HIN, JA + HIN, SA + HIN, HIN and the untreated control plants (Fig. 4.4.1.1e). The SOD activity was significantly greater in PJA + HIN and JA + HIN treated plants of ICGV 86031, ICG 2271 and JL 24 ( $F_{(5,17)} = 11.7, 21.4$  and  $13.7$ , respectively,  $P < 0.01$ ) as compared to the respective PSA + HIN, SA + HIN, HIN and the untreated control plants. Across the genotypes, ICGV 86699 had significantly greater SOD activity in PJA + HIN treated plants ( $F_{(4,14)} = 38.5$ ,  $P < 0.05$ ) than in ICGV 86031, ICG 2271, ICG 1697 and JL 24. ICGV 86699 and ICGV 86031 exhibited significantly greater SOD activity in JA + HIN treated plants ( $F_{(4,14)} = 21.4$ ,  $P < 0.05$ ) as compared to ICG 2271, ICG 1697 and JL 24. Insect-resistant genotypes showed significantly greater SOD activity in PSA + HIN, SA + HIN, HIN and the untreated control plants ( $F_{(4,14)} = 17.4, 19.2, 25.6$  and  $13.6$ , respectively,  $P < 0.05$ ) than the corresponding treatments in JL 24.

**(f). APX activity:** Alteration of APX activity in groundnut plants was observed following exogenous application of JA and SA, and insect infestation (Fig. 4.4.1.1f). The APX activity of plants treated with PJA + HIN, PSA+ HIN and JA + HIN was significantly greater than SA + HIN, HIN and the control plants ( $F_{(5,17)} = 38.5, 21.7, 37.3, 18.6$  and  $24.9$  for ICGV 86699, ICGV 86031, ICGV 2271, ICG 1697, and JL 24, respectively,  $P < 0.05$ ). Across the genotypes, insect-resistant genotypes showed significantly higher APX activity in PJA + HIN, PSA + HIN and JA + HIN treated plants than in JL 24 ( $P < 0.05$ ). Constitutive levels of APX activity were significantly greater ( $P < 0.05$ ) in insect-resistant genotypes as compared to the susceptible check, JL 24.

**(g). CAT activity:** The CAT showed altered expression in various treatments and in different genotypes (Fig. 4.4.1.1g). Among the treatments, significantly greater CAT activity was observed in plants treated with PJA + HIN and JA + HIN in all the genotypes ( $F_{(5,17)} = 33.9, 39.9, 28.5, 31.9$  and  $17.3$  for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL24, respectively,  $P < 0.01$ ) as compared to the PSA + HIN, SA + HIN, HIN and the untreated control plants. When comparing the genotypes, PJA + HIN, PSA + HIN and JA + HIN treated plants of the insect-resistant genotypes showed significantly greater CAT activity ( $F_{(4,14)} = 11.3, 15.2$  and  $10.5$ , respectively,  $P < 0.05$ ) than in JL 24, however, the SA + HIN and HIN treated plants of ICG 1697 exhibited significantly greater CAT activity than that of ICGV 86699, ICGV 86031, ICG 2271 and JL 24 ( $F_{(4,14)} = 43.5$ ,  $P < 0.01$ ). Untreated control plants of the insect-resistant genotypes had significantly greater CAT activity as compared to those of the susceptible check, JL 24 ( $P < 0.05$ ).

**(h). PI activity:** Significantly higher *in vitro* PI activity (%) was shown by PJA + HIN and JA + HIN treated plants of ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 (47.2, 37.9, 32.2 and 34.5, respectively) than that of PSA + HIN, SA + HIN, HIN, and the untreated control plants (Fig. 4.4.1.1h). Across the genotypes, ICGV 86699 had significantly greater

PI activity in PJA + HIN and JA + HIN treated plants than that of ICGV 86031, ICG 2271, ICG 1697 and JL 24. No significant difference was observed in PI activity in rest of the treatments across the genotypes.

**(i). Total phenols:** The PJA + HIN and JA + HIN treated plants of ICGV 86699 showed significantly greater phenolic content ( $F_{(5,17)} = 30.4$ ,  $P < 0.05$ ) as compared to the plants treated with PSA + HIN, SA + HIN, HIN and the untreated control plants (Fig. 4.4.1.1i). There was no significant difference in phenolic content of the plants treated with PJA + HIN, PSA + HIN, JA + HIN, SA + HIN and HIN in ICGV 86031, ICG 2271, ICG 1697 and JL 24 ( $F_{(5,17)} = 30.4, 45.9, 28.3$  and  $39.8$  for respectively,  $P < 0.01$ ). Among the genotypes, the phenolic content in the insect-resistant genotypes was significantly greater in PJA + HIN, PSA + HIN, JA + HIN, SA + HIN and the control plants ( $F_{(4,14)} = 25.4, 36.5, 29.7, 42.5$  and  $31.2$ , respectively,  $P < 0.01$ ) as compared to that of JL 24. The HIN infested plants of ICGV 86699 had significantly higher phenolic content ( $F_{(4,14)} = 33.6$ ,  $P < 0.05$ ) than in the ICGV 86031, ICG 2271, ICG 1697 and JL 24.

**(j). Flavonoids:** Flavonoid content was significantly higher in plants treated with PJA + HIN and JA + HIN treated plants ( $F_{(5,17)} = 12.3, 17.5, 10.9$  and  $11.4$  for ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697, respectively,  $P < 0.01$ ) than in PSA + HIN, SA + HIN and the control plants; however, in JL 24, no differences were observed in flavonoid content in plants treated with PJA + HIN, PSA + HIN, JA + HIN, SA + HIN and the HIN plants (Fig. 4.4.1.1j). Across the genotypes, insect-resistant plants showed greater levels of flavonoid content ( $P < 0.05$ ) than in JL 24.

**(k). Condensed tannins:** There were significant differences in condensed tannin content across the treatments and the genotypes (Fig. 4.4.1.1k). PJA + HIN plants exhibited greater levels of tannins in all the genotypes [ICGV 86699 ( $F_{(5,17)} = 35.7$ ,  $P < 0.01$ ), ICGV 86031

( $F_{(5,17)} = 59.2$ ,  $P < 0.001$ ), ICG 2271 ( $F_{(5,17)} = 27.9$ ,  $P < 0.05$ ), ICG 1697 ( $F_{(5,17)} = 21.3$ ,  $P < 0.05$ ), JL 24 ( $F_{(5,17)} = 19.8$ ,  $P < 0.05$ )] as compared to PSA + HIN, JA + HIN, SA + HIN and HIN treated plants. Among the genotypes, insect-resistant genotypes had significantly greater amounts of condensed tannins in all the treatments ( $F_{(4,14)} = 21.8$ , 11.7, 10.8, 16.5, 32.5 and 13.3 for PJA+HIN, PSA+HIN, JA+HIN, SA+HIN, HIN and the untreated control,  $P < 0.05$ ) than the respective treatments in JL 24.

**(l). H<sub>2</sub>O<sub>2</sub> content:** The H<sub>2</sub>O<sub>2</sub> levels increased in plants in response to various treatments (Fig. 4.4.1.11). The PJA + HIN, PSA + HIN and JA + HIN treated plants had significantly higher H<sub>2</sub>O<sub>2</sub> content in all the tested genotypes [ICGV 86699 ( $F_{(5,17)} = 27.9$ ,  $P < 0.001$ ), ICGV 86031 ( $F_{(5,17)} = 15.6$ ,  $P < 0.01$ ), ICG 2271 ( $F_{(5,17)} = 18.3$ ,  $P < 0.05$ ), ICG 1697 ( $F_{(5,17)} = 9.3$ ,  $P < 0.05$ ) and JL 24 ( $F_{(5,17)} = 11.1$ ,  $P < 0.05$ ) than the respective SA + HIN, HIN and the untreated control plants. When comparing the genotypes, insect-resistant genotypes showed considerable increase in H<sub>2</sub>O<sub>2</sub> content in all the treatments ( $F_{(4,14)} = 10.4$ , 15.7, 21.4, 13.9, 11.6 and 23.1 for PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and the untreated control,  $P < 0.01$ ) as compared to JL 24.

**(m). MDA content:** MDA content varied between plants treated with JA and SA, and insect infested plants (Fig. 4.4.1.1m). The PSA + HIN, SA + HIN and HIN treated plants exhibited greater MDA content in ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 ( $F_{(5,17)} = 9.7$ , 10.3, 7.5 and 11.6, respectively,  $P < 0.05$ ) as compared to PJA + HIN, JA + HIN and the untreated control plants. In JL 24, PSA + HIN treated plants had significantly greater MDA content ( $F_{(5,17)} = 18.3$ ,  $P < 0.05$ ) than that of PJA + HIN, JA + HIN, SA + HIN, HIN and the untreated control plants. Across the genotypes, PSA + HIN, PJA + HIN and JA + HIN treated plants of JL 24 exhibited significantly higher MDA content ( $F_{(4,14)} = 8.6$ , 11.1 and 7.8, respectively,  $P < 0.05$ ) than that of ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697.

(n). **Protein content:** There was a tremendous increase in total protein content in JA and SA treated and insect infested plants (Fig. 4.4.1.1n). The plants pretreated with JA and SA and infested with *H. armigera*, and the plants treated with JA + HIN had greater protein content than the plants treated with SA + HIN, HIN and the untreated plants ( $F_{(5,17)} = 12.6, 25.5$  and  $21.3$ , for ICGV 86699, ICGV 86031 and ICG 2271, respectively,  $P < 0.01$ ). However, in ICG 1697 and JL 24, the protein content of plants treated with SA + HIN was at par with those treated with PJA + HIN, PSA + HIN and JA + HIN ( $F_{(5,17)} = 34.6$  and  $27.7$  for ICG 1697 and JL 24, respectively,  $P < 0.05$ ). Across the genotypes tested, insect-resistant genotypes showed significantly higher accumulation of proteins than that of the susceptible check, JL 24.

#### **4.4.1.2. Effect of JA and SA induced resistance on *H. armigera***

##### **4.4.1.2.1. Plant damage rating, larval survival and larval weight**

The plant damage by *H. armigera* was significantly reduced in plants pretreated with JA in all the genotypes [ICGV 86699 (2.0), ICGV 86031(2.5), ICG 2271(3.2), ICG 1697 (3.0), and JL 24 (5.5)] as compared to the PSA + HIN, JA + HIN, SA + HIN and the insect-infested plants (Table 4.4.1.2.1a). Pre-treatment with SA also reduced the insect damage, but was not at par with that of the JA pretreated plants. Among the genotypes, insect-resistant genotypes showed reduced damage in all the treatments as compared to that of JL 24. The larval weights and larval survival showed significant differences in different treatments. Larval survival was significantly lower in PJA + HIN treated plants in all the genotypes. Across the genotypes, larvae fed on ICGV 86699 and ICGV 86031 showed less survival, whereas the larvae fed on JL 24 exhibited greater survival as compared to those fed on insect-resistant genotypes in all the treatments. Larvae fed on PJA + HIN treated plants showed reduced weights as compared to those fed on PSA + HIN, PSA + HIN, SA +



HIN, JA + HIN and SIN (Table 4.4.1.2.1b). In all the treatments, larvae fed on the insect-resistant genotypes had lower weights than the larvae fed on the susceptible check, JL 24.

#### 4.4.1.2.2. Effect on insect midgut enzymes

**(a). Total serine protease activity:** The serine protease activity of *H. armigera* larvae fed on plants treated with PJA + HIN and JA + HIN was significantly lower than the larvae fed on PSA + HIN, SA + HIN and the untreated plants in all the genotypes ( $F_{(4,14)} = 16.8, 13.6, 19.2, 14.3,$  and  $11.9$  for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24, respectively,  $P < 0.05$ ) (Fig. 4.4.1.2.2a). Among the genotypes, larvae fed on untreated JL 24 plants had significantly greater serine protease activity ( $F_{(4,14)} = 13.4, P < 0.05$ ) as compared to the larvae fed on untreated plants of ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697. There were no significant differences in rest of the treatments across the genotypes.

**(b). Trypsin activity:** Significant differences in trypsin activity were observed in *H. armigera* larvae fed on groundnut genotypes with different treatments (Fig. 4.4.1.2.2b). Significantly lower trypsin activity was recorded in the larvae fed on plants treated with PJA + HIN, PSA + HIN and JA + HIN in ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24 ( $F_{(4,14)} = 7.8, 10.4, 9.9, 11.3,$  and  $8.5,$  respectively,  $P < 0.05$ ) than the larvae fed on SA + HIN treated and untreated plants. In ICG 1697, significantly lower trypsin activity was recorded in larvae fed on PJA + HIN treated plants ( $F_{(4,14)} = 23.8, P < 0.01$ ) than those fed on the plants treated with PSA + HIN, JA + HIN, SA + HIN and on untreated plants. Across the genotypes, larvae fed on PJA + HIN, PSA + HIN and JA + HIN treated plants of ICGV 86699 had significantly lower trypsin activity ( $F_{(4,14)} = 35.6, 27.8$  and  $32.6,$  respectively,  $P < 0.01$ ) than those fed on the respective treatments of ICGV 86031, ICG 2271, ICG 1697 and JL 24. Larvae fed on SA + HIN treated plants of the insect-resistant

genotypes had significantly lower trypsin activity than the larvae fed on respective treated plants of JL 24 ( $P < 0.05$ ). The trypsin activity of the larvae fed on untreated plants of ICGV 86699, ICGV 86031 and ICG 2271 was lower than those fed on untreated plants of ICG 1697 and JL 24 ( $F_{(4,14)} = 14.2$ ,  $P < 0.05$ ).

**(c). Glutathione-S-transferase (GST) activity:** The *H. armigera* larvae fed on PJA + HIN treated plants of ICGV 86699 had greater GST activity ( $F_{(4,14)} = 13.9$ ,  $P < 0.05$ ) than those fed on plants treated with PSA + HIN, JA + HIN, SA + HIN and HIN (Fig. 4.4.1.2.2c). There was no significant difference in GST in larvae fed on PJA + HIN and JA + HIN treated plants of ICG 2271 and JL 24 ( $P > 0.05$ ). In ICG 1697, larvae fed on plants treated with PJA + HIN, PSA+HIN, JA+HIN and SA+HIN had more GST activity than those fed on HIN treated plants.

**(d). Esterase (EST) activity:** The *H. armigera* larvae fed on groundnut plants with different treatments did not show any significant differences in EST activity across the treatments (Fig. 4.4.1.2.2d). However, in JL 24, larvae fed on untreated plants had significantly higher EST activity than the larvae fed on treated plants ( $P < 0.05$ ). Across the genotypes, no significant differences were recorded in different treatments (all,  $P > 0.05$ ).

**(e). Total protein content:** The protein content of the larvae fed on plants treated with PJA + HIN and JA + HIN was significantly lower ( $5.5 \text{ mg mL}^{-1}$ ) than those fed on PSA + HIN, SA + HIN and untreated plants of ICGV 86699 and ICG 2271 ( $F_{(4,14)} = 21.3$  and  $17.2$ , respectively,  $P < 0.05$ ) (Table 4.4.1.2.2). In ICGV 86031, ICG 1697 and JL 24, larvae fed on PJA + HIN plants had significantly lower protein ( $F_{(4,14)} = 11.6$ ,  $P < 0.05$ ) content than those fed on PSA + HIN, JA + HIN, SA + HIN and HIN plants. Across the genotypes, no significant differences were observed in total protein content of the larvae fed on various

treatments in insect-resistant genotypes; however, the differences were significant as compared to that of the susceptible check, JL 24 (all,  $P < 0.05$ ).

#### 4.4.2. JA and SA induced resistance in groundnut against *S. litura*

Like *H. armigera* infested plants, *S. litura* infested plants showed almost similar response to different treatments in all the biochemical parameters.

##### 4.4.2.1. Plant defensive traits

**(a). POD activity:** The POD activity was significantly increased in plants treated with PJA + SIN and JA + SIN in all the genotypes ( $F_{(5,17)} = 41.1, 57.8, 32.3, 41.3$  and  $17.7$  for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24, respectively,  $P < 0.05$ ) than those of the PSA + SIN, SA + SIN, SIN and the untreated control plants (Fig. 4.4.2.1a). When comparing the genotypes, ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 showed significantly greater POD activity in PJA + SIN ( $F_{(4,14)} = 19.3, P < 0.001$ ), PSA + SIN ( $F_{(4,14)} = 25.8, P < 0.05$ ), JA + SIN ( $F_{(4,14)} = 31.1, P < 0.01$ ), SIN ( $F_{(4,14)} = 29.5, P < 0.01$ ) and the untreated control plants ( $F_{(4,14)} = 21.7, P < 0.05$ ) than that of JL 24.

**(b). PPO activity:** The plants treated with PJA + SIN exhibited greater PPO activity than the plants treated with PSA + SIN, JA + SIN, SA + SIN, SIN and the untreated control plants [ICGV 86699 ( $F_{(5,17)} = 25.7, P < 0.01$ ), ICGV 86031 ( $F_{(5,17)} = 45.6, P < 0.01$ ), ICG 2271 ( $F_{(5,17)} = 21.4, P < 0.05$ ), ICG 1697 ( $F_{(5,17)} = 11.7, P < 0.05$ ) and JL 24 ( $F_{(5,17)} = 18.4, P < 0.05$ )] (Fig. 4.4.2.1b). In JL 24, no significant differences were recorded in PPO activity among PJA + HIN, PSA + HIN and SIN treated plants. Across the genotypes, insect-resistant plants had significantly higher PPO activities in almost all the treatments ( $F_{(4,14)} = 10.2, 16.5, 28.3, 21.6, 32.5$  and  $9.8$  for PJA + SIN, PSA + SIN, JA + SIN, SA + SIN, HIN and untreated control, respectively,  $P < 0.01$ ) than that of JL 24.

**(c). PAL activity:** The PAL activity increased in plants treated with JA and SA (Fig. 4.4.2.1c). There was a considerable increase in the PAL activity of the plants treated with PJA + SIN in all the genotypes ( $F_{(5,17)} = 33.9, 42.6$  and  $26.9$ , for ICGV 86699, ICGV 86031 and ICG 2271, respectively,  $P < 0.05$ ) as compared to PSA + SIN, JA + SIN, SA + SIN, SIN and the untreated control plants. However, in ICG 1697 and JL 24, significantly greater PAL activity was observed in PJA + SIN and JA + SIN treated plants ( $F_{(5,17)} = 22.5$  and  $34.7$ , respectively,  $P < 0.05$ ) as compared to PSA + SIN, SA + SIN, SIN and the untreated control plants. Across the genotypes, ICGV 86699, ICGV 86031 and ICG 2271 had significantly greater PAL activity in PJA + SIN, PSA + SIN and JA + SIN treated plants ( $F_{(4,14)} = 34.4, 17.8$  and  $21.5$ , respectively,  $P < 0.01$ ) as compared to ICG 1697 and JL 24. Treatments with SA + SIN and SIN did not show significant differences in PAL activity across the genotypes ( $P > 0.05$ ). However, constitutive levels of PAL were significantly greater in insect-resistant genotypes ( $F_{(4,14)} = 29.1$ ,  $P < 0.05$ ) than in the susceptible check, JL 24.

**(d). LOX activity:** The LOX activity was significantly greater in plants treated with PJA + SIN, JA + SIN and SIN in all the genotypes ( $F_{(5,17)} = 56.7, 44.5, 34.6, 31.8$  and  $18.1$  for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24, respectively,  $P < 0.01$ ) (Fig. 4.4.2.1d). When comparing the genotypes, ICGV 86699 and ICGV 86031 showed greater LOX activity in PJA + SIN, PSA + SIN and JA + SIN ( $F_{(4,14)} = 32.4, 10.2$ , and  $8.9$ , respectively,  $P < 0.05$ ) than ICG 2271, ICG 1697 and JL 24. The insect-resistant genotypes had significantly greater LOX activity in SIN treated ( $F_{(4,14)} = 19.8$ ,  $P < 0.01$ ) and untreated control ( $F_{(4,14)} = 12.5$ ,  $P < 0.05$ ) plants than that in the susceptible check, JL 24.

**(e). SOD activity:** The *S. litura* infestation and JA treatment induced greater SOD activity in groundnut genotypes (Fig. 4.4.2.1e). Plants treated with PJA + SIN and JA + SIN exhibited greater SOD activity as compared to the plants treated with PSA + SIN, SA +

SIN, SIN and uninfested control plants in the insect-resistant genotypes ( $F_{(5,17)} = 18.9, 11.3, 21.9$  and  $23.2$  for ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697, respectively,  $P < 0.05$ ). In JL 24, no significant differences in SOD activity were recorded among the PJA + SIN, JA + SIN, PSA + SIN and SIN treated plants, however, the SOD activity was greater than the untreated control plants ( $F_{(5,17)} = 41.2$ ,  $P < 0.05$ ). When comparing the genotypes, PJA + SIN, PSA + SIN, JA + SIN and SIN treated plants of the insect-resistant genotypes had significantly greater SOD activity ( $F_{(4,14)} = 38.3, 26.5, 15.6$  and  $17.9$ , respectively,  $P < 0.01$ ) than the respective treatments of JL 24. Constitutive SOD activity of the insect-resistant genotypes was also significantly greater ( $F_{(4,14)} = 9.2$ ,  $P < 0.05$ ) than that of JL 24.

**(f). APX activity:** The PJA + SIN, PSA + SIN and JA + SIN treated plants exhibited significantly greater APX activity than that of SA + SIN, SIN and the untreated control plants in all the genotypes ( $F_{(5,17)} = 23.2, 18.4, 29.6, 11.3$  and  $14.5$ , respectively, for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24,  $P < 0.05$ ) (Fig. 4.4.2.1f). Across the genotypes, ICGV 86699 and ICGV 86031 showed greater APX activity in PJA + SIN, JA + SIN and PSA + SIN treated plants ( $F_{(4,14)} = 10.5, 19.5$  and  $13.4$ , respectively,  $P < 0.05$ ) than ICG 2271, ICG 1697 and JL 24. The ICGV 86699 plants treated with SA + SIN and SIN had significantly greater APX activity ( $F_{(4,14)} = 7.3$  and  $14.3$ , respectively,  $P < 0.01$ ) than the respective treatments of ICGV 86031, ICG 2271, ICG 1697 and JL 24. The plants treated with JA + HIN and also the constitutive levels of APX were significantly greater in insect-resistant genotypes ( $F_{(4,14)} = 15.8$  and  $24.5$ , respectively,  $P < 0.05$ ) than in the susceptible check, JL 24.

**(g). CAT activity:** Plants pretreated with JA showed increased CAT in response to *S. litura* infestation (Fig. 4.4.2.1g). In all the genotypes, PJA + SIN and JA + SIN treated plants had significantly higher CAT activity ( $F_{(5,17)} = 11.4, 8.9, 17.3, 21.3$  and  $6.7$  for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24, respectively,  $P < 0.05$ ) than the PSA + SIN,

SA + SIN and SIN treated plants. Across the genotypes, ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 had significantly greater CAT activity in PJA + SIN, PSA + SIN, JA + SIN, SA + SIN, SIN and the untreated control plants ( $F_{(4,14)} = 4.6, 10.3, 7.4, 8.9, 11.4$  and  $12.5$ , respectively,  $P < 0.05$ ) than in the susceptible check, JL 24.

**(h). PI activity:** The PJA + SIN and JA + SIN treated ICGV 86699 and ICGV 86031 plants showed significantly greater inhibition of protease activity under *in vitro* conditions (40 and 47%, respectively) than the plants treated with PSA + HIN, SA + HIN, SIN and the untreated control plants (Fig. 4.4.2.1h). Across the genotypes, ICGV 86699, ICGV 86031 and ICG 2271 exhibited significantly greater PI activity in PJA + SIN, PSA + SIN, JA + SIN and SIN treated plants than the respective treatments of ICG 1697 and JL 24.

**(i). Phenolic content:** Plants treated with PJA + SIN, PSA + SIN and JA + SIN had significantly greater amounts of phenols than the SIN and uninfested control plants in ICGV 86699 and ICGV 86031 ( $F_{(5,17)} = 34.1$ , and  $31.8$ , respectively,  $P < 0.01$ ) (Fig. 4.4.2.1i). Phenolic content was significantly greater in PJA + SIN, PSA + SIN, JA + SIN and SA + SIN treated plants of ICG 2271, ICG 1697 and JL 24 ( $F_{(5,17)} = 32.3, 18.2$  and  $21.9$ , respectively,  $P < 0.01$ ) than SIN and the untreated control plants. Among the genotypes, insect-resistant genotypes showed significantly greater phenolic content in all the treatments [PJA + SIN ( $F_{(4,14)} = 52.4$ ,  $P < 0.001$ ), PSA + SIN ( $F_{(4,14)} = 38.6$ ,  $P < 0.01$ ), JA + SIN ( $F_{(4,14)} = 25.9$ ,  $P < 0.05$ ), SA + SIN ( $F_{(4,14)} = 32.3$ ,  $P < 0.05$ ), SIN ( $F_{(4,14)} = 19.8$ ,  $P < 0.05$ ) and the uninfested control plants ( $F_{(4,14)} = 27.4$ ,  $P < 0.05$ )] than that of JL 24.

**(j). Flavonoids:** A significant increase in flavonoids content was observed in plants treated with PJA + SIN, and JA + SIN ( $F_{(5,17)} = 23.6, 16.4, 35.3$  and  $17.9$  for ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697, respectively,  $P < 0.01$ ) as compared to the PSA + SIN, SA + SIN, SIN and the untreated control plants (Fig. 4.4.2.1j). In JL 24, no significant

differences were recorded in flavonoid content in PJA + SIN, PSA + SIN, JA + SIN and SIN treated plants. Across the genotypes, the insect-resistant genotypes had greater amounts of flavonoids in different treatments ( $F_{(4,14)} = 45.7, 52.1, 26.3, 22.2, 11.6$  and  $19.8$  for PJA+SIN, PSA+SIN, JA+SIN, SA+SIN, SIN and control,  $P < 0.01$ ) than in the susceptible check, JL 24.

**(k). Condensed tannins:** Plants treated with PJA + SIN had significantly greater tannin content than the plants treated with PSA + SIN, JA + SIN, SA + SIN, SIN and the untreated control plants ( $F_{(5,17)} = 16.4, 12.3, 27.8$  and  $13.7$ , respectively, for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24,  $P < 0.01$ ) (Fig. 4.4.2.1k). In JL 24, there were no significant differences in tannin content of PJA + SIN, JA + SIN and SIN treated plants. The condensed tannin levels of insect-resistant genotypes were significantly higher in all the treatments as compared to the susceptible check, JL 24. Across the genotypes, insect-resistant genotypes had significantly greater amounts of condensed tannins in all the treatments ( $F_{(4,14)} = 12.4, 18.4, 13.4, 10.6, 7.4$  and  $6.3$  for PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and the untreated plants,  $P < 0.05$ ) than in the respective treatments of JL 24.

**(l). H<sub>2</sub>O<sub>2</sub> content:** Plants treated with PJA + SIN, PSA + SIN and JA + SIN showed significantly greater H<sub>2</sub>O<sub>2</sub> content [ICGV 86699 ( $F_{(5,17)} = 7.8, P < 0.01$ ), ICGV 86031 ( $F_{(5,17)} = 10.5, P < 0.01$ ), ICG 2271 ( $F_{(5,17)} = 11.8, P < 0.05$ ) and ICG 1697 ( $F_{(5,17)} = 5.8, P < 0.05$ ) than their respective SA + HIN, HIN treated and the untreated control plants (Fig. 4.4.2.1l). In JL 24, PJA + SIN and PSA + SIN treated plants showed greater H<sub>2</sub>O<sub>2</sub> content ( $F_{(5,17)} = 7.1, P < 0.05$ ) than that of the JA + SIN, SA + SIN, SIN and the untreated control plants. When comparing the genotypes, insect-resistant genotypes showed a considerable increase in H<sub>2</sub>O<sub>2</sub> content in almost all the treatments ( $F_{(4,14)} = 9.4, 12.6, 6.9, 14.7, 10.1$  and

13.2 for PJA + HIN, PSA + HIN, JA + HIN, SA + HIN, HIN and the untreated control,  $P < 0.05$ ) as compared to JL 24.

**(m). MDA content:** Malondialdehyde content showed considerable variation across the treatments (Fig. 4.4.2.1m). PJA + SIN, PSA + SIN, SA + SIN and SIN treated plants had greater MDA content as compared to JA + SIN and the untreated control plants in ICGV 86699, ICG 227 and ICG 1697 ( $F_{(5,17)} = 7.6, 11.3$  and  $10.4$ , respectively,  $P < 0.05$ ); however, in ICGV 86031, PJA + SIN, JA + SIN and the untreated control plants had lower MDA content than that of the PSA + SIN, SA + SIN and SIN treated plants. In JL 24, PJA + SIN and PSA + SIN treated plants had significantly greater MDA content ( $F_{(5,17)} = 11.9$ ,  $P < 0.05$ ) than that of JA + SIN and SA + SIN, SIN treated and the untreated control plants. There were no significant differences in MDA content across the genotypes and the treatments ( $P > 0.05$ ).

**(n). Protein content:** PJA + SIN, PSA + SIN and JA + SIN treated plants exhibited greater protein content in ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 ( $F_{(5,17)} = 23.5, 38.7, 24.1$  and  $21.6$ , respectively,  $P < 0.01$ ) than SA + SIN, SIN and the untreated control plants (Fig. 4.4.2.1n). Across the tested genotypes, insect-resistant genotypes had significantly higher protein contents than the susceptible check, JL 24 in all the treatments.

#### **4.4.2.2. Effect of JA and SA induced resistance to *S. litura***

##### **4.4.2.2.1. Damage rating, larval survival and larval weight**

The plants treated with PJA + SIN suffered relatively lesser damage as compared to the plants treated with PSA + SIN, SA + SIN and SIN in almost all the genotypes [ICGV 86699 (2.4), ICGV 86031 (3.2), ICG 2271 (2.8), ICG 1697 (3.3), and JL 24 (5.7)] (Table 4.4.2.2.1a). ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 suffered lower damage in



all the treatments as compared to JL 24. There were significant differences in larval weights and larval survival across treatments and genotypes (Table 4.4.2.2.1b).

#### **4.4.2.2.2. Effect on insect midgut enzymes**

**(a). Total serine protease activity:** The *S. litura* larvae fed on treated plants showed considerably lower levels of serine protease activity than those fed on untreated plants in all the genotypes ( $F_{(4,14)} = 8.3, 6.4, 9.3, 11.4$  and  $16.1$  for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24, respectively,  $P < 0.05$ ) (Fig. 4.4.2.2.2a). Among the genotypes, no significant differences were observed in serine protease activity in larvae fed on different treatments. The interaction effects between treatments and genotypes were not significant.

**(b). Trypsin activity:** Larvae fed on plants treated with PJA + SIN, PSA + SIN, JA + SIN and SA + SIN exhibited lower trypsin activity ( $F_{(4,14)} = 11.9, 5.4, 16.3$  and  $6.7$  for ICGV 86699, ICGV 86031, ICG 2271 and JL 24, respectively,  $P < 0.05$ ) than the larvae fed on SIN and the untreated control plants across genotypes, except in ICG 1697, where, larvae fed on PJA + SIN and JA + SIN treated plants had lower trypsin activity than the larvae fed on PSA + SIN, SA + SIN treated and the untreated plants (Fig. 4.4.2.2.2b). Across the genotypes, no significant differences were observed in trypsin activity of the larvae fed on different treatments in insect-resistant genotypes than those fed on the respective treatments in JL 24.

**(c). GST activity:** The *S. litura* larvae fed on ICGV 86699 and JL 24 plants treated with PJA + SIN had significantly higher GST activity ( $F_{(4,14)} = 28.1, P < 0.01$ ) than the larvae fed on PSA + SIN, JA + SIN, SA + SIN and the untreated plants (Fig. 4.4.2.2.2c). In ICGV 86031, larvae fed on plants treated with PJA + SIN, and JA + SIN had higher GST activity ( $F_{(4,14)} = 33.5, P < 0.05$ ) as compared to rest of the treatments. Larvae fed on PJA + SIN, PSA + SIN and JA + SIN treated plants of ICG 2271 had higher GST activity ( $F_{(4,14)} = 16.5,$

$P < 0.05$ ) than those fed on SA + SIN and SIN treated plants. However, no significant difference was observed in GST activity of the larvae fed on various treatments of ICG 1697. Across the genotypes, GST activity of the larvae fed on PJA + SIN was greater ( $F_{(4,14)} = 11.5$ ,  $P < 0.05$ ) than those fed on corresponding treatments of ICGV 86031, ICG 2271, ICG 1697 and JL 24. Larvae fed on PSA + SIN, JA + SIN, SA + SIN, and SIN treated plants of insect-resistant genotypes had higher GST activity ( $F_{(4,14)} = 9.7, 12.2, 16.8$  and  $8.2$ , respectively,  $P < 0.05$ ) than those fed on the respective treatments of JL 24.

**(d). EST activity:** There were no significant differences in EST activity in the larvae fed on plants with different treatments across genotypes (Fig. 4.4.2.2.d). Lower EST activity was observed in larvae fed on PJA + SIN, PSA + SIN and JA + SIN treated plants of ICGV 86699 and ICGV 86031 ( $F_{(4,14)} = 11.7, 16.4$  and  $9.9$  for PJA + SIN, PSA + SIN and JA + SIN, respectively,  $P < 0.05$ ) than in ICG 2271, ICG 1697 and JL 24.

**(e). Total protein content:** The *S. litura* larvae fed on PJA + SIN and JA + SIN treated plants had lower total protein content in all the genotypes than those fed on PSA + SIN, SA + SIN and SIN treated plants ( $F_{(4,14)} = 17.3, 15.7, 11.3, 23.5$  and  $19.8$  for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24, respectively,  $P < 0.05$ ) (Table 4.4.2.2.2). Across the genotypes, larvae fed on insect-resistant plants had significantly lower protein content than those fed on the susceptible check, JL 24 in all the treatments ( $F_{(4,14)} = 15.5, 9.6, 22.2, 11.1$  and  $9.9$  for PJA + SIN, PSA + SIN, JA + SIN and SIN, respectively,  $P < 0.05$ ).

#### **4.5. Induced resistance of pre-infested plants on *H. armigera* and *S. litura***

##### **4.5.1. Effect of induced resistance of pre-infested plants on *H. armigera***

###### **4.5.1.1. Effect on larval weight gain**

The *H. armigera* larvae fed on plants previously infested with *H. armigera* larvae exhibited slower growth and development (Table 4.5.1.1). There were significant differences in weight gain between the larvae fed on preinfested plants and those fed on the control plants in ICGV 86699 ( $F_{(1,5)} = 15.6$ ,  $P < 0.01$ ), ICGV 86031 ( $F_{(1,5)} = 19.4$ ,  $P < 0.05$ ) and ICG 2271 ( $F_{(1,5)} = 16.8$ ,  $P < 0.01$ ). Across the genotypes, larvae fed on the control and preinfested plants of JL 24 exhibited significantly higher weight gain than those fed on ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 [preinfested ( $F_{(4,14)} = 21.3$ ,  $P < 0.01$ ) and control plants ( $F_{(4,14)} = 11.4$ ,  $P < 0.05$ )].

#### 4.5.1.2. Effect on insect midgut enzymes

**(a). Serine protease activity:** A significantly reduced activity of serine protease was observed in larvae fed on the preinfested plants of ICGV 86699 ( $F_{(1,5)} = 12.2$ ,  $P < 0.05$ ) and ICG 86031 ( $F_{(1,5)} = 9.1$ ,  $P < 0.05$ ) (Table 4.5.1.2). Across the genotypes, the larvae fed on JL 24 showed significantly greater levels of serine protease activity, both in control and the preinfested plants than those fed on ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 ( $F_{(4,14)} = 8.5$  and  $4.8$  for larvae fed on control and preinfested plants, respectively,  $P < 0.05$ ).

**(b). Trypsin activity:** There were significant differences in trypsin activity between the larvae fed on preinfested and control plants in all the genotypes ( $F_{(1,5)} = 9.5$ ,  $8.5$ ,  $5.2$ ,  $12.4$  and  $16.6$  for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24, respectively,  $P < 0.05$ ) (Table 4.5.1.2). Larvae fed on the control and preinfested plants of JL 24 had significantly greater trypsin activity than those fed on ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697.

**(c). GST activity:** Although there was an increase in GST activity in the larvae fed on plants preinfested with *H. armigera*, the differences between the larvae fed on control and infested groundnut plants were not significantly different in ICGV 86699, ICGV 86031,

ICG 2271 and ICG 1697 ( $F_{(1,5)} = 18.7, 12.4, 16.3$  and  $9.4$ , respectively,  $P < 0.05$ ) (Table 4.5.1.2). Also, across the genotypes, no significant difference was observed in GST activity of both, the larvae fed on control plants and those fed on preinfested plants (both,  $P > 0.05$ ).

**(d). EST activity:** There was no significant effect of preinfestation on the EST activity of *H. armigera* larvae within the genotypes, except in ICGV 86699 and ICG 2271, where larvae fed on preinfested plants had significantly lower EST activity ( $F_{(1,5)} = 6.2$  and  $5.9$ , respectively,  $P = 0.05$ ) (Table 4.5.1.2). Among the genotypes, significantly greater EST activity was observed in larvae fed on JL 24 control and preinfested plants ( $F_{(4,14)} = 4.4$  and  $7.8$ , respectively,  $P < 0.05$ ).

**(e). Total protein content:** The *H. armigera* larvae fed on preinfested plants showed lower levels in protein content in all the genotypes as compared to those fed on the control plants; however, the differences were significant in ICGV 86699, ICGV 86031 and ICG 2271 ( $F_{(1,5)} = 16.7, 11.3$  and  $10.1$ , respectively,  $P < 0.05$ ) (Table 4.5.1.3). Across the genotypes, larvae fed on the insect-resistant control and preinfested plant had significantly lower protein content ( $F_{(4,14)} = 23.2$  and  $17.9$ , respectively,  $P < 0.05$ ) than those fed on the corresponding treatments of JL 24.

#### **4.5.2. Effect of induced resistance of preinfested plants on *S. litura***

##### **4.5.2.1. Effect on larval weight**

Larvae fed on preinfested plants showed reduced weight as compared to those fed on the untreated control plants in ICGV 86699 and ICGV 86031 ( $F_{(1,5)} = 8.5$  and  $11.4$ , respectively,  $P < 0.05$ ) (Table 4.5.1.1). Across the genotypes, larvae fed on control plants of ICGV 86699 and ICGV 86031 had lower larval weights ( $F_{(4,14)} = 7.6$ ,  $P < 0.05$ ) than those fed on ICG 2271, ICG 1697 and JL 24. The larvae fed on preinfested plants of ICGV 86699

and ICGV 86031 and ICG 2271 had significantly lower weights ( $F_{(4,14)} = 11.0, 8.2$  and  $7.6$ , respectively,  $P < 0.05$ ) as compared to those fed on ICGV 1697 and JL 24.

#### **4.5.2.2. Effect on insect midgut enzymes**

**(a). Serine protease activity:** Total serine protease activity was reduced in insects fed on the preinfested plants in ICGV 86699, ICGV 86031 and ICG 2271 ( $F_{(1,5)} = 4.3, 7.6, 8.1$ , respectively,  $P < 0.05$ ) (Table 4.5.2.1). Across the genotypes, larvae fed on the preinfested plants of the insect-resistant genotypes had significantly lower serine protease activity (all,  $P < 0.05$ ) than those fed on JL 24.

**(b). Trypsin activity:** A reduction in trypsin activity was observed in larvae fed on the preinfested plants of ICGV 86699 and ICG 2271 ( $F_{(4,5)} = 9.3$  and  $4.5$ , respectively,  $P < 0.05$ ) than the respective control plants (Table 4.5.2.1). Among the genotypes, control and preinfested plants of ICGV 86699 and ICG 2217 showed strong inhibitory effect [control ( $F_{(4,5)} = 3.5$  and  $8.3$ , respectively,  $P < 0.05$ ; infested ( $F_{(4,5)} = 11.4$  and  $16.8$ , respectively,  $P < 0.05$ )] on the trypsin activity of *S. litura* than ICGV 86031, ICG 1697 and JL 24.

**(c). GST activity:** There was some increase in GST activity of the larvae fed on plants pretreated with insects (Table 4.5.2.1); however, the significance varied. Significant differences in GST activity were observed between the larvae fed on preinfested and control plants in ICGV 86699 ( $F_{(1,5)} = 13.1$ ,  $P < 0.01$ ), ICGV 86031 ( $F_{(4,5)} = 8.5$ ,  $P < 0.05$ ) and ICG 2271 ( $F_{(1,5)} = 16.8$ ,  $P < 0.01$ ); however, the differences were not significant between ICG 1697 and JL 24 ( $P > 0.05$ ). Among the genotypes, larvae fed on control and treated plants did not show any significant difference across each other.

**(d). EST activity:** There were significant differences in EST between the larvae fed on preinfested and control plants in ICGV 86699 and ICG 2271 ( $F_{(1,5)} = 31.8$  and  $21.4$ , respectively,  $P < 0.05$ ) (Table 4.5.2.1). Across the genotypes, larvae fed on control plants of

ICGV 86031 showed significantly reduced EST activity ( $F_{(4,14)} = 31.4$ ,  $P < 0.01$ ) than those fed on the control plants of ICGV 86699, ICG 2271, ICG 1697 and JL 24. However, larvae fed on preinfested plants of ICGV 86031, ICGV 86031 and ICG 2271 showed more effect on EST activity ( $F_{(4,14)} = 26.1$ ,  $P < 0.05$ ) than those fed on ICG 1697 and JL 24.

**(e). Total protein content:** The protein content of *S. litura* larvae showed significant difference when fed on the control and preinfested plants of ICGV 86699 and ICGV 86031 ( $F_{(1,5)} = 9.7$  and  $14.1$ , respectively,  $P < 0.05$ ) (Table 4.5.1.3). No significant difference was observed between the larvae fed on control and preinfested plants in ICG 2271, ICG 1697 and JL 24. Across the genotypes, protein activity of the larvae fed on control plants of insect-resistant genotypes was significantly lower ( $P < 0.05$ ) than those fed on control plants of JL 24. The larvae fed on preinfested plants of ICGV 86699 and ICGV 86031 exhibited significantly lower total protein content ( $F_{(4,14)} = 18.9$ ,  $P < 0.05$ ) than those fed on the preinfested plants of ICG 2271, ICG 1697 and JL 24.

#### **4. 6. Effect of JA, SA and insect infestation on trichome density**

Alteration in number and density of trichomes was observed in plants at five and 10 days after treatment with JA, SA and *H. armigera* infestation (Fig. 4.6). The JA treated plants of ICG 1697 showed significantly greater number of trichomes at 10 DAT ( $F_{(3,11)} = 34.5$ ,  $P < 0.01$ ) as compared to SA, HIN and untreated control plants. There was no significant differences in trichome numbers between JA, SA and HIN treated and control plants at 5 DAT in ICGV 86699 ( $P > 0.05$ ). However, at 10 DAT, a significant increase in trichome count was observed in JA and SA treated, and insect infested plants ( $F_{(3,11)} = 21.4$ ,  $P < 0.01$ ) as compared to the untreated control plants. In ICGV 86031 and ICG 2271, JA treated plants showed a significant increase in trichome density both at 5 ( $F_{(3,11)} = 14.5$  and  $27.9$ , respectively,  $P < 0.05$ ) and 10 DAT ( $F_{(3,11)} = 12.4$  and  $10.7$ , respectively,  $P < 0.05$ )

than SA and HIN treated and untreated control plants. Across the genotypes, at 5 DAT, ICG 2271 and ICG 1697 plants treated with JA exhibited significantly higher trichome number ( $F_{(4,14)} = 36.9, P < 0.01$ ) than ICGV 86699, ICGV 86031, and JL 24; while at 10 DAT, the JA treated plants of ICG 1697 exhibited significantly greater number of trichomes ( $F_{(4,14)} = 49.8, P < 0.001$ ) than ICGV 86699, ICGV 86031, ICG 2271 and JL 24. The SA and HIN treated plants of ICG 1697 showed greater number of trichomes at 5 DAT ( $F_{(4,14)} = 10.3$  and  $7.8$ , respectively,  $P < 0.05$ ) and 10 DAT ( $F_{(4,11)} = 29.7$  and  $15.4$ , respectively,  $P < 0.05$ ) than ICGV 86699, ICGV 86031, ICG 2271 and JL 24. Constitutive levels of trichomes were greater in ICG 1697 ( $F_{(4,14)} = 12.6, P < 0.05$ ) as compared to rest of the genotypes tested.

#### **4.7. Effect of JA, SA and insect infestation on the ovipositional behavior of *H. armigera***

The susceptible check, JL 24 was preferred for oviposition by the *H. armigera* females in all the treatments as compared to ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 (Table 4.7). However, the numbers of eggs laid differed across the treatments. Among the treatments, PJA + HA, PHI + HA and JA + HA treated plants were less preferred for oviposition across genotypes ( $F_{(5,17)} = 64.3, 33.2, 36.5, 28.7$ , and  $49.6$  for ICGV 86699, ICGV 86031, ICG 2271, ICG 1697 and JL 24, respectively,  $P < 0.01$ ) than the PSA + HA, SA + HA and HA treated plants. Among the resistant genotypes, ICG 1697 plants was least preferred for egg laying in PSA + HIN and HA treated plants ( $F_{(4,14)} = 29.6$  and  $16.1$ , respectively,  $P < 0.01$ ) as compared to ICGV 86699, ICGV 86031, ICG 2271 and JL 24. Plants of ICGV 86699 and ICG 1697 treated with PJA + HA, PHI + HA and JA + HA were less preferred for egg laying ( $F_{(4,14)} = 32.4, 24.5, 19.8$ , respectively,  $P < 0.01$ ) as compared to ICGV 86031, ICG 2271 and JL 24. Across the genotypes, significantly greater number of eggs was laid on JL 24 in all the treatments ( $P < 0.01$ ).

#### **4.8. Orientation of parasitoids towards different groundnut genotypes**

#### 4.8.1. Orientation of *Campoletis chlorideae*

The *Campoletis chlorideae* females exhibited significant attraction to groundnut leaves than to blank in all the genotypes, and the differences between groundnut leaves and blank were significant in ICGV 86699 ( $\chi^2 = 8.4$ ,  $P < 0.01$ ), ICG 2271 ( $\chi^2 = 4.5$ ,  $P < 0.05$ ) and ICG 1697 (3.9,  $P < 0.05$ ). There was no significant difference between samples and blank in ICGV 86031 ( $\chi^2 = 2.1$ ,  $P > 0.05$ ) and JL 24 ( $\chi^2 = 2.5$ ,  $P > 0.05$ ) (Fig. 4.8.1). The time taken to reach the sample varied across the genotypes (Table 4.8.1). The average total time taken by *C. chlorideae* to choose groundnut genotypes was ICGV 86699 (0.5 min) and ICG 2271 (1.0 min), ICGV 86031 (1.1 min), ICG 1697 (1.3 min) and JL 24 (1.5). In a comparison between the test genotypes and the susceptible check, JL 24, the *C. chlorideae* females showed greater attraction towards ICGV 86699 ( $\chi^2 = 4.2$ ,  $P < 0.05$ ) and ICGV 86031 ( $\chi^2 = 6.1$ ,  $P < 0.05$ ) than to JL 24. The time taken to reach the test genotype was 1.0, 1.3, 1.3 and 1.4 min, respectively, for ICGV 86031, ICGV 86699, ICG 2271 and ICG 1697. Under choice conditions between infested and uninfested samples, the significant differences were observed in ICGV 86699 ( $\chi^2 = 5.4$ ,  $P < 0.05$ ), ICGV 86031 ( $\chi^2 = 4.7$ ,  $P < 0.05$ ) and ICG 2271 ( $\chi^2 = 5.1$ ,  $P < 0.05$ ). No significant differences in parasitoid attraction between infested and uninfested plants were observed in ICG 1697 and JL 24. The time taken was less to reach the infested samples (0.5, 0.5, 1.0 and 1.3 min for ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697, respectively) than the uninfested plants.

#### 4.8.2. Orientation of *Trichogramma chilonis*

The *Trichogramma chilonis* females showed more preference towards the groundnut samples as compared to the blank; however, significant differences were observed between test sample and blank in case of ICGV 86699 ( $\chi^2 = 6.05$ ,  $P < 0.05$ ), ICGV 86031 ( $\chi^2 = 3.9$ ,  $P < 0.05$ ), and ICG 2271 ( $\chi^2 = 4.8$ ,  $P < 0.05$ ) (Fig. 4.8.2), and the mean time taken to reach the



sample varied. Differences were not significant between the test sample and the blank in case of ICG 1697 ( $\chi^2 = 2.45$ ,  $P > 0.05$ ) and JL 24 ( $\chi^2 = 0.45$ ,  $P > 0.05$ ). Under choice conditions between the test genotypes and JL 24, the *T. chilonis* females were attracted more towards the resistant genotypes; however, the differences were significant only in ICGV 86699 ( $\chi^2 = 6.4$ ,  $P < 0.05$ ). The time taken to reach the test sample differed across the genotypes (5.3 – 8.3 min) (Table 4.8.2). Under the choice conditions between the infested and uninfested samples of the same genotype, significant differences were observed between insect infested and uninfested samples of ICGV 86699 ( $\chi^2 = 8.4$ ,  $P < 0.01$ ), ICGV 86031 ( $\chi^2 = 4.2$ ,  $P < 0.05$ ) and ICG 1697 ( $\chi^2 = 3.9$ ,  $P < 0.05$ ) and the time taken was 6.8 min.

#### **4.9. Effect of flavonoids on survival, development and midgut enzymes of *H. armigera* and *S. litura***

##### **4.9.1. *Helicoverpa armigera***

Among the flavonoids tested, higher larval mortality of *H. armigera* was observed in larvae fed on diet treated with 1000 ppm of chlorogenic acid (42.5%), caffeic acid (37.2%) and protocatechuic acid (34.5%), followed by quercetin (27.5%), cinnamic acid (25.8%), catechin (25.0%) and ferulic acid (23.3%) (Table 4.9.1a). At 500 ppm, significantly higher mortality was observed in larvae fed on the diets treated with caffeic acid (26.0%) and protocatechuic acid (25.5%) as compared to the rest of the treatments. There was no significant effect on larval mortality at 100 ppm.

At 5 days after treatment (DAT), the larval weights (mg per five larvae) of *H. armigera* larvae fed on diets treated with flavonoids at 100, 500 and 1000 ppm were lower in protocatechuic acid (135.7, 73.3 and 24.4), gentic acid (103.2, 59.9 and 27.6), chlorogenic acid (101.5, 76.7 and 30.8), caffeic acid (0.04, 0.07 and 0.13), ferulic acid

(132.9, 77.5 and 33.8), and trihydroxyflavone (0.02, 0.16 and 0.19), respectively (Table 4.9.1b). However, at 10 DAT, caffeic acid showed significantly greater reduction in larval weight (mg per five larvae) (483.1, 250.4 and 129.9), followed by protocatechuic acid (491.2, 273.6 and 181.4) at 100, 500 and 1000 ppm, respectively, than the rest of the treatments. As compared to the larvae fed on untreated control diet, larvae fed on flavonoid treated diets showed reduced larval weights.

A considerable effect of flavonoids at 1000 ppm concentration was observed on the serine protease and trypsin activities of *H. armigera* larvae; however, the levels of significance varied (Fig. 4.9.1a,b). Across the treatments larvae fed on genistic and protocatechuic acids at 1000 ppm showed lower serine protease activity than those fed at 1000 ppm of rest of the treatments. GST activity of *H. armigera* larvae was significantly higher, when fed on the diets treated with chlorogenic acid, caffeic acid, genistic acid, ferulic acid, protocatechuic acids, trihydroxyflavone, catechin, cinnamic acid and quercetin at 1000 ppm than at 500 ppm and larvae fed on control diet (Fig. 4.9.1c). Across the treatments, protocatechuic acids, trihydroxyflavone and caffeic acid induced higher levels than rest of the treatments at 1000 ppm. Larvae fed on untreated control diet had lower levels of GST activity. *H. armigera* larvae fed on flavonoid treated diets showed lower levels of EST activity at 1000 ppm in chlorogenic acid, caffeic acid, ferulic acid, genistic acid, ferulic acid, Catechin, cinnamic acid, quercetin and umbellifeone (4.9.1d).

#### **4.9.2. *Spodoptera litura***

The *S. litura* larvae fed on diets treated with chlorogenic acid, protocatechuic acid and caffeic acid at 1000 ppm showed significantly higher mortality (40.3, 37.2 and 33.1%, respectively) as compared to the rest of the treatments at 10 DAT (Table 4.9.2a). The larval weights were significantly reduced in the larvae fed on diets with protocatechuic acid,

caffeic acid and chlorogenic acid at 5 DAT at 1000 ppm (34.5, 35.8 and 37.6 mg per five larvae), followed by the larvae fed on the diets treated with catechin, ferulic acid, trihydroxyflavone, umbelliferone and cinnamic acid (42.4, 45.4, 47.7, 51.9 and 54.2 mg per five larvae, respectively) (Table 4.9.2b). At 10 DAT, larval weights (mg per five larvae) were significantly reduced in the larvae fed on diet treated with 1000 ppm of chlorogenic (204.8), ferulic (219.9), caffeic (226.7) and protocatechuic (231.8) acids.

The total serine protease and trypsin activities were lower in larvae fed on the diets treated with 1000 ppm of chlorogenic acid, caffeic acid, ferulic acid, trihydroxyflavone, gensitic acids, cinnamic acid and umbelliferone (Fig. 4.9.2a,b). No significant difference was observed in serine protease activity of larvae fed on different flavonoid treated diets at 100 and 500 ppm concentrations. Among the concentrations, all the treatments significantly reduced the trypsin activity of *S. litura* larvae at 1000 ppm concentration. Across the treatments, lower levels of trypsin activity were observed in larvae fed on chlorogenic acid, ferulic acid, protocatechuic acid, catechin and cinnamic acid at 1000 ppm. All the treatments induced GST activity of *S. litura* larvae at 1000 ppm (Fig. 4.9.2c). Across treatments, protocatechuic acid, catechin, cinnamic acid and quercetin induced higher GST activity than the rest of the treatments. The EST activity showed reduction in larvae fed on flavonoid treated diets at 1000 ppm (Fig. 4.9.2d).

#### **4.10. Effect of lectins and phenyl $\beta$ - glucoside on survival, development and physiology of *H. armigera* and *S. litura***

Semisynthetic diet was incorporated with different concentrations of groundnut leaf lectin (GLL), concavalin (ConA) and phenyl  $\beta$ - glucoside and evaluated for their toxicity, effects on growth and development and physiological activities of *H. armigera* and *S. litura* larvae.

#### **4.10.1. Effect on *H. armigera***

##### **4.10.1.1. Effect on larval weight**

The semi-synthetic diet did not cause significant larval mortality in both *H. armigera* and *S. litura* (data not shown). However the larval body weights were reduced when these insects were fed on the treated diets. At 5 DAT, *H. armigera* larvae fed on the diets treated with GLL, ConA and phenyl  $\beta$ - glucoside ( $5 \mu\text{g mL}^{-1}$  each) showed body weight reduction by 4.1-, 3.8- and 2.4- fold, respectively, as compared to those fed on the control diet (Table 4.10.1.1). At 10 DAT, larval weights were reduced by 4.0-, 2.4- and 2.1- fold, when fed on the diets treated with GLL, ConA and phenyl  $\beta$ - glucoside ( $5 \mu\text{g mL}^{-1}$  each), respectively.

**4.10.1.2. Total serine protease and trypsin activities:** The *H. armigera* larvae fed on the diets containing 5 and  $2.5 \mu\text{g mL}^{-1}$  GLL showed significantly lower levels of total serine protease and trypsin activities ( $F_{(2,8)} = 19.9$  and  $17.3$ , respectively,  $P < 0.05$ ) than those fed on diets with  $1.25 \mu\text{g mL}^{-1}$  GLL (Table 4.10.1.2). Similarly, larvae fed on ConA treated diets at 5 and  $2.5 \mu\text{g mL}^{-1}$  concentration had significantly reduced total serine protease and trypsin activities ( $P < 0.05$ ). The larvae fed on phenyl  $\beta$ - glucoside treated diet showed reduced serine protease activity at 5 and  $2.5 \mu\text{g mL}^{-1}$  concentrations ( $F_{(2,8)} = 11.2$ ,  $P < 0.05$ ), but did not exhibit any significant difference in trypsin activity across the concentrations. Across the treatments, larvae fed on GLL treated diet at 5 and  $2.5 \mu\text{g mL}^{-1}$  concentrations had significantly reduced serine protease and trypsin activities ( $P < 0.05$ ).

**4.10.1.3 GST and EST activities:** The *H. armigera* larvae fed on GLL showed increased GST activity at 2.5 and  $5 \mu\text{g mL}^{-1}$  concentrations as compared to those fed on diet with  $1.25 \mu\text{g mL}^{-1}$  concentrations ( $F_{(2,8)} = 14.5$ ,  $P < 0.05$ ) (Table 4.10.1.3). Although there was an increase in GST activity of the larvae fed on ConA and phenyl  $\beta$ - glucoside treated diets, the

differences were not statistically significant ( $P > 0.05$ ). Across the treatments, no significant differences were observed in GST activity of *H. armigera* larvae in all the concentrations used ( $P > 0.05$ ).

The *H. armigera* larvae fed on GLL and ConA treated diet showed reduced EST activity at  $5 \mu\text{g mL}^{-1}$  concentration ( $F_{(2,8)} = 7.8$  and  $9.9$ , respectively,  $P < 0.05$ ) than those fed on diet with  $2.5$  and  $1.25 \mu\text{g mL}^{-1}$  concentrations (Table 4.10.1.3). The larvae fed on diet containing phenyl  $\beta$ - glucoside did not show any significant effect on EST activity of *H. armigera* larvae ( $P > 0.05$ ). Across the treatments, larvae fed on GLL treated diet at  $5 \mu\text{g mL}^{-1}$  showed significantly reduced EST activity ( $F_{(3,11)} = 23.5$ ,  $P < 0.05$ ) as compared to those fed on ConA and phenyl  $\beta$ - glucoside treated diets. In concentrations,  $2.5$  and  $1.25 \mu\text{g mL}^{-1}$ , no significant differences were recorded in EST activity of the larvae across the treatments ( $P > 0.05$ ).

**4.10.1.4. Total protein content in midgut:** The *H. armigera* larvae fed on diet treated with GLL showed significantly reduced protein content at  $5 \mu\text{g mL}^{-1}$  concentration ( $F_{(2,8)} = 10.1$ ,  $P < 0.05$ ) than those fed on diet with  $2.5$  and  $1.25 \mu\text{g mL}^{-1}$  concentrations (Table 4.10.1.4). No significant differences were observed in total protein content between the larvae fed on diet treated with different concentrations of ConA and phenyl  $\beta$ - glucoside ( $P > 0.05$ ). Across the treatments, larvae fed on GLL treated diet at  $5$  and  $2.5$  had significantly reduced protein content ( $F_{(3,11)} = 13.1$  and  $9.1$ , respectively,  $P < 0.05$ ) than the larvae fed on the corresponding treatments of ConA and phenyl  $\beta$ - glucoside and on untreated control diet.

#### **4.10.2. Effect on *S. litura***

##### **4.10.2.1. Effect on larval weight**

At 5 DAT, the *S. litura* larvae exhibited reduction in weight by 2.8-, 1.8- and 1.5-folds, respectively, on diets with GLL, ConA and phenyl  $\beta$ - glucoside ( $5 \mu\text{g mL}^{-1}$  each)

(Table 4.10.2.1). AT 10 DAT, the reduction in larval weights was 2.7-, 2.0- and 1.7-folds, respectively, in larvae fed on diets treated with GLL, ConA and phenyl  $\beta$ - glucoside ( $5 \mu\text{g mL}^{-1}$  each).

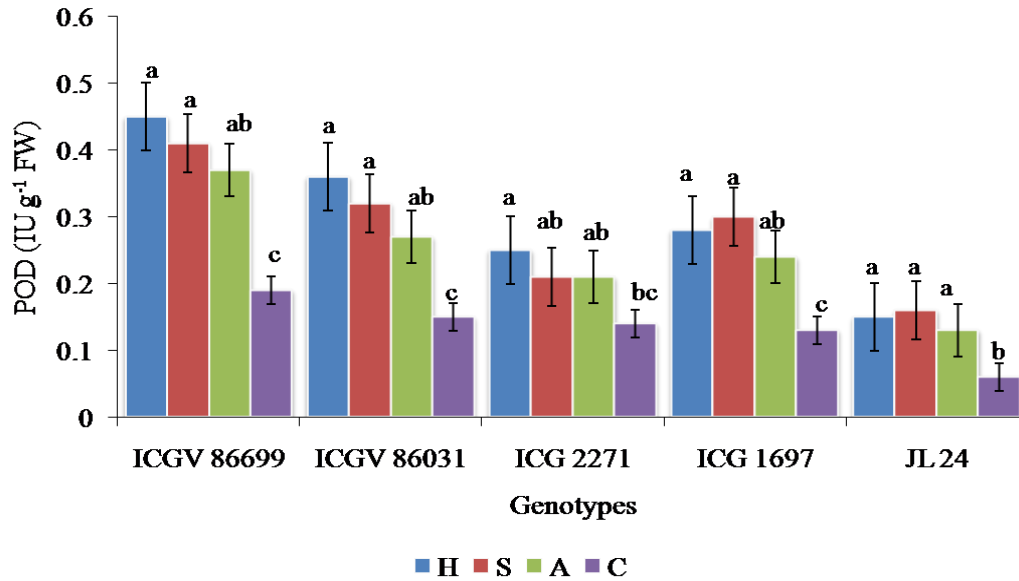
**4.10.2.2. Total serine protease and trypsin activities:** The *S. litura* larvae showed almost similar response to all the enzymes as in *H. armigera*. The larvae fed on diet treated with GLL, ConA and phenyl  $\beta$ - glucoside at  $5 \mu\text{g mL}^{-1}$  showed significantly reduced serine protease activity ( $F_{(2,8)} = 21.4$  and  $33.4$ , respectively,  $P < 0.05$ ) than those fed at  $2.5$  and  $1.25 \mu\text{g mL}^{-1}$  concentrations in each treatment (Table 4.10.2.2). Across the treatments, reduced serine protease activity was observed in larvae fed on GLL treated diet at  $5 \mu\text{g mL}^{-1}$  ( $F_{(2,8)} = 14.1$  and  $17.3$ , respectively,  $P < 0.05$ ) than the rest of the treatments. However, at  $2.5$  and  $1.25 \mu\text{g mL}^{-1}$  concentrations, serine protease activity was significantly lower in larvae fed on treated diets as compared to those fed on untreated control diet. The trypsin activity of the larvae fed on different treatments at  $5 \mu\text{g mL}^{-1}$  concentrations was significantly lower than those fed on diet at  $2.5$  and  $1.25 \mu\text{g mL}^{-1}$  concentrations. Across the treatments, larvae fed on diets treated with GLL, ConA and phenyl  $\beta$ - glucoside exhibited reduced trypsin activity at  $5 \mu\text{g mL}^{-1}$  as compared to those fed on untreated control diet. No significant differences were observed in trypsin activity of larvae fed on diet treated with GLL, ConA and phenyl  $\beta$ - glucoside at  $2.5$  and  $1.25 \mu\text{g mL}^{-1}$  concentrations and the larvae fed on untreated control diet.

**4.10.2.3. GST and EST activities in insects:** The GST activity of larvae fed on the diet treated with GLL at  $5 \mu\text{g mL}^{-1}$  concentrations was significantly higher than those fed at  $1.25 \mu\text{g mL}^{-1}$  treated diet (Table 4.10.2.3). No significant difference was recorded in GST activity of the larvae fed on rest of the diets at different concentrations. Moreover, across the treatments, there was no significant difference in GST activity. The EST activity of the larvae differed significantly across the concentrations through the treatment. Across the

treatments, larvae fed on GLL, ConA and phenyl  $\beta$ - glucoside treated diets had significantly lower EST activity than those fed on the untreated control diet.

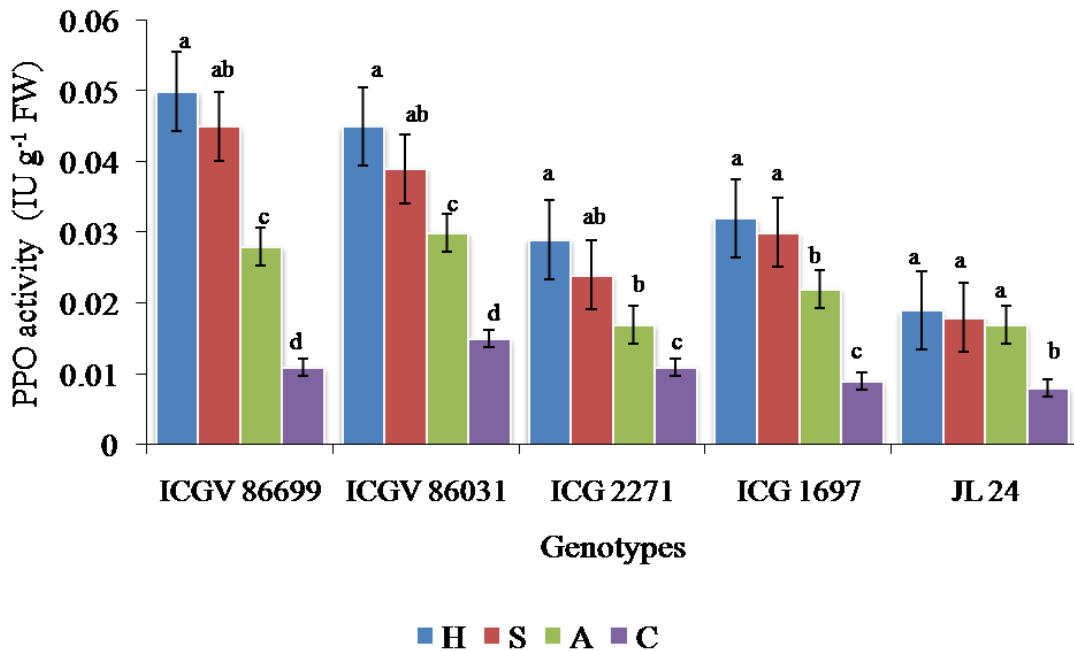
**4.10.1.3 Total protein content in midgut:** *S. litura* larvae fed on GLL and ConA treated diet at 5  $\mu\text{g mL}^{-1}$  concentration exhibited lower total protein content ( $F_{(2,8)} = 13.7$  and 10.8, respectively,  $P < 0.05$ ) as compared to those fed on the diet with corresponding 2.5 and 1.25  $\mu\text{g mL}^{-1}$  concentrations (Table 4.10.1.4). The larvae fed on the diet treated with phenyl  $\beta$ -glucoside did not show any significant difference across the concentrations. Among the treatments, significantly lower protein content was recorded in the larvae fed on GLL treated diet at 5 ( $F_{(3,11)} = 21.3$ ,  $P < 0.01$ ) and 2.5  $\mu\text{g mL}^{-1}$  ( $F_{(3,11)} = 17.6$ ,  $P < 0.05$ ) as compared to those fed on the respective treatments of ConA and phenyl  $\beta$ - glucoside and on untreated control diet.

**Fig. 4.3.1a: Peroxidase (POD) activity (IU g<sup>-1</sup>FW) of groundnut genotypes infested with *Helicoverpa armigera*, *Spodoptera litura* and *Aphis craccivora*.**



Bars (Mean  $\pm$  SD) of same colors with similar alphabets are not statistically different at ( $P < 0.05$ ).

**Fig. 4.3.1b: Polyphenol oxidase (PPO) activity (IU g<sup>-1</sup>FW) of groundnut genotypes infested with *Helicoverpa armigera*, *Spodoptera litura* and *Aphis craccivora*.**

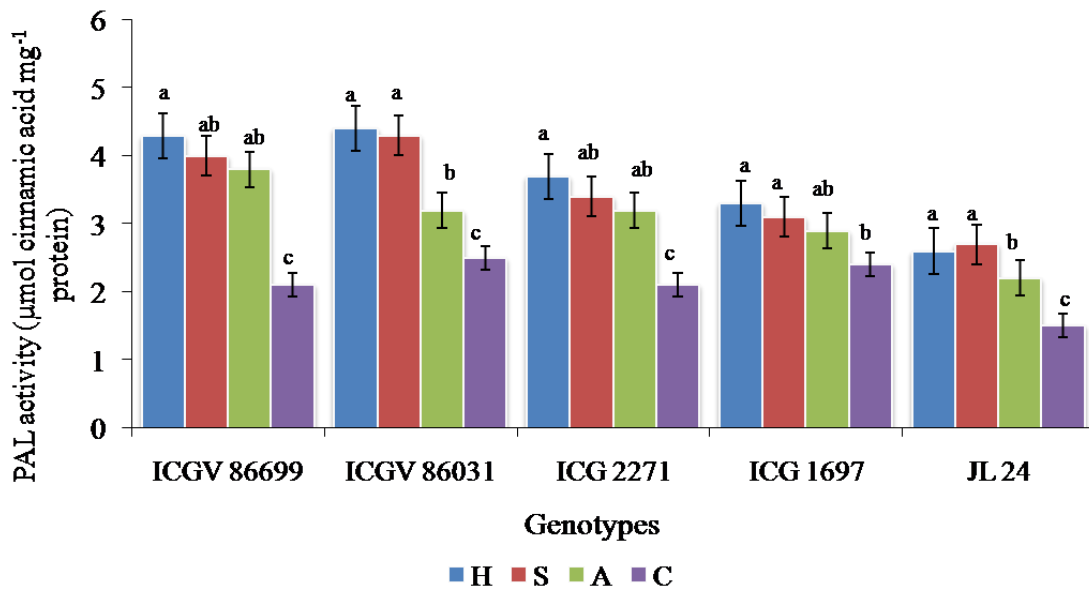


Bars (Mean  $\pm$  SD) of same colors with similar alphabets are not statistically different at ( $P < 0.05$ ).

H = *H. armigera* infested; S = *S. litura* infested; A = *A. craccivora* infested; C= non-infested control; FW = fresh weight of leaf tissue.

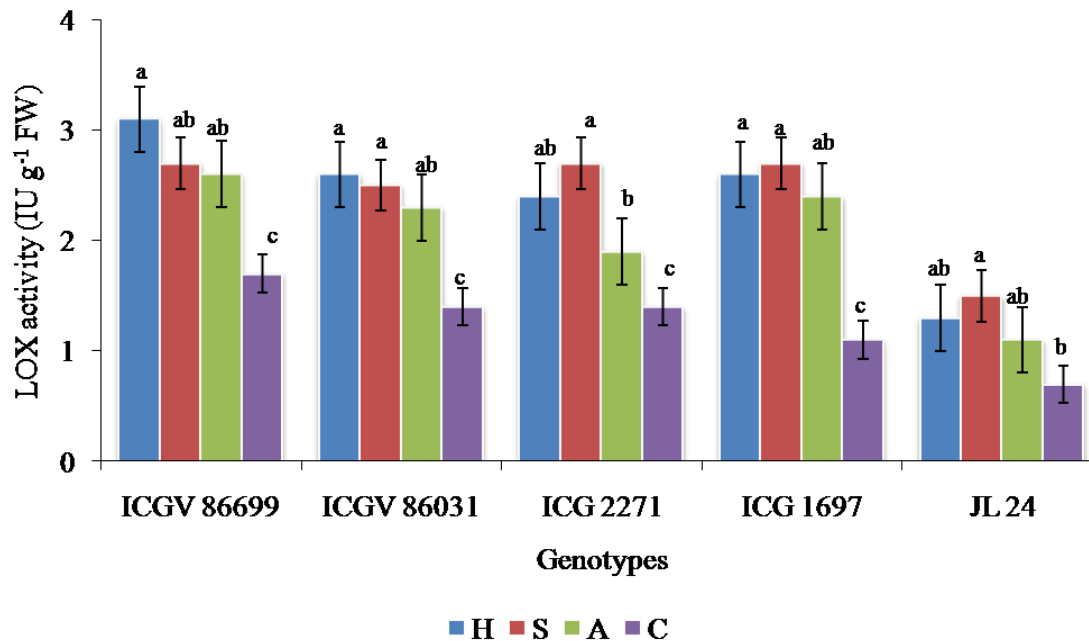


Fig. 4.3.1c: Phenylalanine ammonia lyase (PAL) activity ( $\mu\text{mol cinnamic acid min}^{-1} \text{mg}^{-1}$  protein) of groundnut genotypes infested with *Helicoverpa armigera*, *Spodoptera litura* and *Aphis craccivora*.



Bars (Mean  $\pm$  SD) of same colors with similar alphabets are not statistically different at ( $P < 0.05$ ).

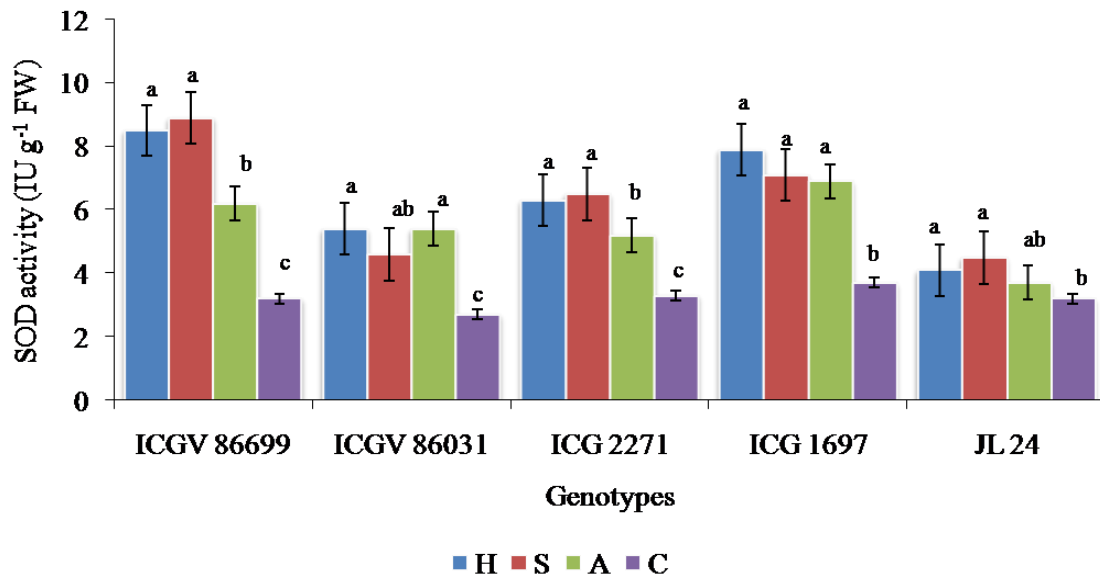
Fig. 4.3.1d: Lipoxygenase (LOX) activity ( $\text{IU g}^{-1}\text{FW}$ ) of groundnut genotypes infested with *Helicoverpa armigera*, *Spodoptera litura* and *Aphis craccivora*.



Bars (Mean  $\pm$  SD) of same colors with similar alphabets are not statistically different at ( $P < 0.05$ ).

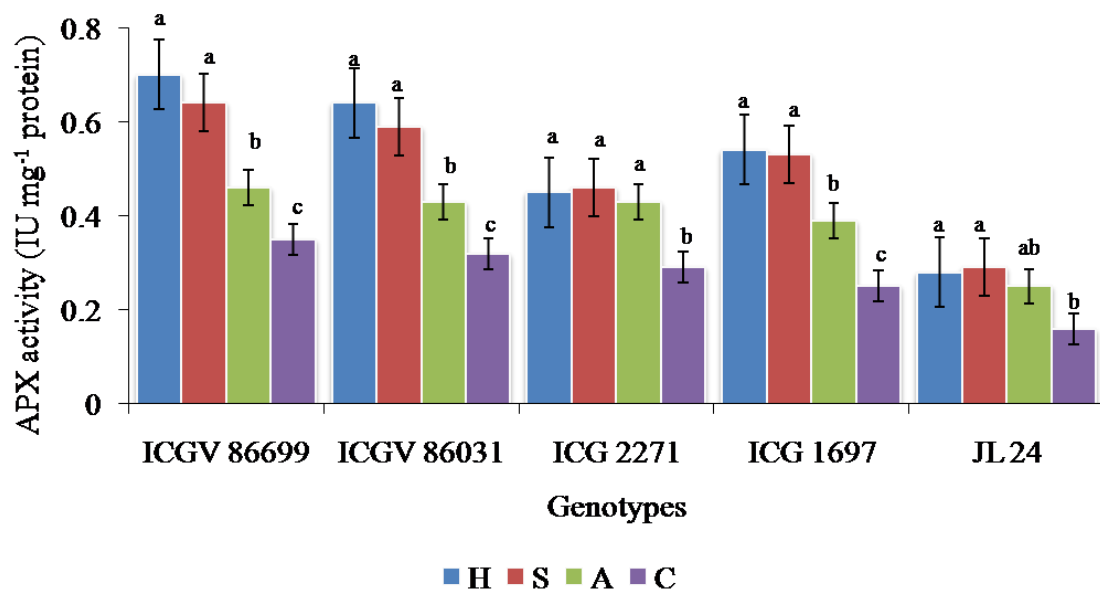
H = *H. armigera* infested; S = *S. litura* infested; A = *A. craccivora* infested; C= non-infested control; FW = fresh weight of leaf tissue.

Fig. 4.3.1e: Superoxide dismutase (SOD) activity (IU g<sup>-1</sup>FW) of groundnut genotypes infested with *Helicoverpa armigera*, *Spodoptera litura* and *Aphis craccivora*.



Bars (Mean  $\pm$  SD) of same colors with similar alphabets are not statistically different at ( $P < 0.05$ ).

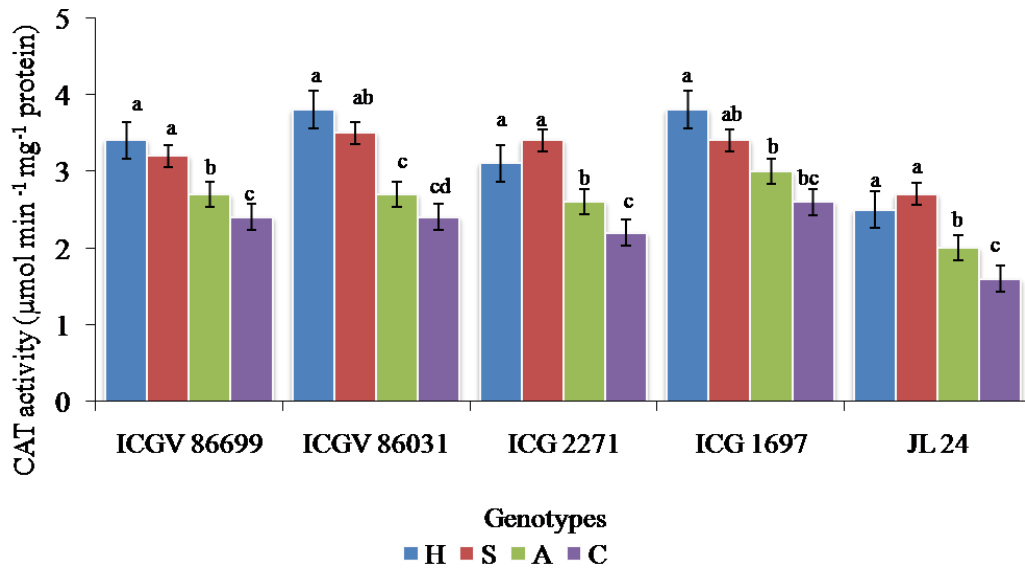
Fig. 4.3.1f: Ascorbate peroxidase (APX) activity (IU mg<sup>-1</sup>protein) of groundnut genotypes infested with *Helicoverpa armigera*, *Spodoptera litura* and *Aphis craccivora*.



Bars (Mean  $\pm$  SD) of same colors with similar alphabets are not statistically different at ( $P < 0.05$ ).

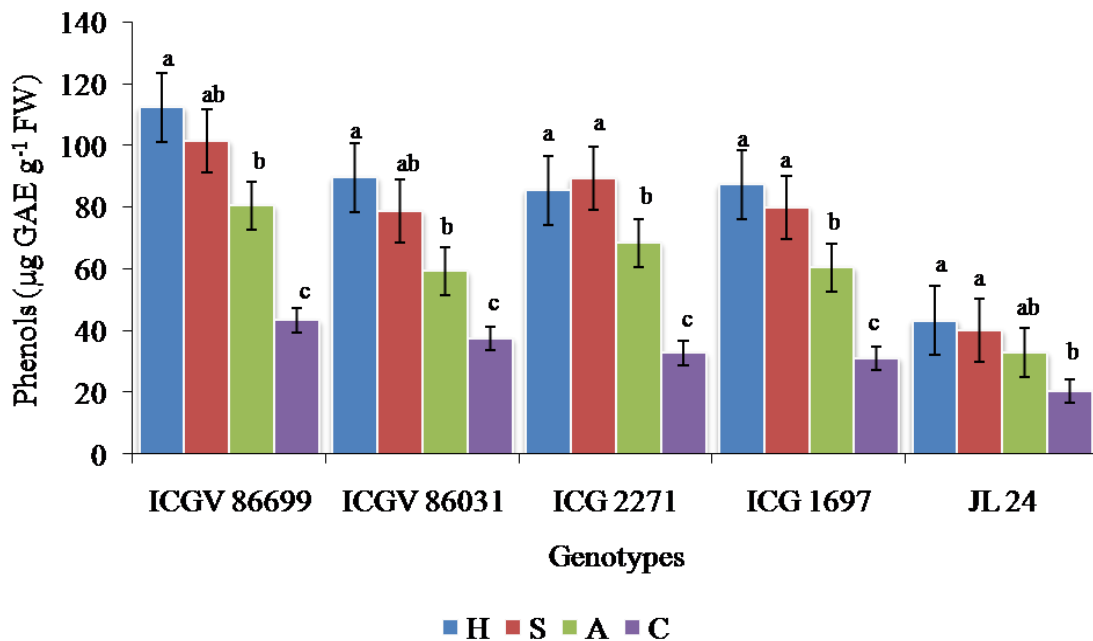
H = *H. armigera* infested; S = *S. litura* infested; A = *A. craccivora* infested; C = non-infested control; FW = fresh weight of leaf tissue.

Fig. 4.3.1g: Catalase (CAT) activity ( $\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$ ) of groundnut genotypes infested with *Helicoverpa armigera*, *Spodoptera litura* and *Aphis craccivora*.



Bars (Mean  $\pm$  SD) of same colors with similar alphabets are not statistically different at ( $P < 0.05$ ).

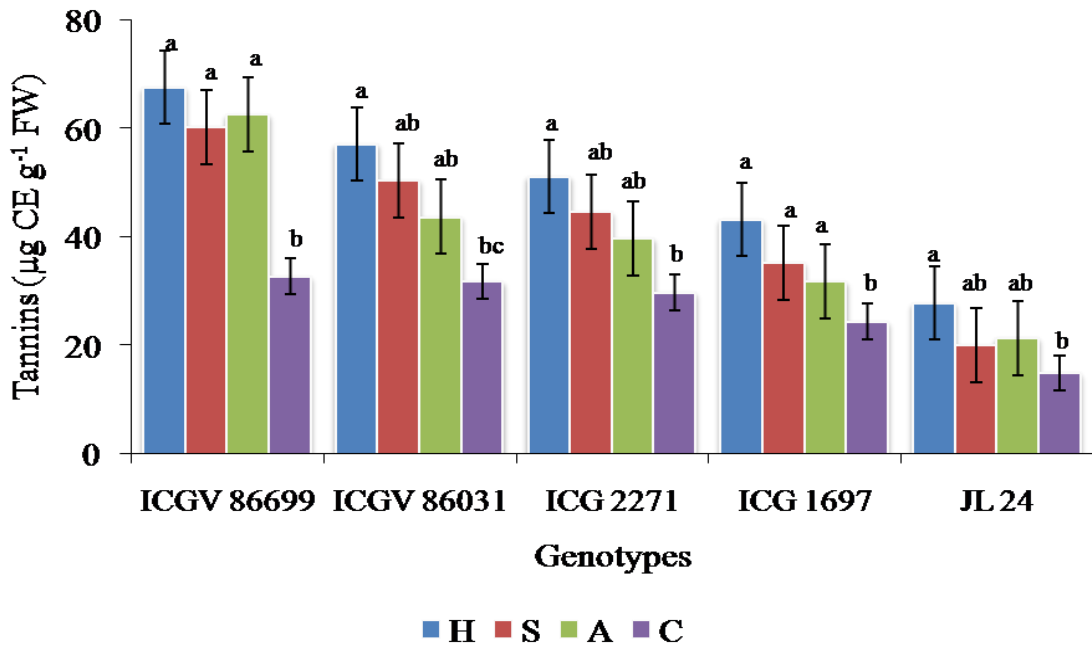
Fig. 4.3.1h: Total phenols ( $\mu\text{g GAE g}^{-1} \text{FW}$ ) of groundnut genotypes infested with *Helicoverpa armigera*, *Spodoptera litura* and *Aphis craccivora*.



Bars (Mean  $\pm$  SD) of same colors with similar alphabets are not statistically different at ( $P < 0.05$ ).

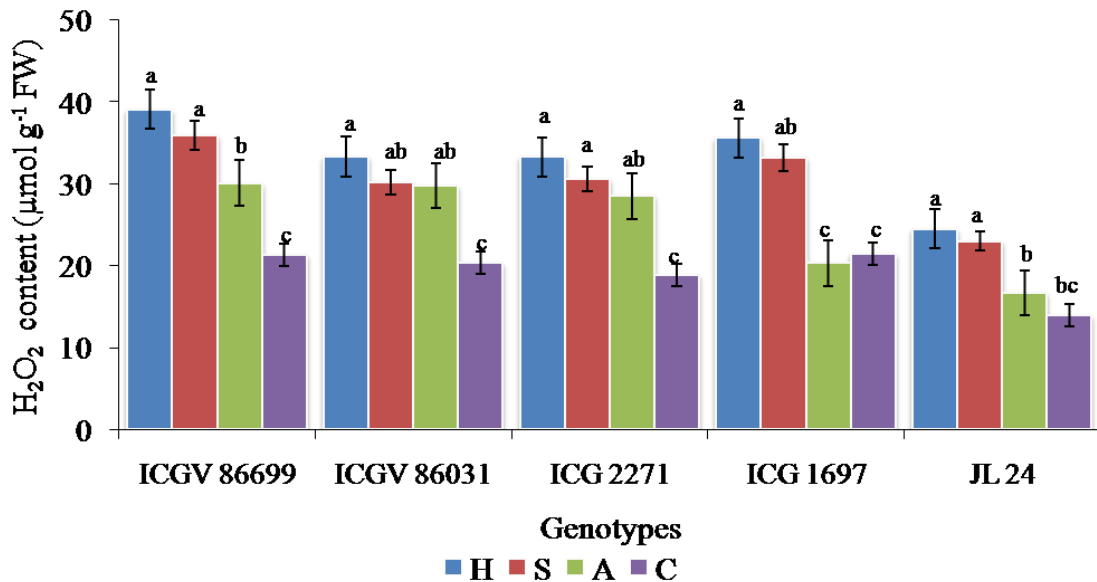
H = *H. armigera* infested; S = *S. litura* infested; A = *A. craccivora* infested; C= non-infested control; FW = fresh weight of leaf tissue; GAE = Gallic acid equivalent.

Fig. 4.3.1i: Tannins ( $\mu\text{g CE g}^{-1}\text{ FW}$ ) of groundnut genotypes infested with *Helicoverpa armigera*, *Spodoptera litura* and *Aphis craccivora*.



Bars (Mean  $\pm$  SD) of same colors with similar alphabets are not statistically different at ( $P < 0.05$ ).

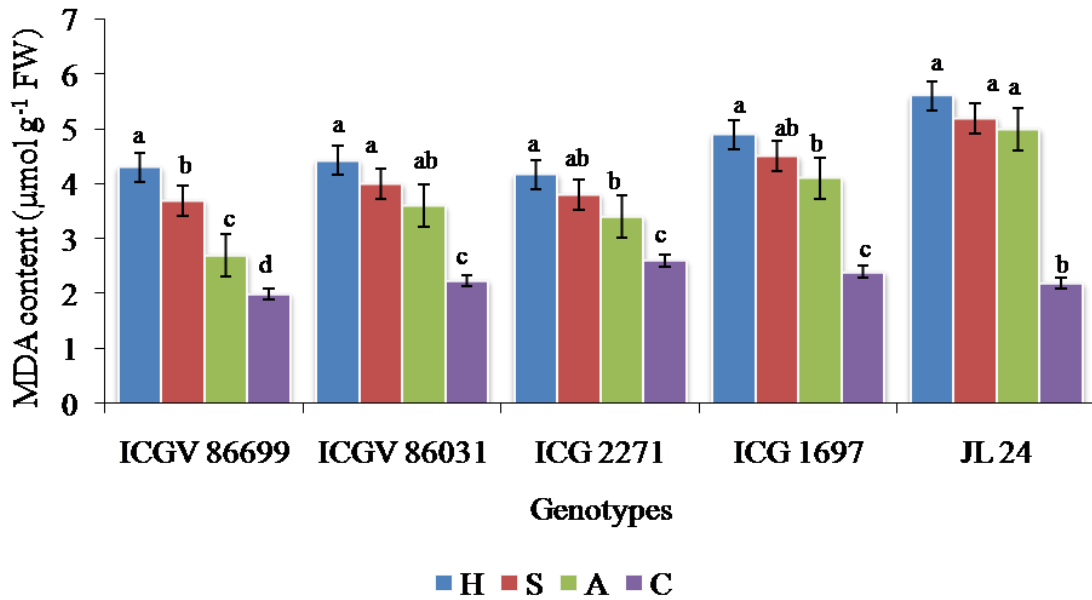
Fig. 4.3.1j: Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) content ( $\mu\text{mol g}^{-1}\text{ FW}$ ) of groundnut genotypes infested with *Helicoverpa armigera*, *Spodoptera litura* and *Aphis craccivora*.



Bars (Mean  $\pm$  SD) of same colors with similar alphabets are not statistically different at ( $P < 0.05$ ).

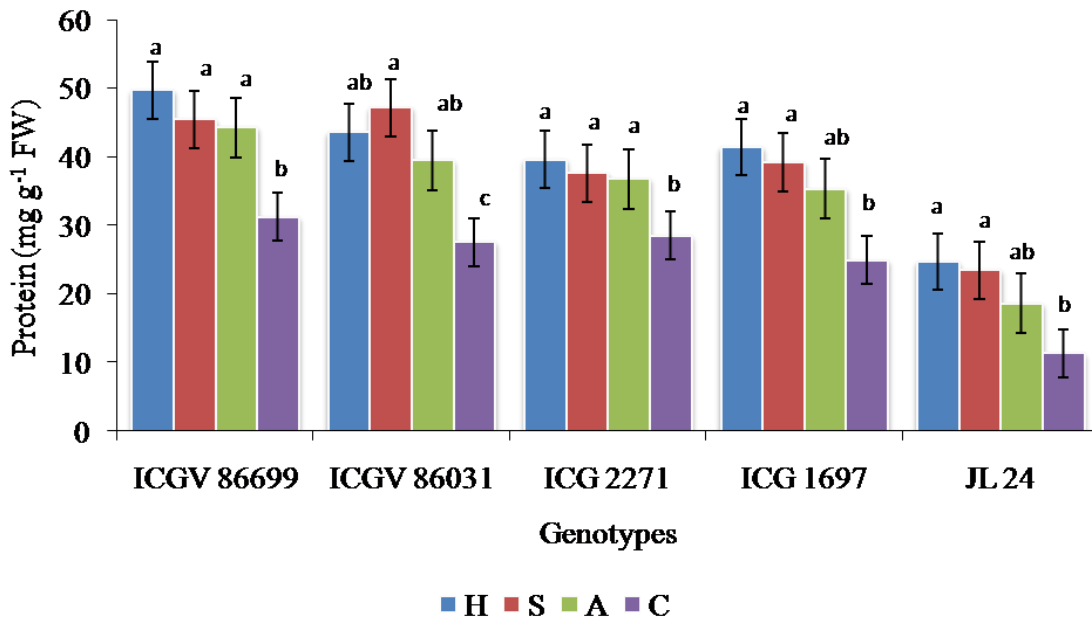
H = *H. armigera* infested; S = *S. litura* infested; A = *A. craccivora* infested; C = non-infested control; FW = fresh weight of leaf tissue; CE = Catechin equivalent.

Fig. 4.3.1k: Malondialdehyde (MDA) content ( $\mu\text{mol g}^{-1}$  FW) of groundnut genotypes infested with *Helicoverpa armigera*, *Spodoptera litura* and *Aphis craccivora*.



Bars (Mean  $\pm$  SD) of same colors with similar alphabets are not statistically different at ( $P < 0.05$ ).

Fig. 4.3.1l: Protein content ( $\text{mg g}^{-1}$  FW) of groundnut genotypes infested with *Helicoverpa armigera*, *Spodoptera litura* and *Aphis craccivora*.



Bars (Mean  $\pm$  SD) of same colors with similar alphabets are not statistically different at ( $P < 0.05$ ).

H = *H. armigera* infested; S = *S. litura* infested; A = *A. craccivora*; C = non-infested control; FW = fresh weight of leaf tissue.

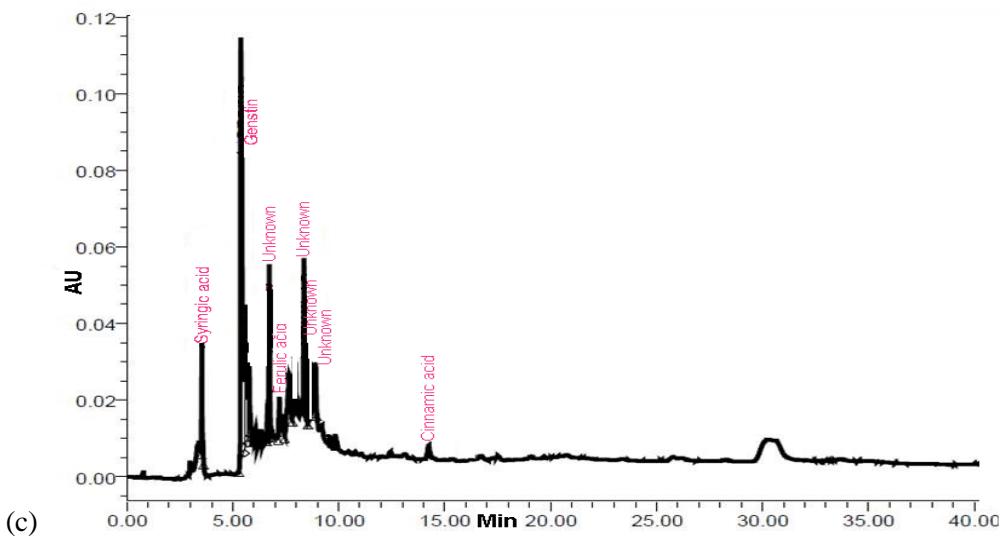
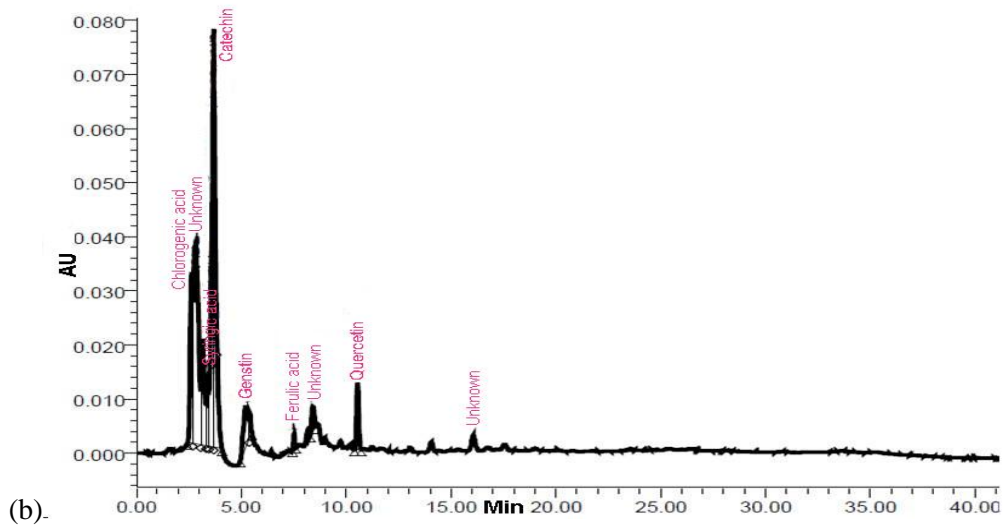
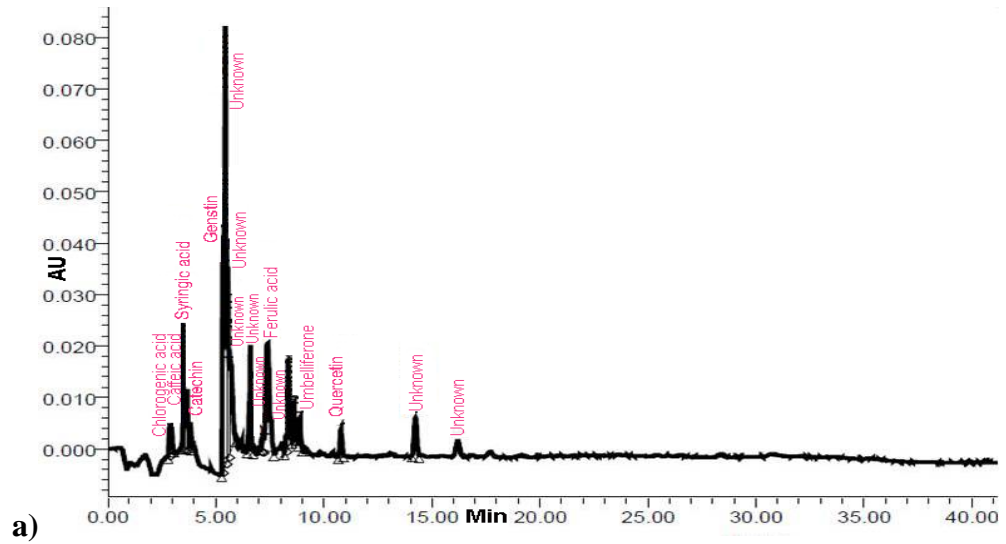


Fig.4.3.3.1. HPLC chromatogram of ICGV 86699 plants infested with; (a) *H. armigera*; (b) *A. craccivora*; and (c) untreated control plants.

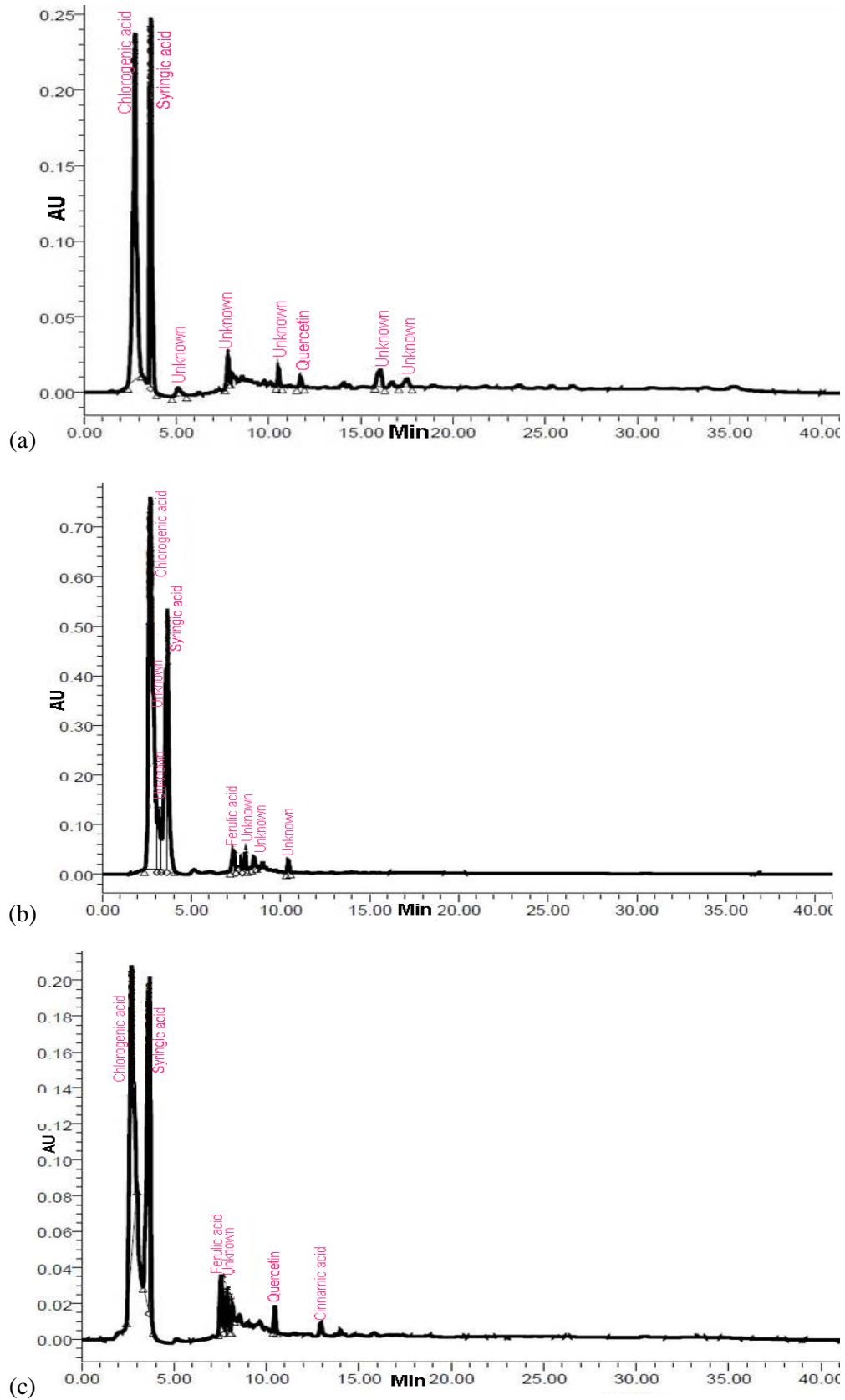


Fig.4.3.3.2. HPLC chromatogram of ICGV 86031 plants infested with; (a) *H. armigera*; (b) *A. craccivora*; and (c) untreated control plants.

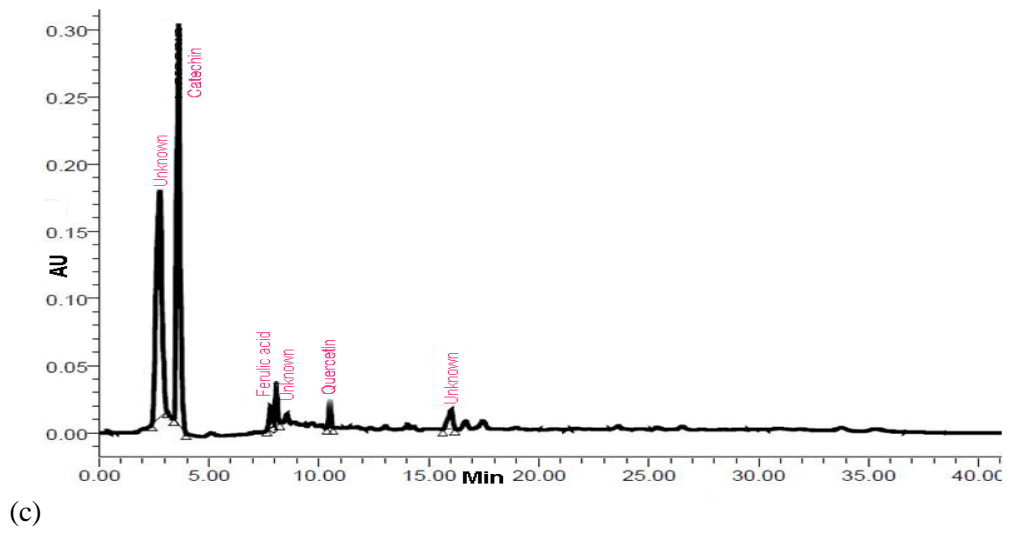
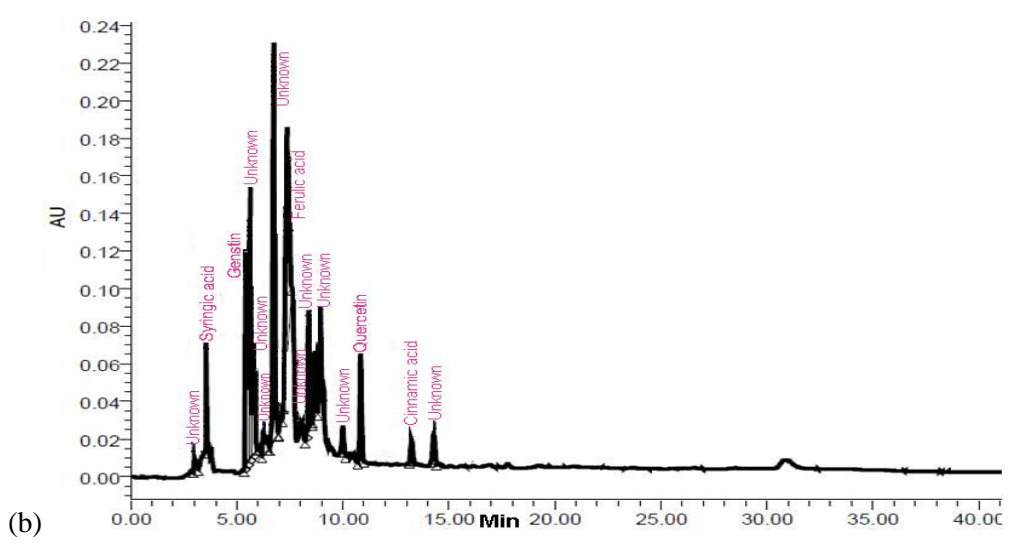
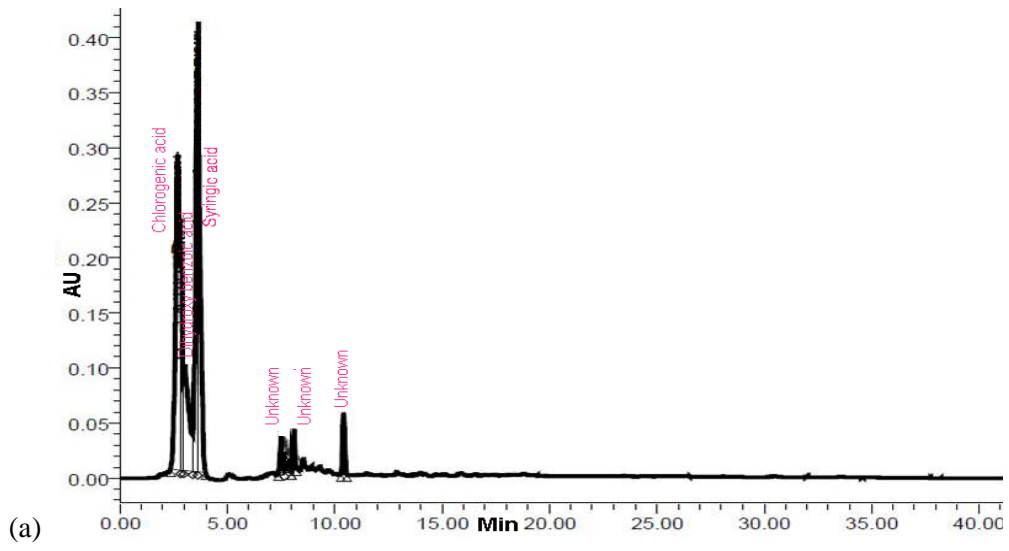


Fig.4.3.3.3. HPLC chromatogram of ICG 2271 plants infested with; (a) *H. armigera*; (b) *A. craccivora*; and (c) untreated control plants.



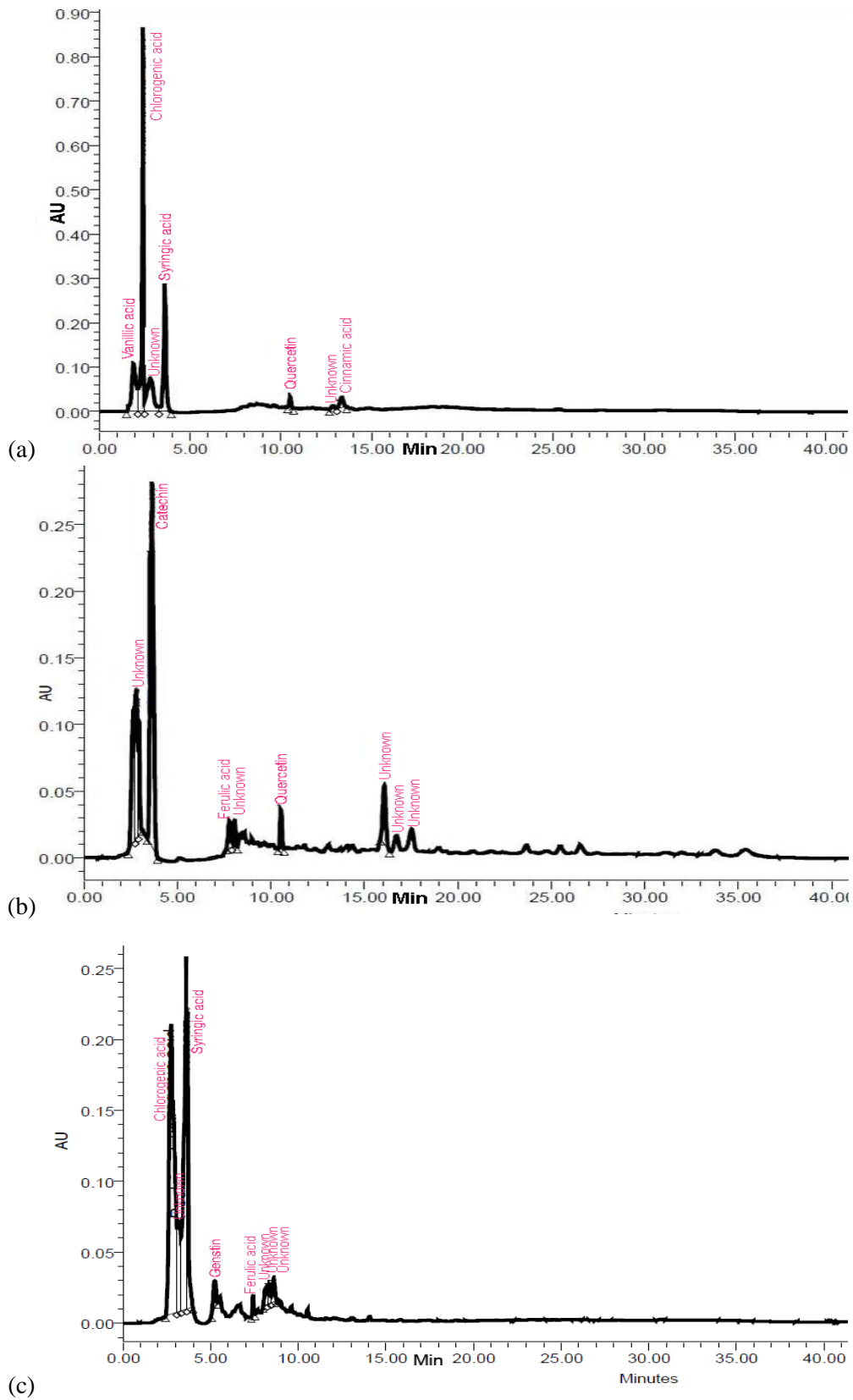


Fig.4.3.3.4. HPLC chromatogram of ICG 1697 plants infested with; (a) *H. armigera*; (b) *A. craccivora*; and (c) untreated control plants.

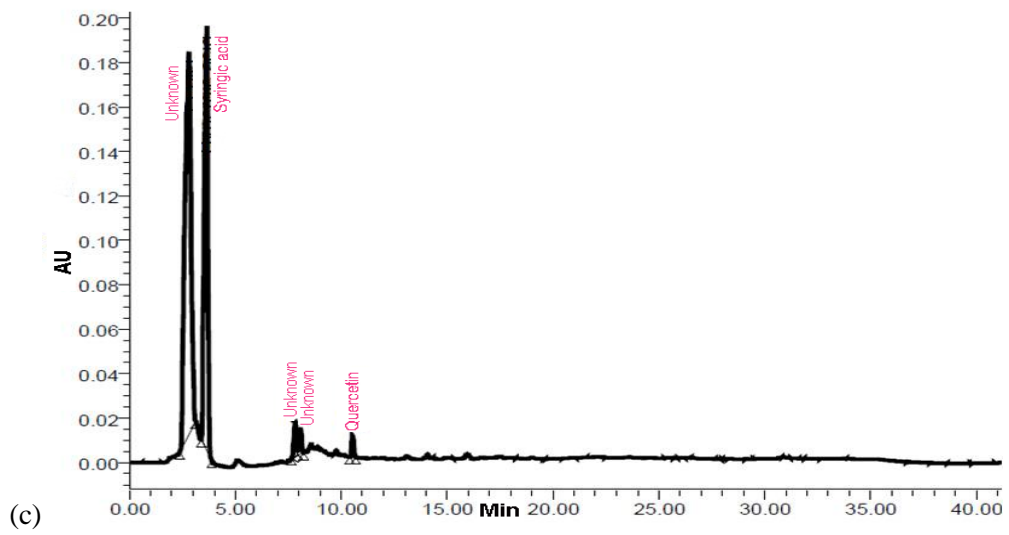
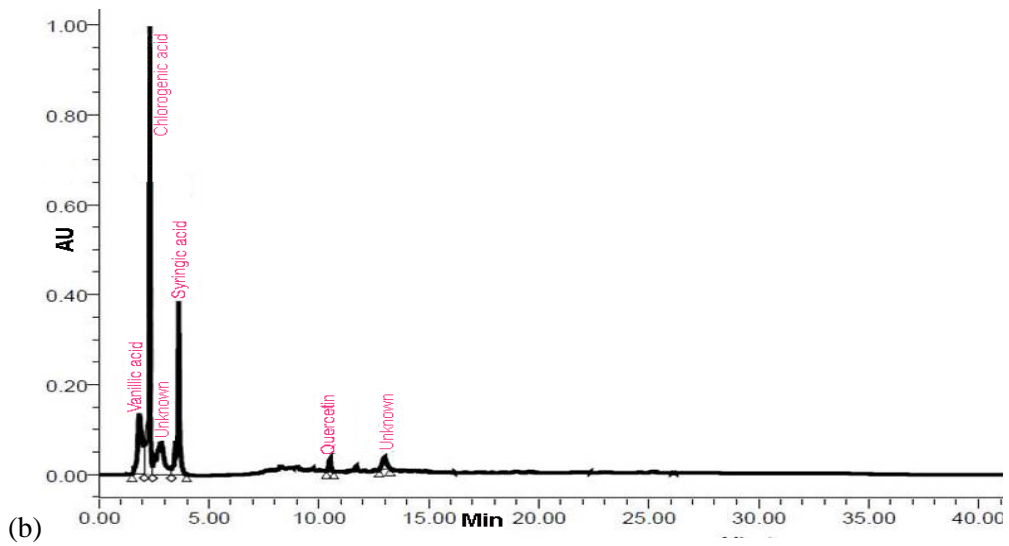
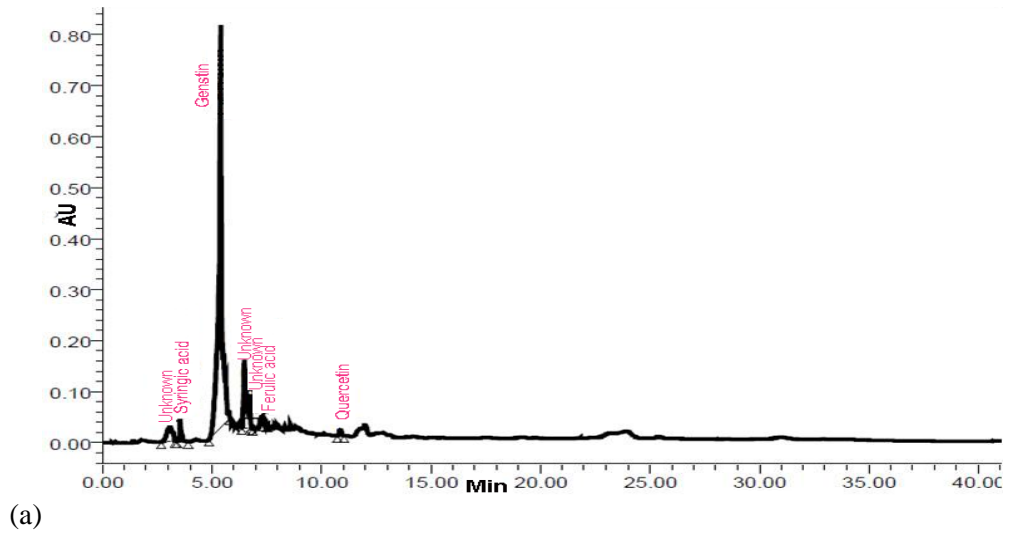
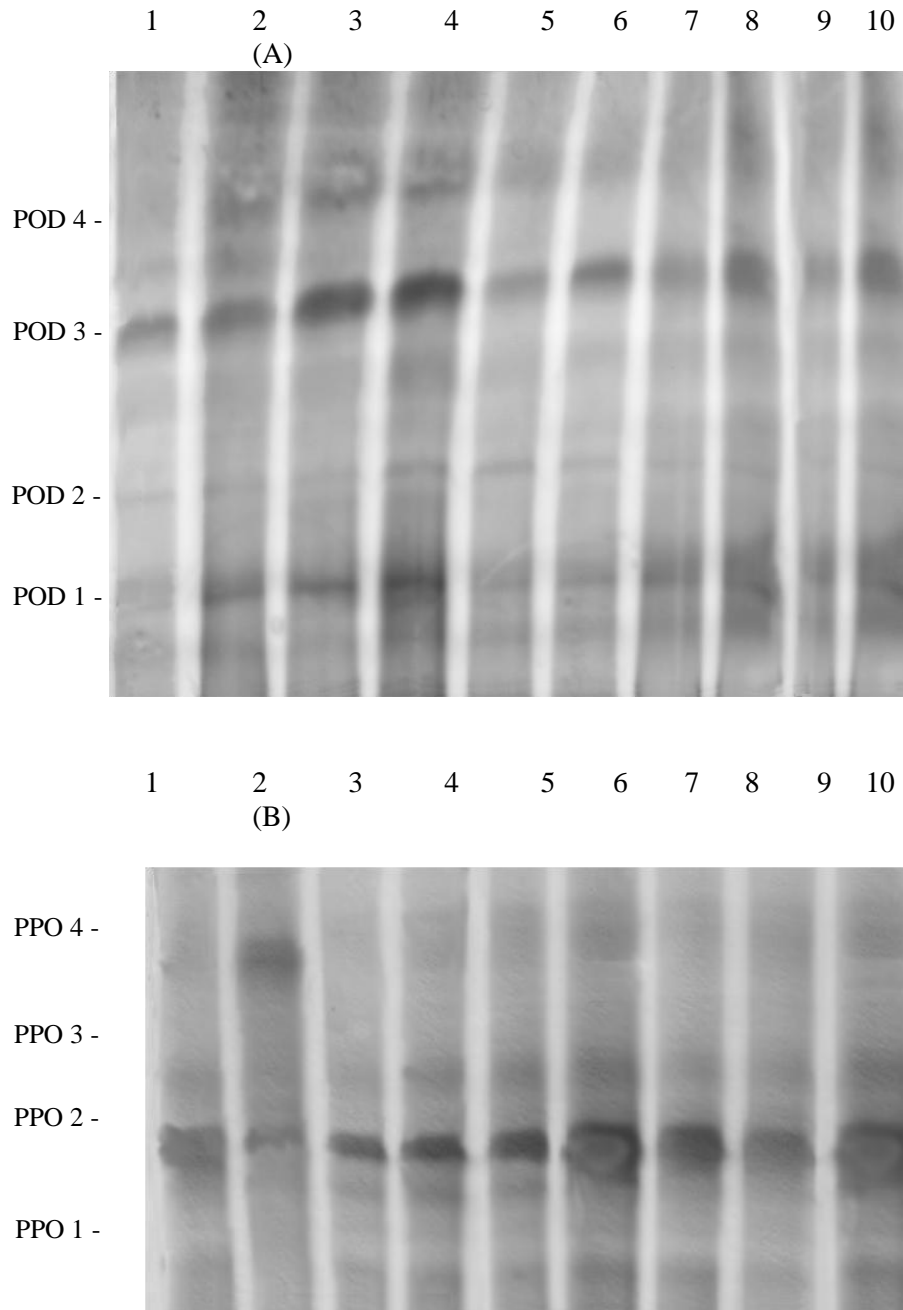


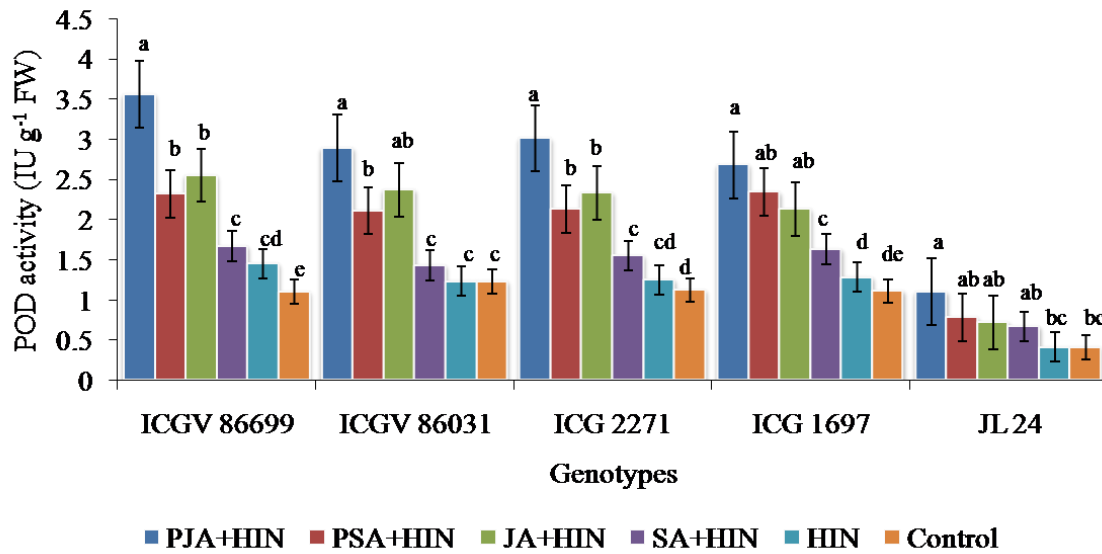
Fig.4.3.3.5. HPLC chromatogram of JL 24 plants infested with; (a) *H. armigera*; (b) *A. craccivora*; and (c) untreated control plants.



plants Fig. 4.3.4. Native PAGE analysis for POD (A) and PPO (B) in groundnut infested with *A. craccivora* and *H. armigera*

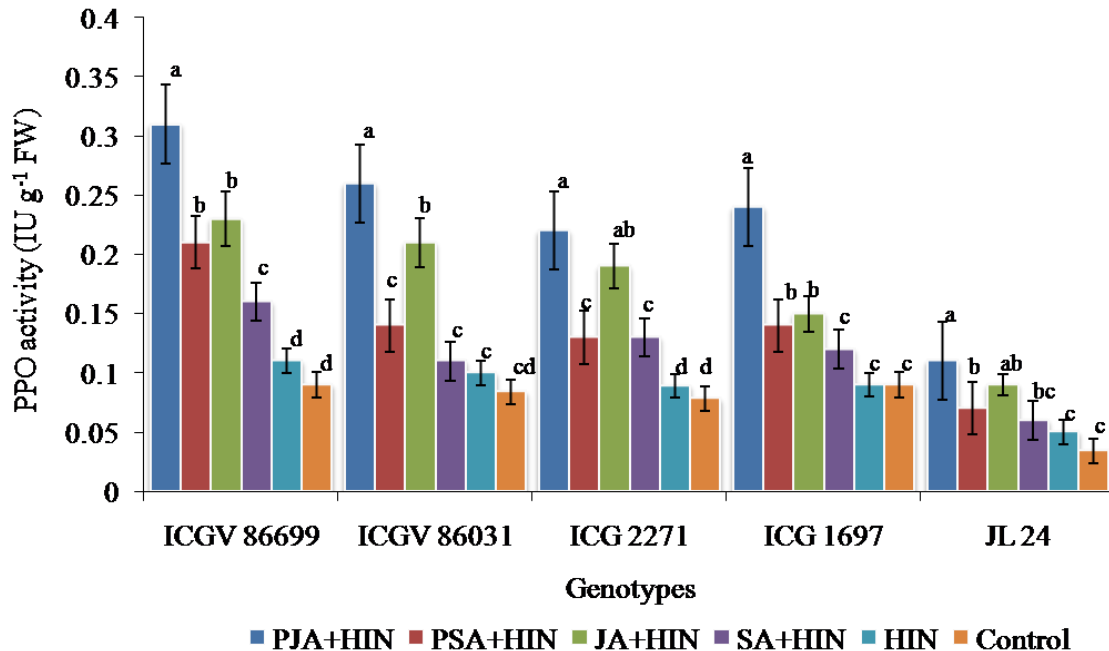
Lane 1= *A. craccivora* infested plants of ICGV 86699; Lane 2 = *H. armigera* infested plants of ICGV 86699; Lane 3 = *A. craccivora* infested plants of ICGV 86031; Lane 4 = *H. armigera* infested plants of ICGV 86031; Lane 5 = *A. craccivora* infested plants of ICG 2271; Lane 6 = *H. armigera* infested plants of ICG 2271; Lane 7 = *A. craccivora* infested plants of ICG 1697; Lane 8 = *H. armigera* infested plants of ICG 1697; Lane 9 = *A. craccivora* infested plants of JL 24; Lane 10 = *H. armigera* infested plants JL 24

**Fig. 4.4.1.1a: Peroxidase (POD) activity (IU g<sup>-1</sup> FW) of groundnut genotypes after *Helicoverpa armigera* infestation and jasmonic acid and salicylic acid application.**



Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

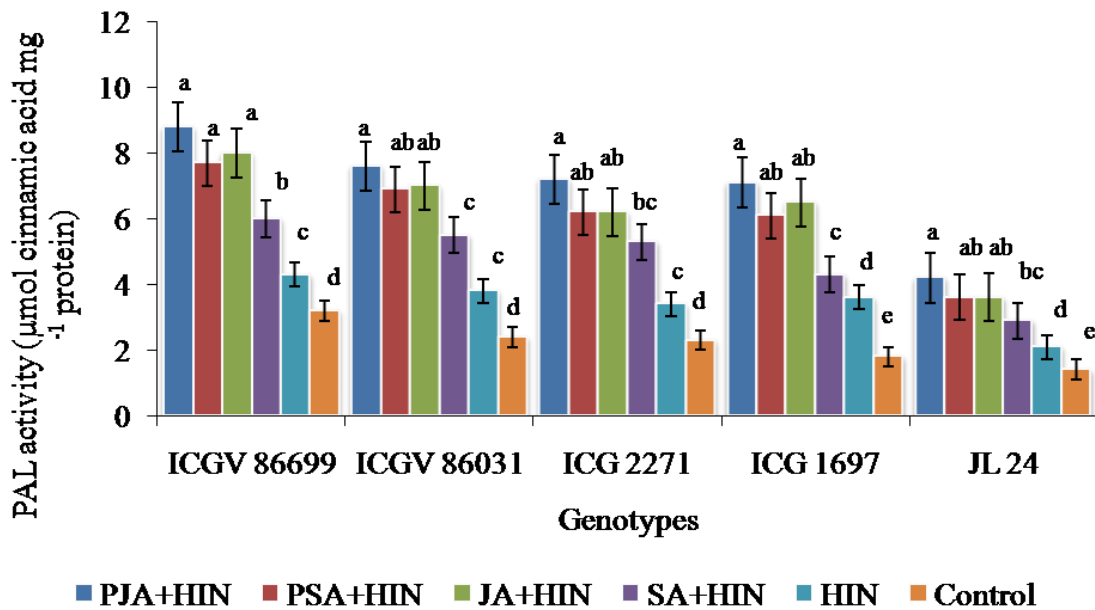
**Fig. 4.4.1.1b: Polyphenol oxidase (PPO) activity (IU g<sup>-1</sup> FW) of groundnut genotypes after *Helicoverpa armigera* infestation and jasmonic acid and salicylic acid application.**



Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

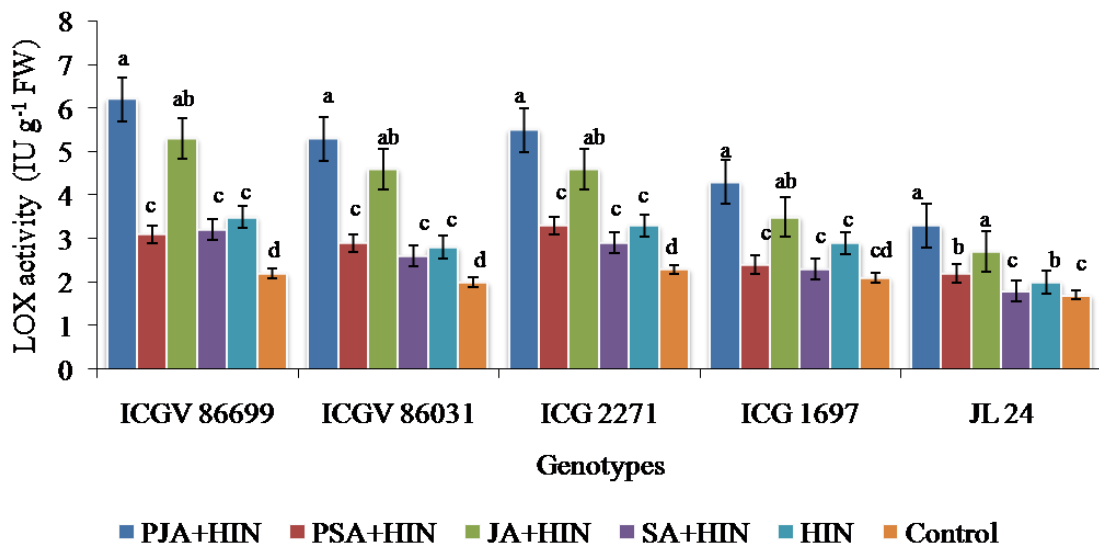
PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN: Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants.

Fig4.4.1.1c: Phenylalanine ammonia lyase (PAL) activity ( $\mu\text{mol cinnamic acid min}^{-1} \text{mg}^{-1}$  protein) of groundnut genotypes after *Helicoverpa armigera* infestation and jasmonic acid and salicylic acid application.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

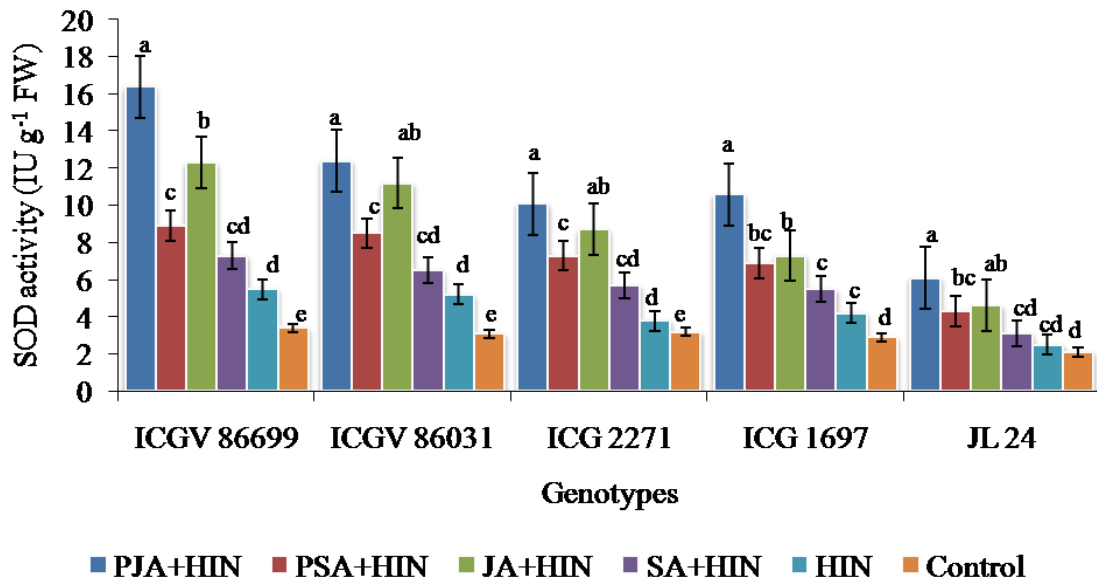
Fig. 4.4.1.1d: Lipoxygenase (LOX) activity ( $\text{IU g}^{-1} \text{FW}$ ) of groundnut genotypes after *Helicoverpa armigera* infestation and jasmonic acid and salicylic acid application.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

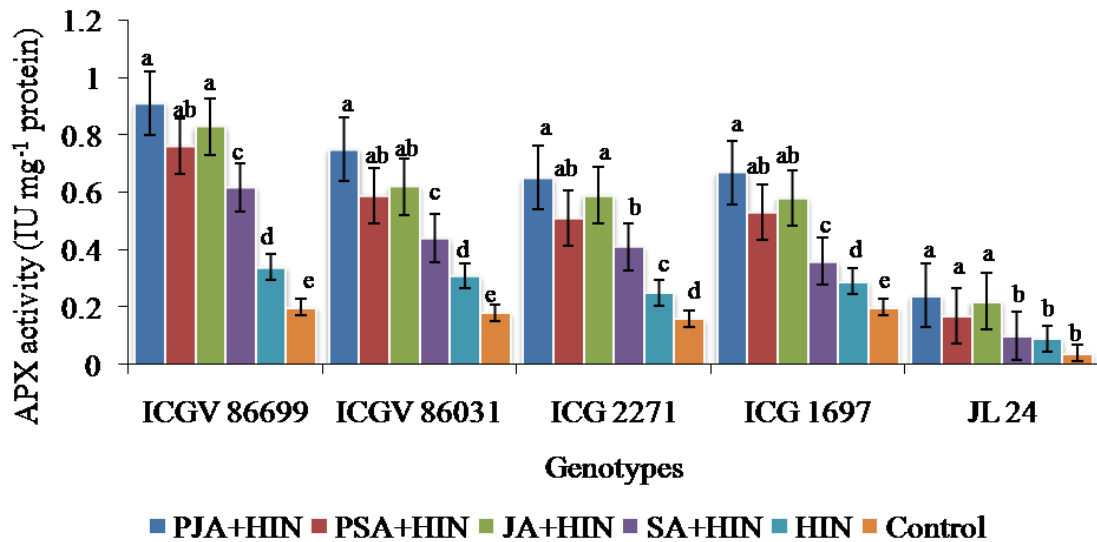
PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN: Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants.

Fig. 4.4.1.1e: Superoxide dismutase (SOD) activity (IU g<sup>-1</sup> FW) of groundnut genotypes after *Helicoverpa armigera* infestation and jasmonic acid and salicylic acid application.



Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

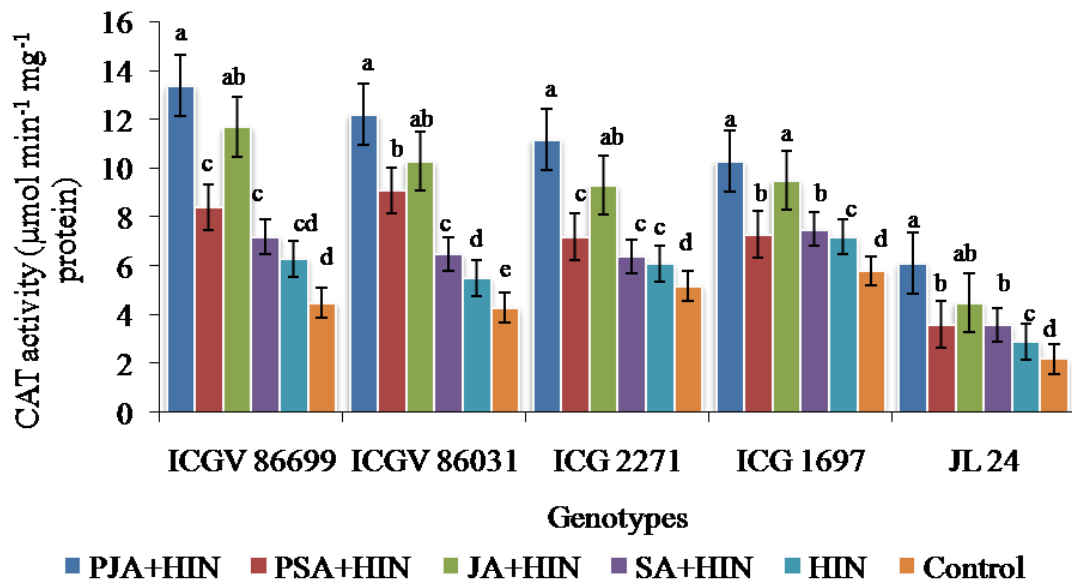
Fig. 4.4.1.1f: Ascorbate peroxidase (APX) activity (IU mg<sup>-1</sup> protein) of groundnut genotypes after *Helicoverpa armigera* infestation and jasmonic acid and salicylic acid application.



Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

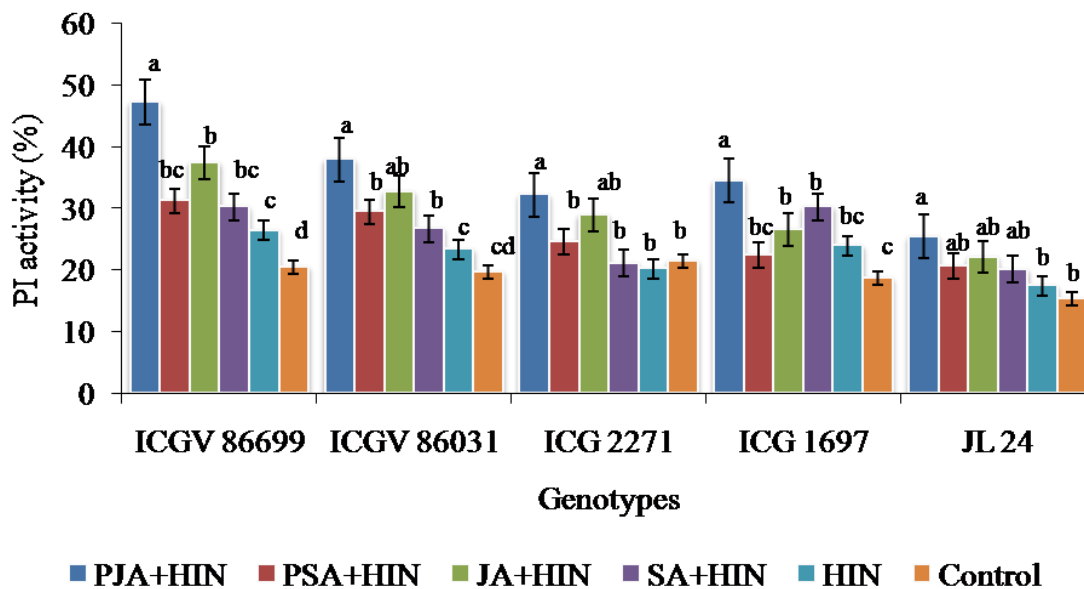
PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN: Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants.

Fig. 4.4.1.1g: Catalase (CAT) activity ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$  protein) of groundnut genotypes after *Helicoverpa armigera* infestation and jasmonic acid and salicylic acid application.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

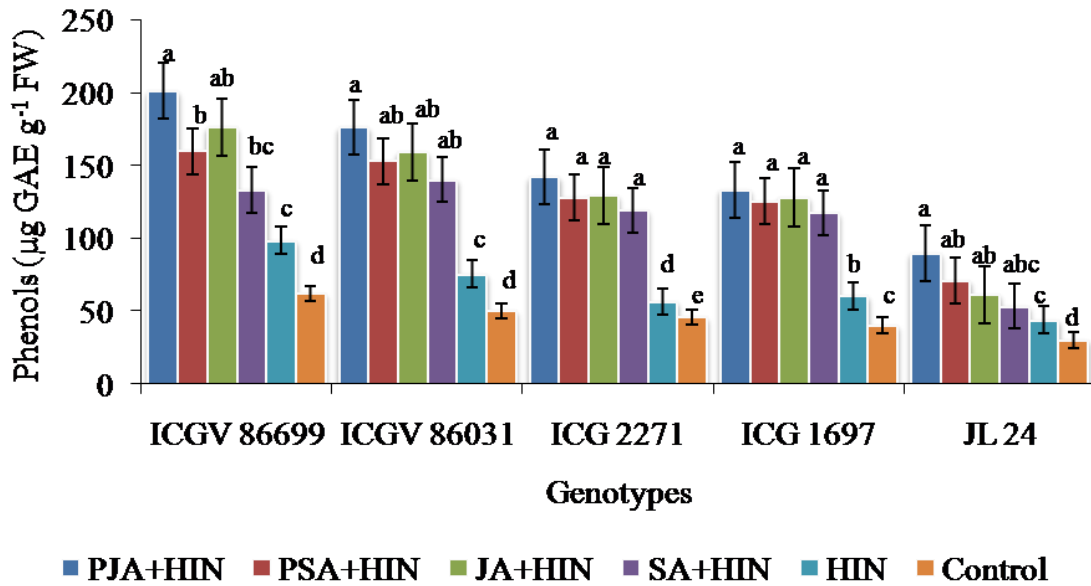
Fig. 4.4.1.1h: The in vitro protease inhibitor (PI) activity (%) of groundnut genotypes after *Helicoverpa armigera* infestation, and jasmonic acid and salicylic acid application.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

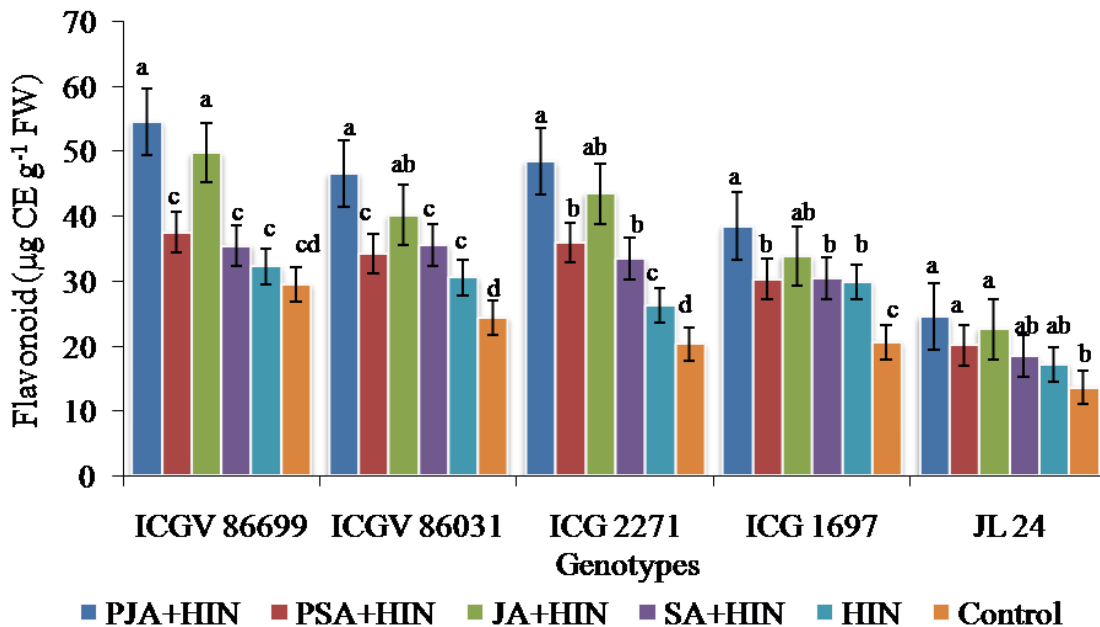
PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN: Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants.

Fig. 4.4.1.1i: Total phenols ( $\mu\text{g GAE g}^{-1}\text{ FW}$ ) of groundnut genotypes after *Helicoverpa armigera* infestation and jasmonic acid and salicylic acid application.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

Fig. 4.4.1.1j: Flavonoid content ( $\mu\text{g CE g}^{-1}\text{ FW}$ ) of groundnut genotypes after *Helicoverpa armigera* infestation and jasmonic acid and salicylic acid application.

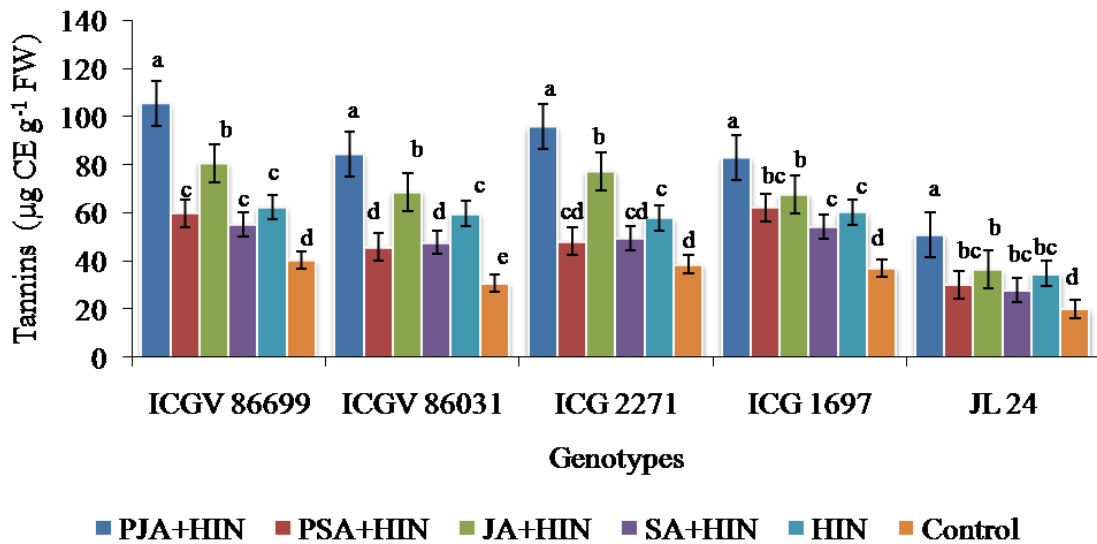


Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN: Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants; CE = Catechin equivalents; GAE = Gallic acid equivalents.

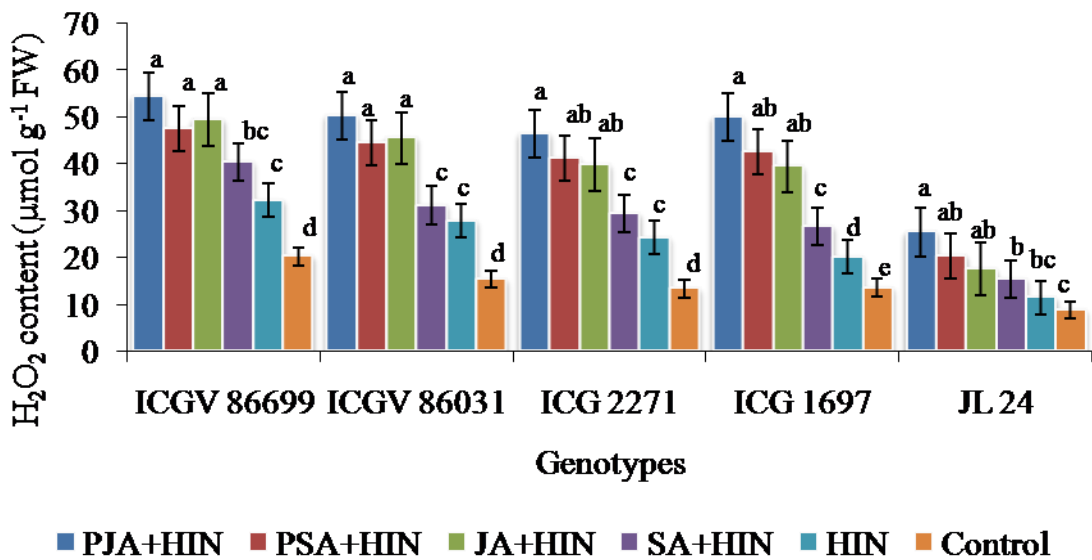


Fig. 4.4.1.1k: Condensed tannins ( $\mu\text{g CE g}^{-1}\text{ FW}$ ) of groundnut genotypes after *Helicoverpa armigera* infestation and jasmonic acid and salicylic acid application.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

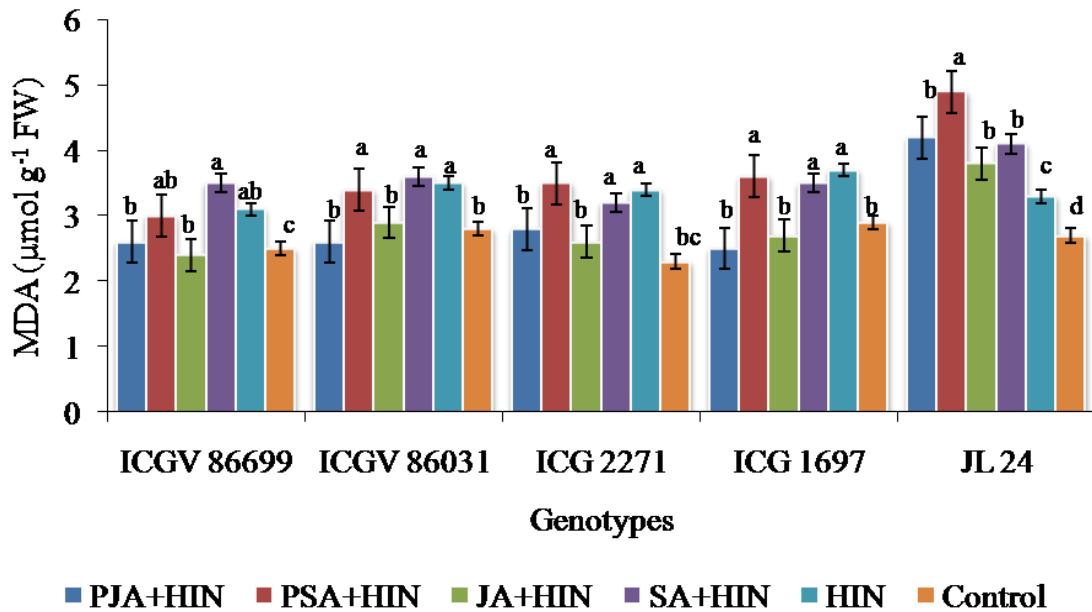
Fig. 4.4.1.1l:  $\text{H}_2\text{O}_2$  content ( $\mu\text{mol g}^{-1}\text{ FW}$ ) of groundnut genotypes after *Helicoverpa armigera* infestation and jasmonic acid and salicylic acid application.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

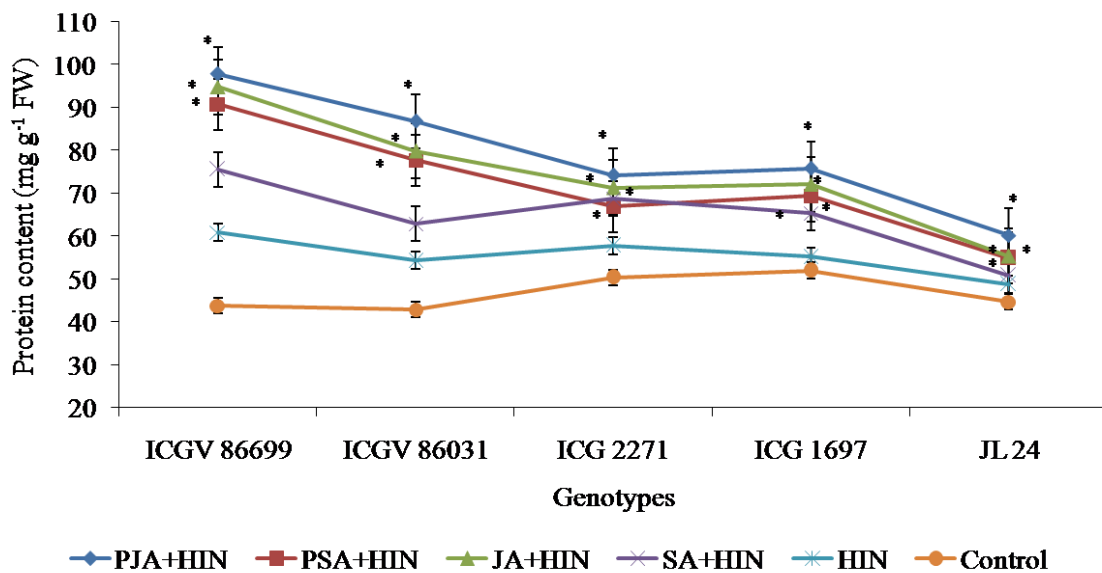
PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN: Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants; CE = Catechin equivalents.

Fig. 4.4.1.1m: Malondialdehyde (MDA) content ( $\mu\text{mol g}^{-1}$  FW) of groundnut genotypes after *Helicoverpa armigera* infestation and jasmonic acid and salicylic acid application.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

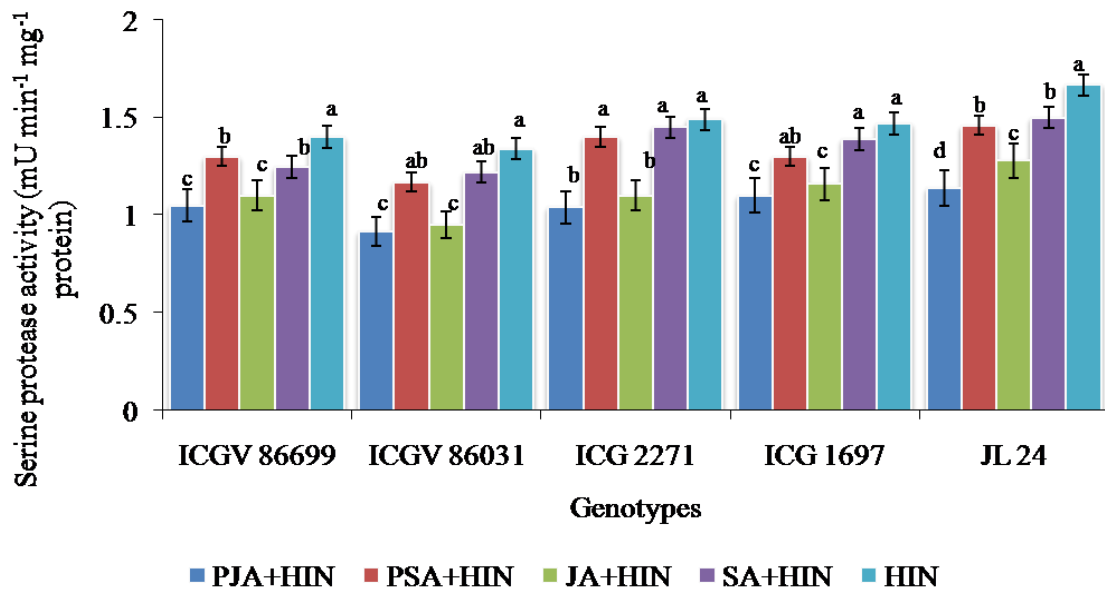
Fig. 4.4.1.1n: Protein content ( $\text{mg g}^{-1}$  FW) of groundnut genotypes after *Helicoverpa armigera* infestation and jasmonic acid and salicylic acid application.



Lines (Mean  $\pm$  SD) with \* within a genotype are not statistically different at  $P \leq 0.05$ .

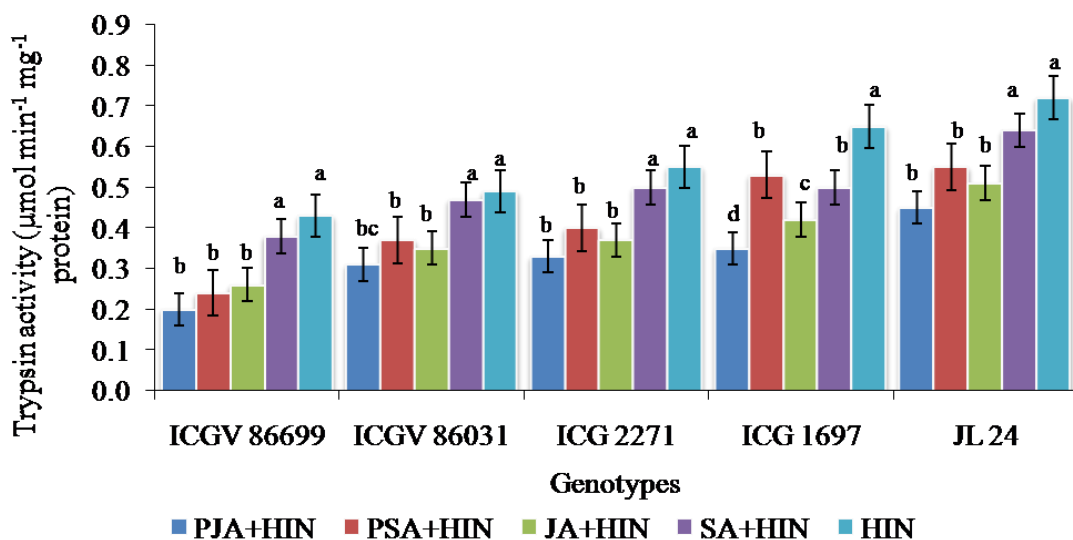
PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN: Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants.

Fig. 4.4.1.2.2a: Total serine protease activity (mU min<sup>-1</sup> mg<sup>-1</sup> protein) of the *Helicoverpa armigera* larvae fed on jasmonic acid and salicylic acid treated groundnut plants.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

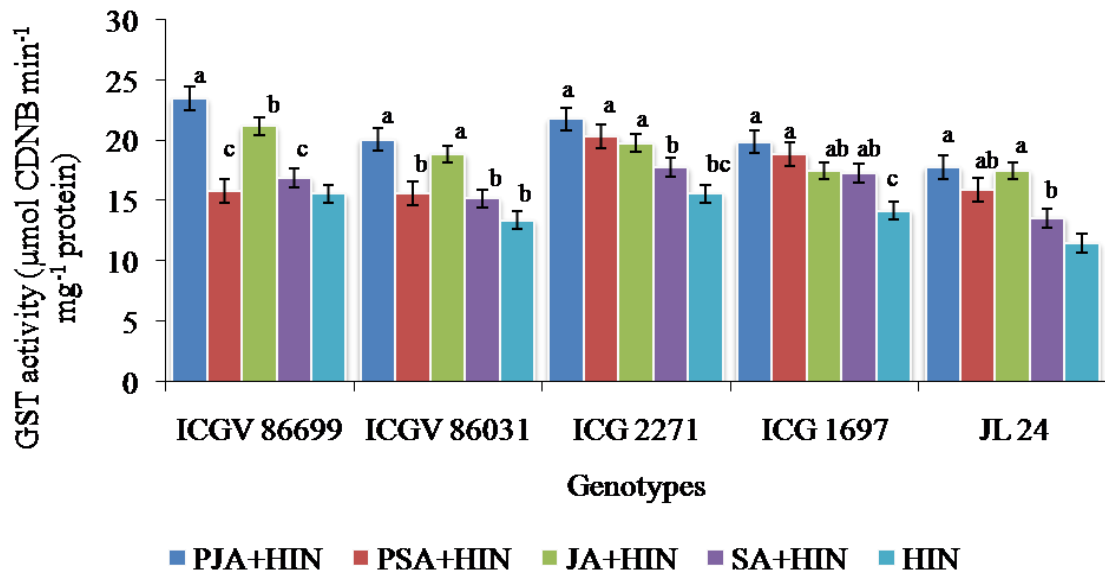
Fig. 4.4.1.2.2b: Trypsin activity (mU min<sup>-1</sup> mg<sup>-1</sup> protein) of the *Helicoverpa armigera* larvae fed on jasmonic acid and salicylic acid treated groundnut plants.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

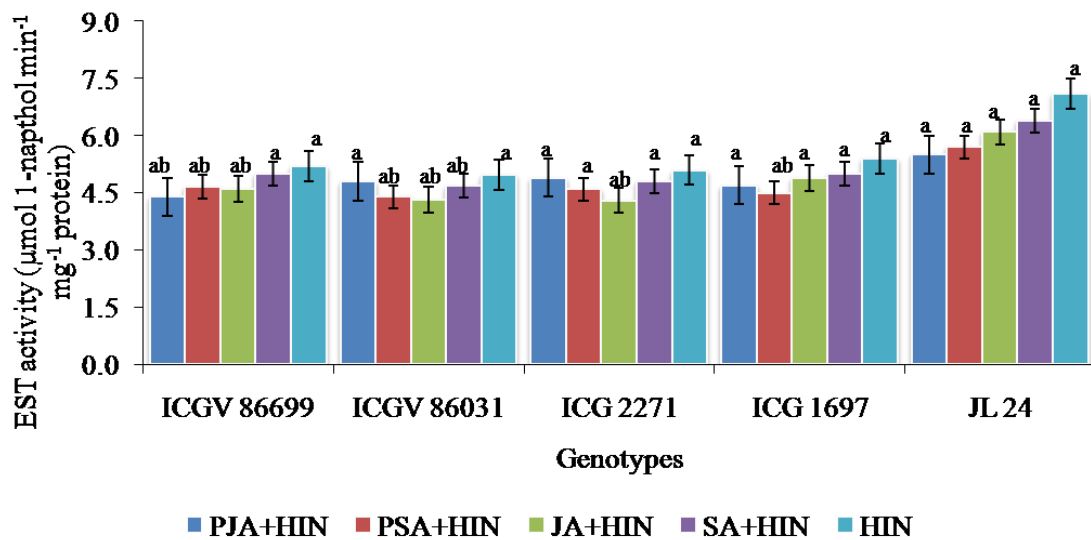
PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN: Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants.

Fig. 4.4.1.2.2c: Glutathione-S-transferase (GST) activity ( $\mu\text{mol CDNB min}^{-1} \text{mg}^{-1} \text{protein}$ ) of the *Helicoverpa armigera* larvae fed on jasmonic acid and salicylic acid treated groundnut plants.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

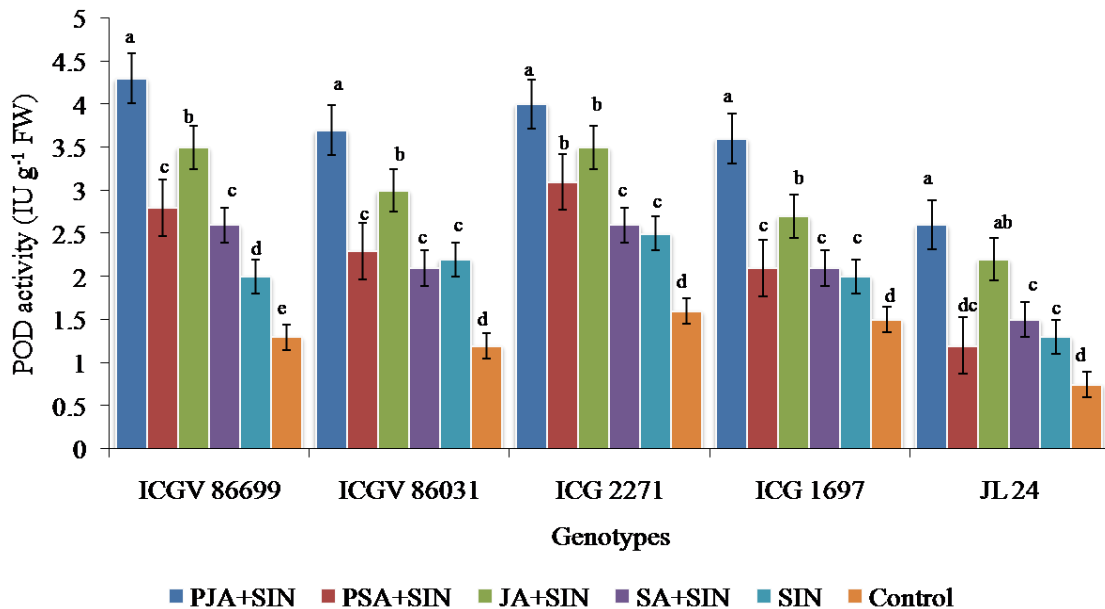
Fig. 4.4.1.2.2d: Esterase (EST) activity ( $\mu\text{mol 1-naphthol min}^{-1} \text{mg}^{-1} \text{protein}$ ) of the *Helicoverpa armigera* larvae fed on jasmonic acid and salicylic acid treated groundnut plants.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

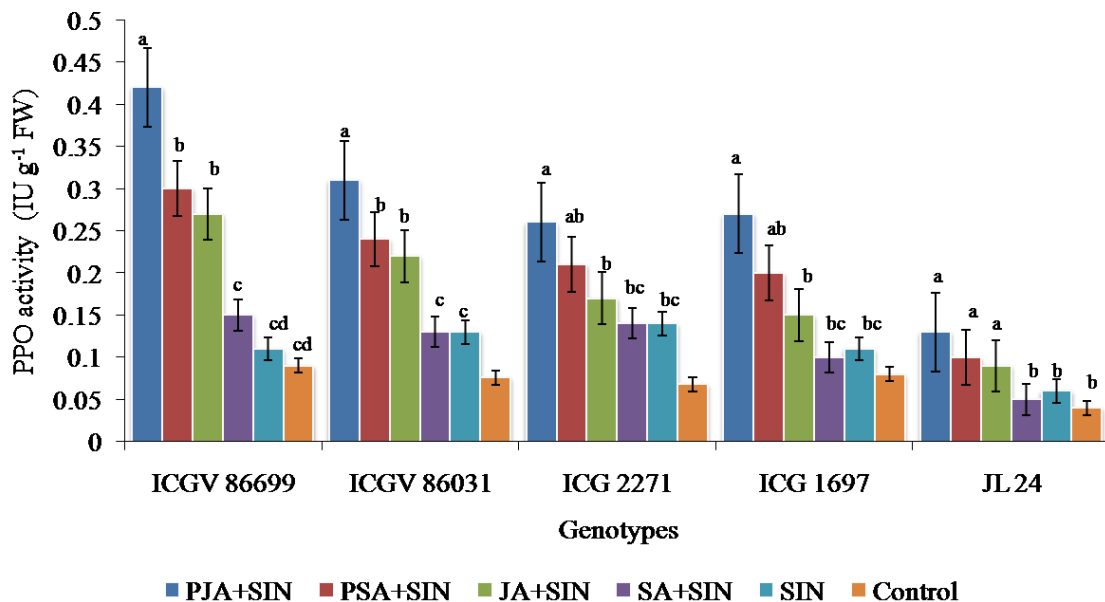
PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN: Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants; CDNB = 1-chloro-2, 4-dinitrobenzene.

**Fig. 4.4.2.1a: Peroxidase (POD) activity (IU g<sup>-1</sup> FW) of groundnut genotypes after *Spodoptera litura* infestation and jasmonic acid and salicylic acid application.**



Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

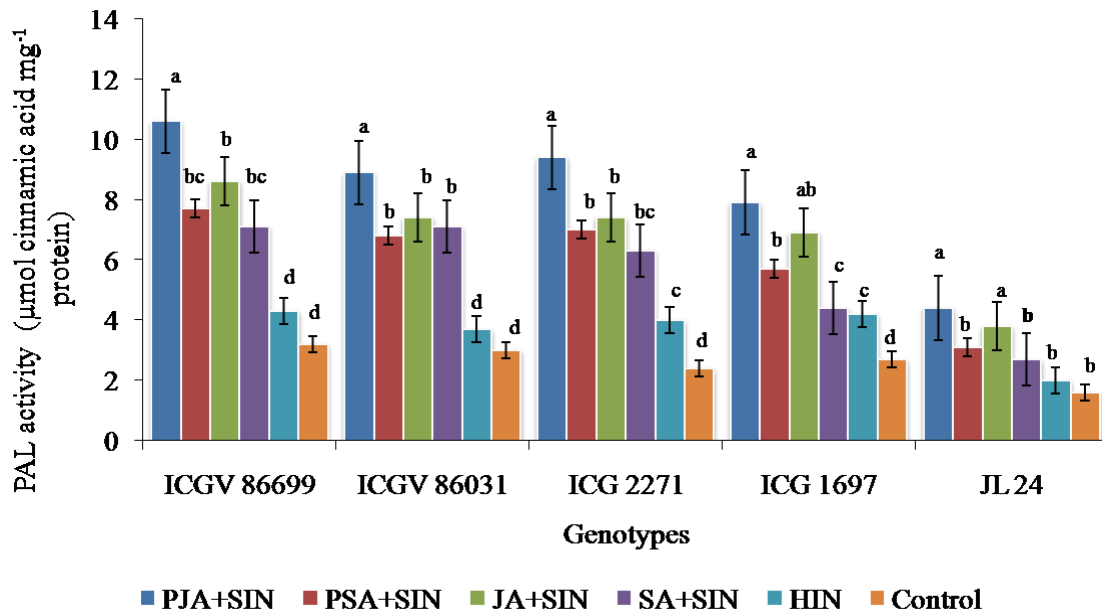
**Fig. 4.4.2.1b: Polyphenol oxidase (PPO) activity (IU g<sup>-1</sup> FW) of groundnut genotypes after *Spodoptera litura* infestation and jasmonic acid and salicylic acid application.**



Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

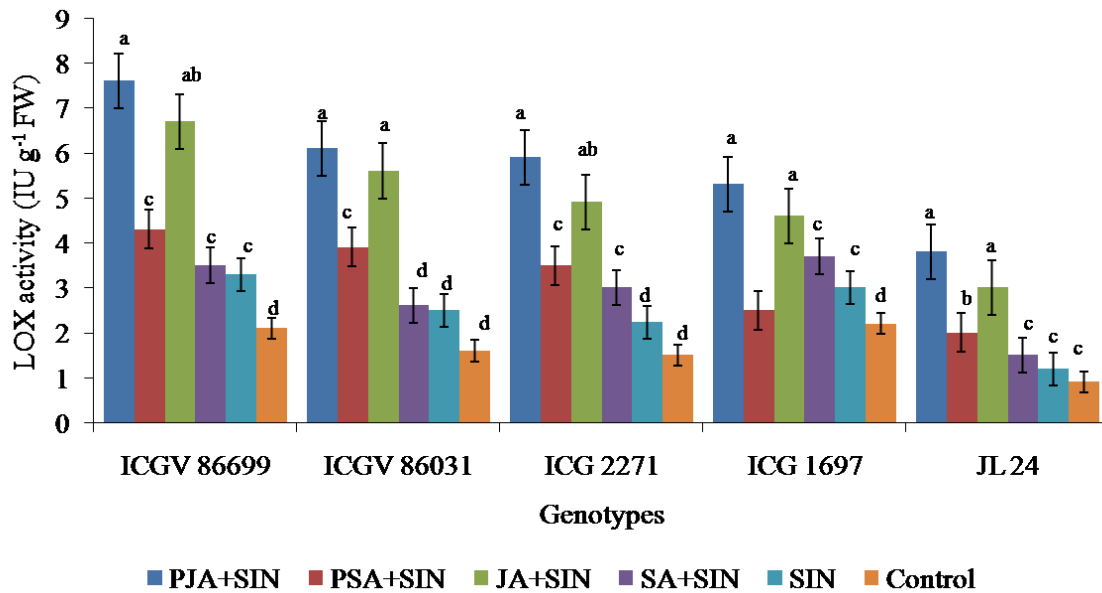
PJA+SIN = Pretreatment with JA one day prior to *S. litura* infestation; PSA+SIN = Pretreatment with SA one day prior to *S. litura* infestation; JA+SIN: Simultaneous application of JA and *S. litura* infestation; SA+SIN = Simultaneous application of SA and *S. litura* infestation; SIN = *S. litura* infested plants; control = Uninfested and untreated plants.

**Fig. 4.4.2.1c: Phenylalanine ammonia lyase (PAL) activity ( $\mu\text{mol cinnamic acid min}^{-1} \text{mg}^{-1}$  protein) of groundnut genotypes after *Spodoptera litura* infestation and jasmonic acid and salicylic acid application.**



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

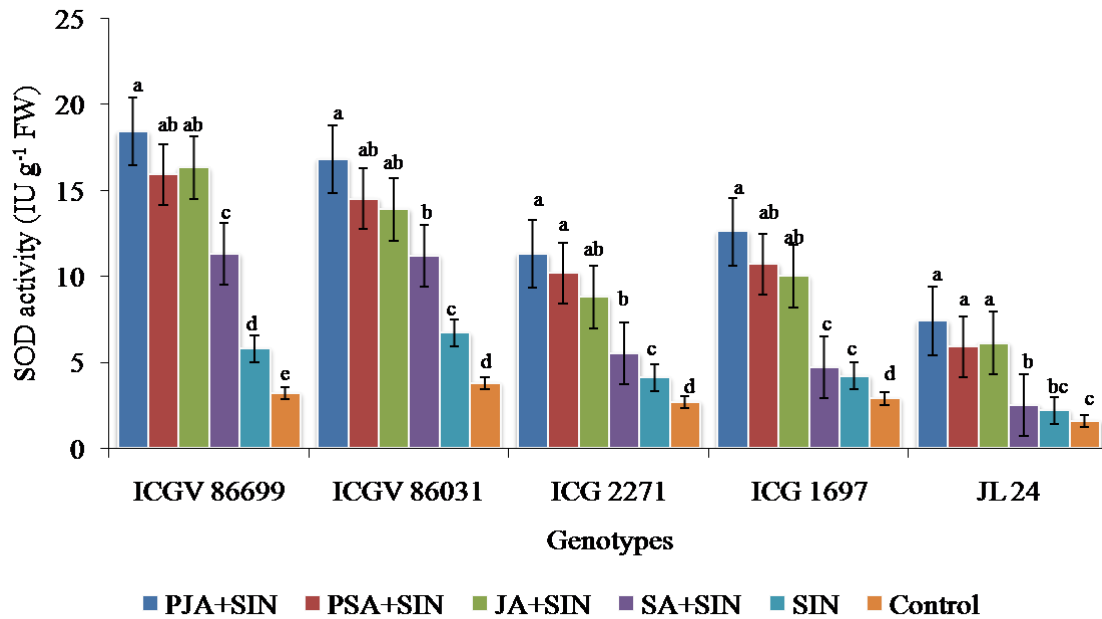
**Fig. 4.4.2.1d: Lipoxygenase (LOX) activity ( $\text{IU g}^{-1} \text{FW}$ ) of groundnut genotypes after *Spodoptera litura* infestation and jasmonic acid and salicylic acid application.**



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

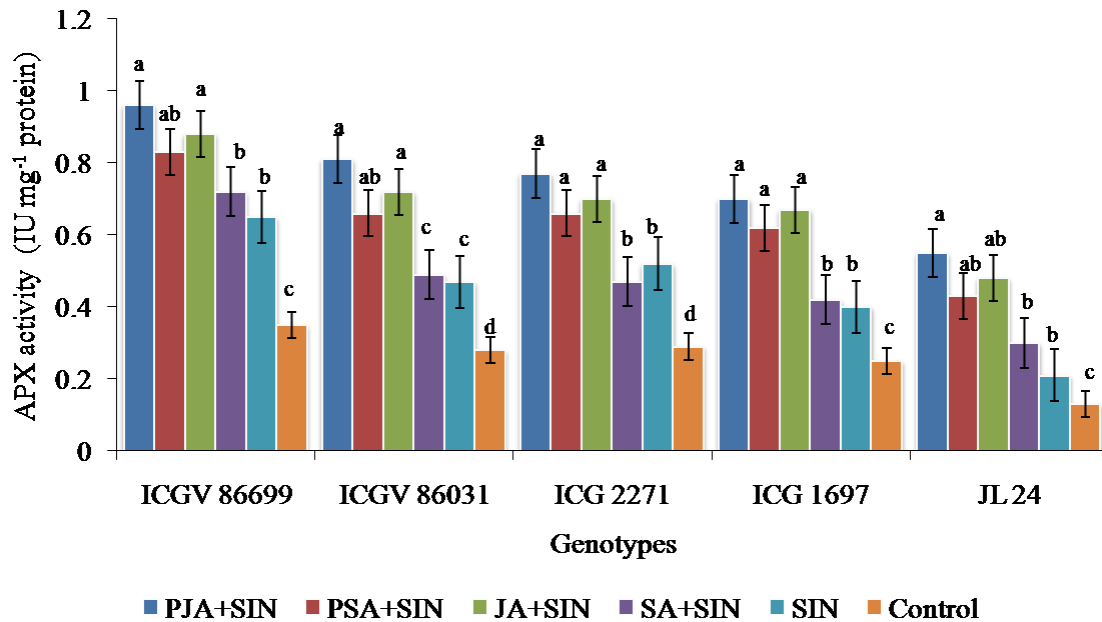
PJA+SIN = Pretreatment with JA one day prior to *S. litura* infestation; PSA+SIN = Pretreatment with SA one day prior to *S. litura* infestation; JA+SIN: Simultaneous application of JA and *S. litura* infestation; SA+SIN = Simultaneous application of SA and *S. litura* infestation; SIN = *S. litura* infested plants; control = Uninfested and untreated plants.

Fig. 4.4.2.1e: Superoxide dismutase (SOD) activity (IU g<sup>-1</sup> FW) of groundnut genotypes after *Spodoptera litura* infestation and jasmonic acid and salicylic acid application.



Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

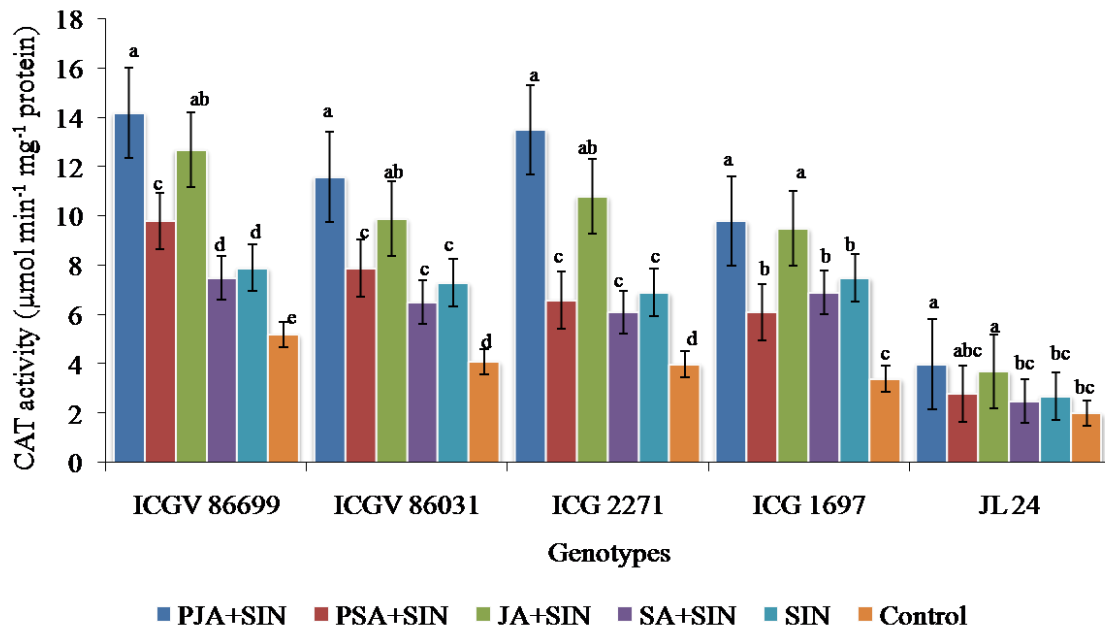
Fig. 4.4.2.1f: Ascorbate peroxidase (APX) activity (IU mg<sup>-1</sup> protein) of groundnut genotypes after *Spodoptera litura* infestation and jasmonic acid and salicylic acid application.



Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

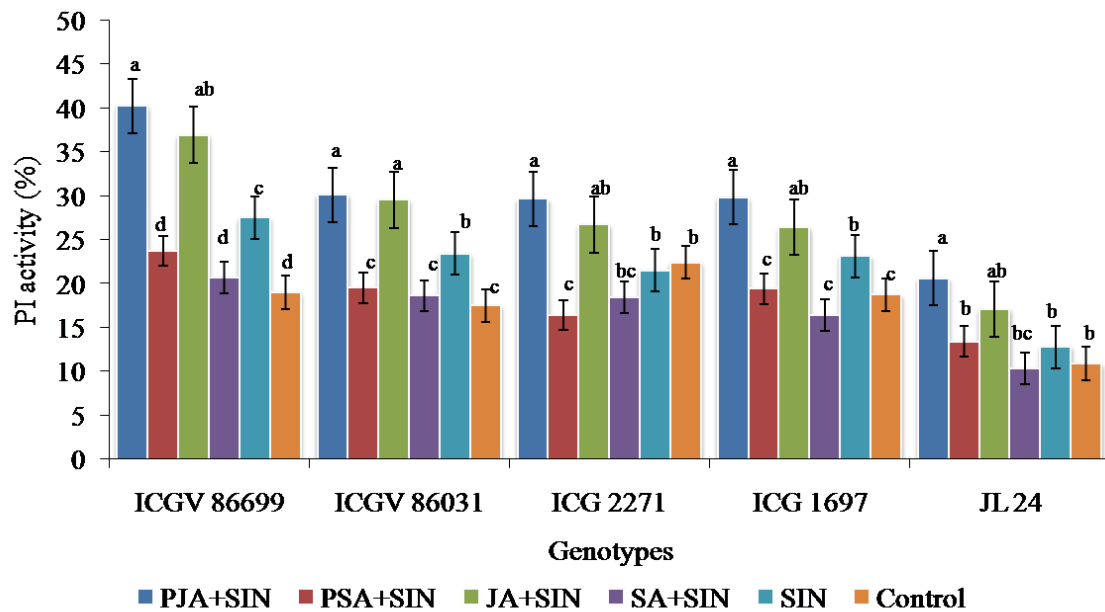
PJA+SIN = Pretreatment with JA one day prior to *S. litura* infestation; PSA+SIN = Pretreatment with SA one day prior to *S. litura* infestation; JA+SIN: Simultaneous application of JA and *S. litura* infestation; SA+SIN = Simultaneous application of SA and *S. litura* infestation; SIN = *S. litura* infested plants; control = Uninfested and untreated plants.

**Fig. 4.4.2.1g: Catalase (CAT) activity ( $\mu\text{mol min}^{-1}\text{mg}^{-1}$  protein) of groundnut genotypes after *Spodoptera litura* infestation and jasmonic acid and salicylic acid application.**



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

**Fig. 4.4.2.1h: The in vitro protease inhibitor (PI) activity (%) of groundnut genotypes after *Spodoptera litura* infestation, and jasmonic acid and salicylic acid application.**

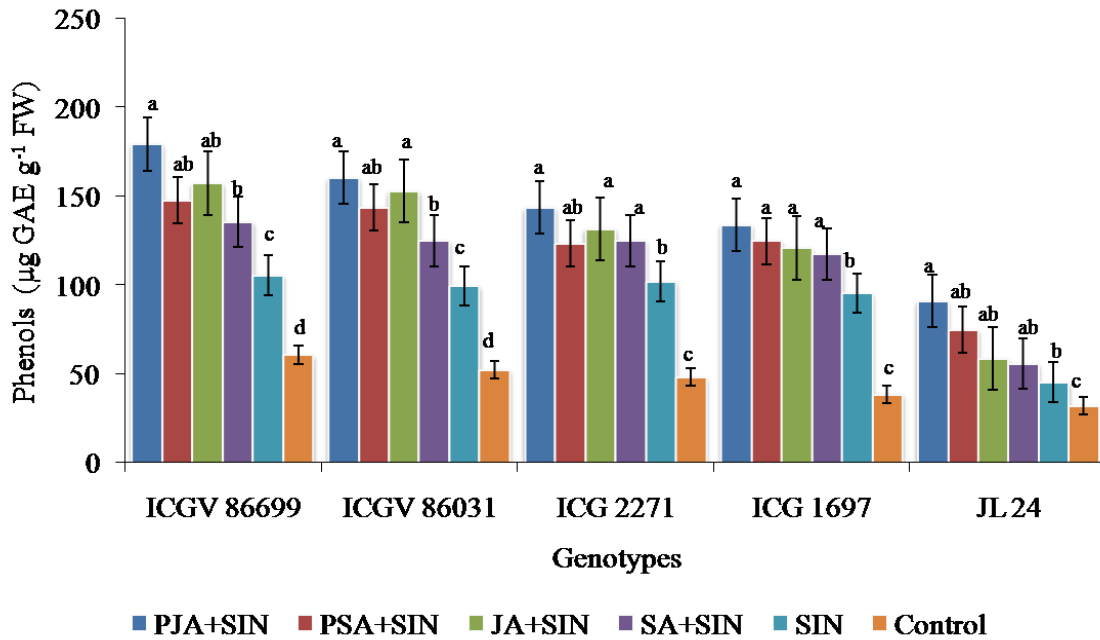


Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

PJA+SIN = Pretreatment with JA one day prior to *S. litura* infestation; PSA+SIN = Pretreatment with SA one day prior to *S. litura* infestation; JA+SIN: Simultaneous application of JA and *S. litura* infestation; SA+SIN = Simultaneous application of SA and *S. litura* infestation; SIN = *S. litura* infested plants; control = Uninfested and untreated plants.

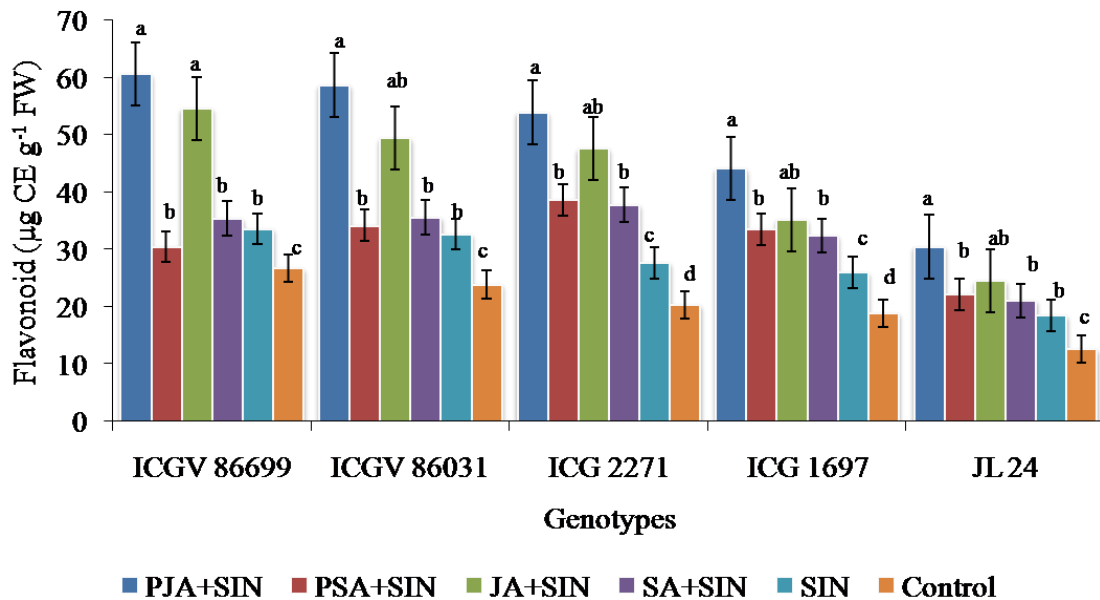


Fig. 4.4.2.1i: Total phenols ( $\mu\text{g GAE g}^{-1}\text{ FW}$ ) of groundnut genotypes after *Spodoptera litura* infestation and jasmonic acid and salicylic acid application.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

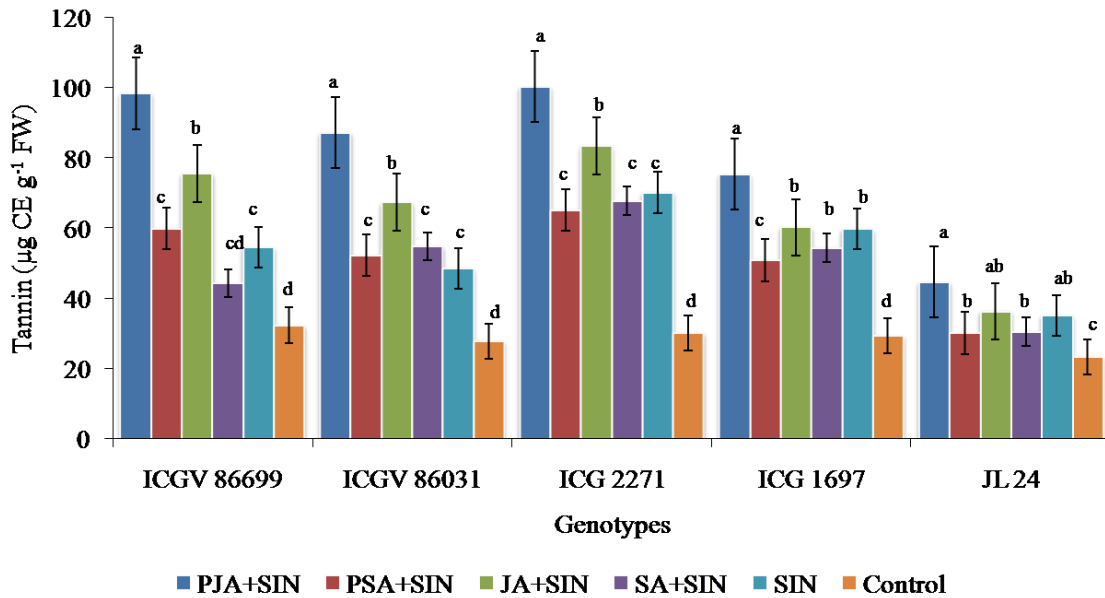
Fig. 4.4.2.1j: Flavonoid content ( $\mu\text{g CE g}^{-1}\text{ FW}$ ) of groundnut genotypes after *Spodoptera litura* infestation and jasmonic acid and salicylic acid application.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

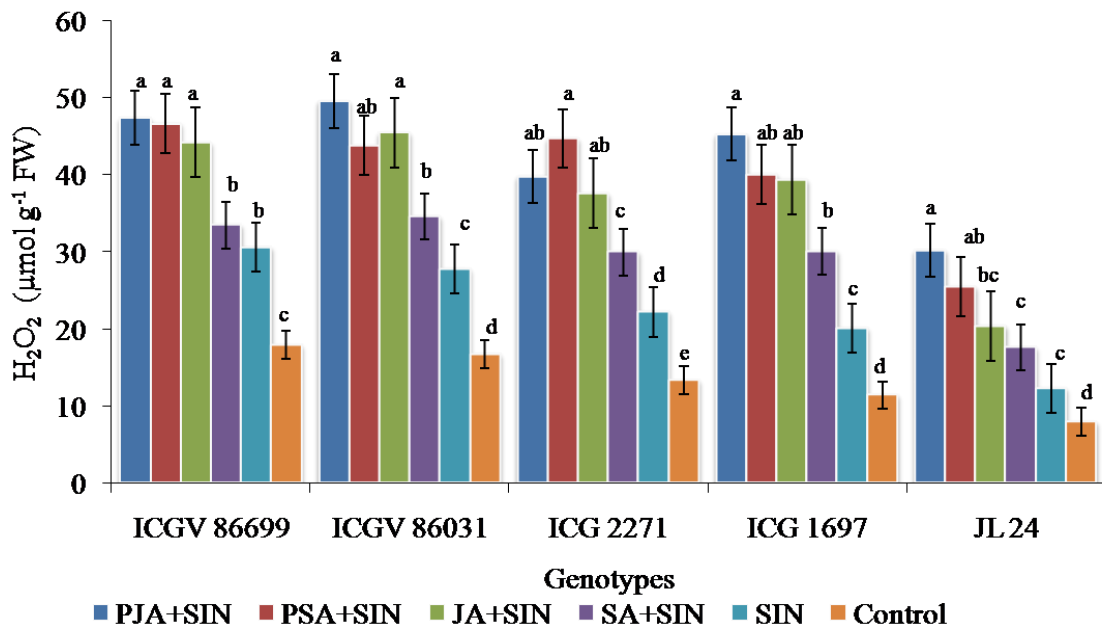
PJA+SIN = Pretreatment with JA one day prior to *S. litura* infestation; PSA+SIN = Pretreatment with SA one day prior to *S. litura* infestation; JA+SIN: Simultaneous application of JA and *S. litura* infestation; SA+SIN = Simultaneous application of SA and *S. litura* infestation; SIN = *S. litura* infested plants; control = Uninfested and untreated plants.

Fig. 4.4.2.1k: Condensed tannins ( $\mu\text{g CE g}^{-1}\text{ FW}$ ) of groundnut genotypes after *Spodoptera litura* infestation and jasmonic acid and salicylic acid application.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

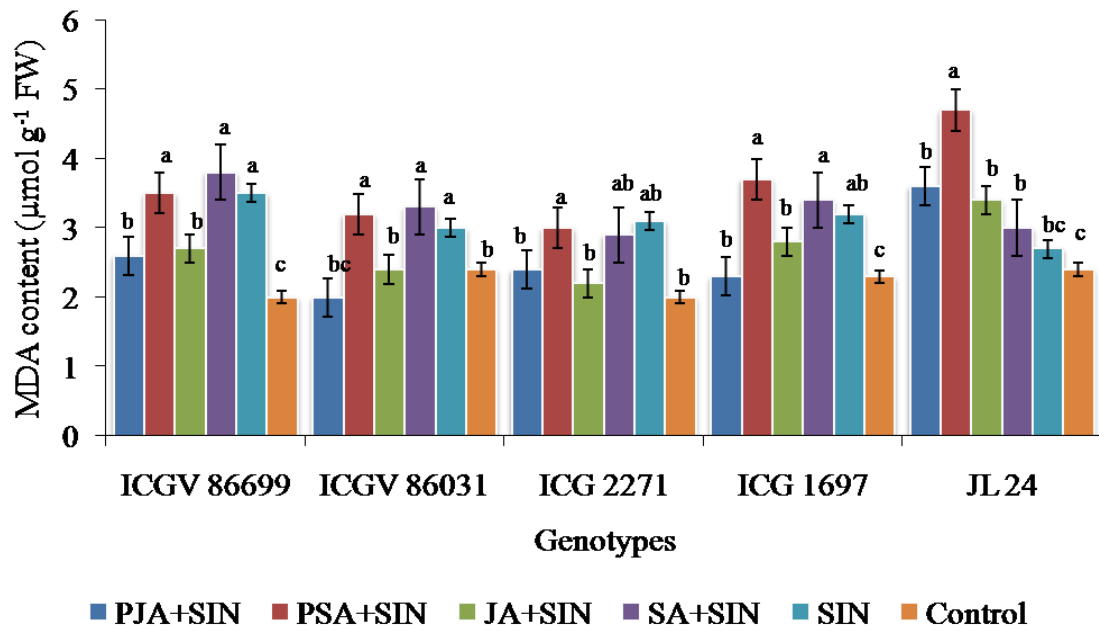
Fig. 4.4.2.1l:  $\text{H}_2\text{O}_2$  content ( $\mu\text{mol g}^{-1}\text{ FW}$ ) of groundnut genotypes after *Spodoptera litura* infestation and jasmonic acid and salicylic acid application.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

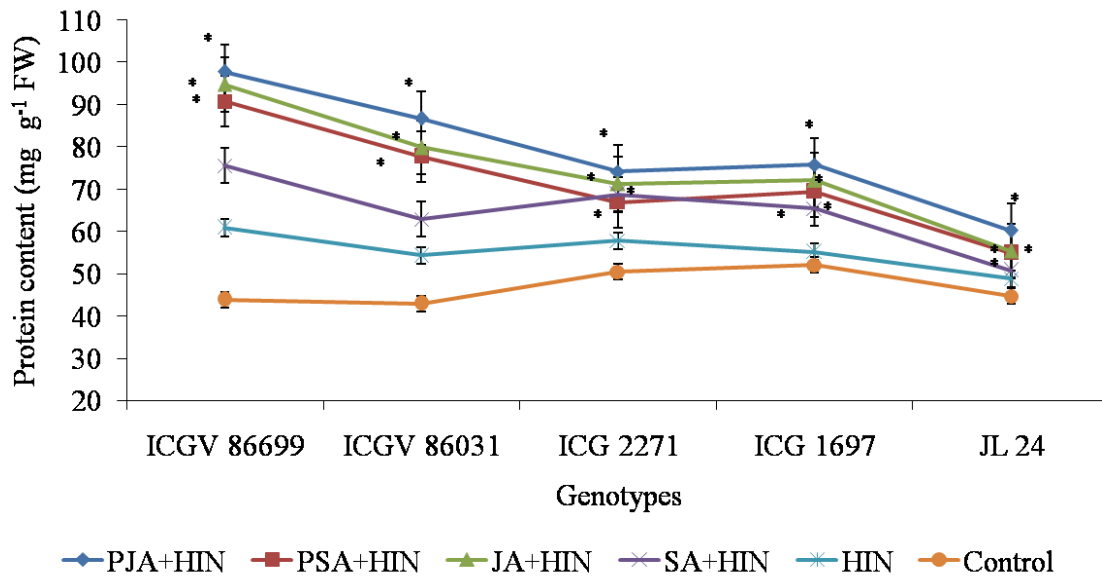
PJA+SIN = Pretreatment with JA one day prior to *S. litura* infestation; PSA+SIN = Pretreatment with SA one day prior to *S. litura* infestation; JA+SIN: Simultaneous application of JA and *S. litura* infestation; SA+SIN = Simultaneous application of SA and *S. litura* infestation; SIN = *S. litura* infested plants; control = Uninfested and untreated plants.

Fig. 4.4.2.1m: Malondialdehyde (MDA) content ( $\mu\text{mol g}^{-1}$  FW) of groundnut genotypes after *Spodoptera litura* infestation and jasmonic acid and salicylic acid application.



Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

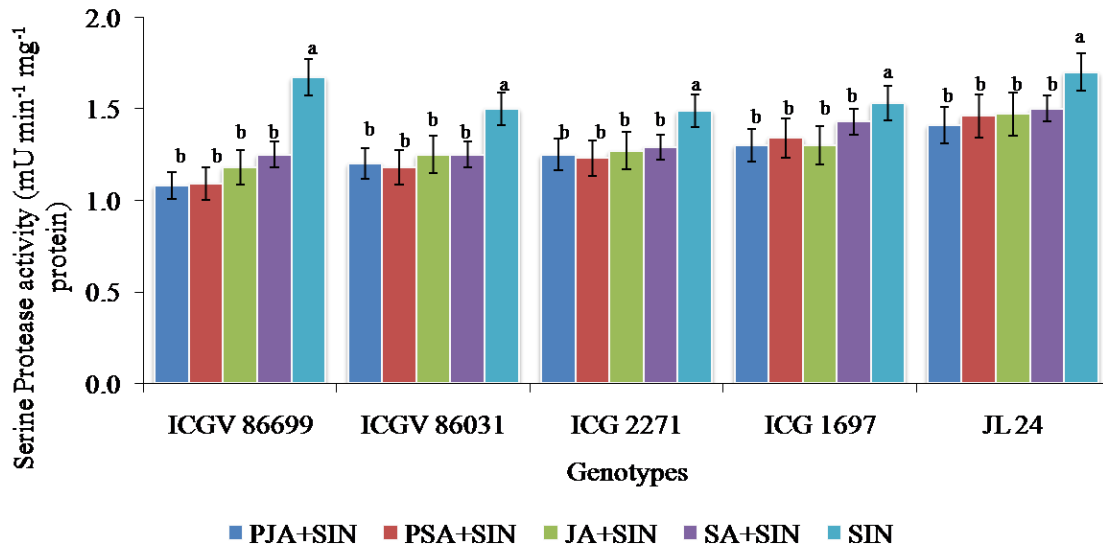
Fig. 4.4.2.1n: Protein content ( $\text{mg g}^{-1}$  FW) of groundnut genotypes after *Spodoptera litura* infestation and jasmonic acid and salicylic acid application.



Lines (Mean  $\pm$  SD) with \* within a genotype are not statistically different at  $P \leq 0.05$ .

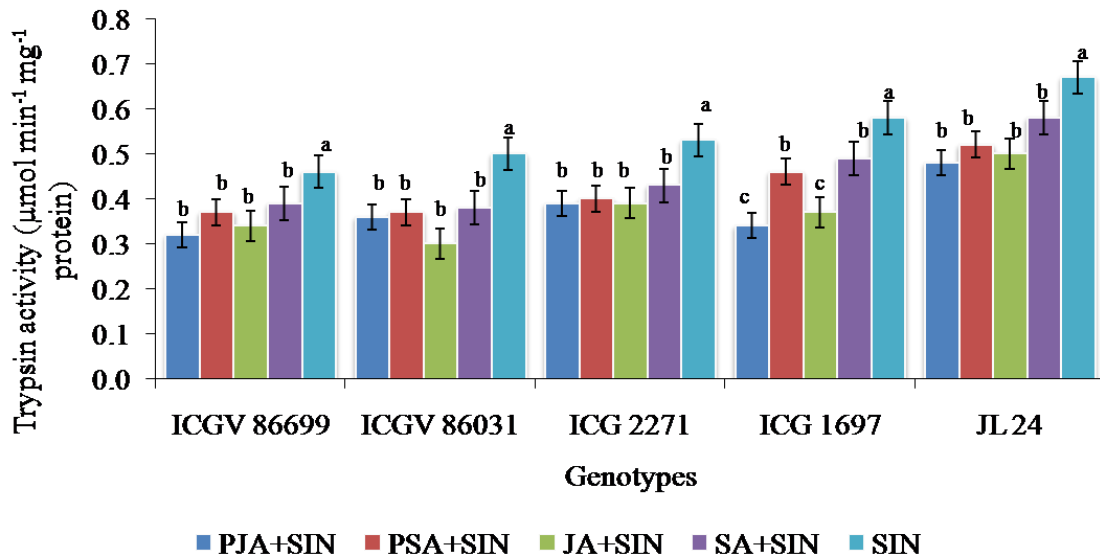
PJA+SIN = Pretreatment with JA one day prior to *S. litura* infestation; PSA+SIN = Pretreatment with SA one day prior to *S. litura* infestation; JA+HIN: Simultaneous application of JA and *S. litura* infestation; SA+SIN = Simultaneous application of SA and *S. litura* infestation; SIN = *S. litura* infested plants; control = uninfested and untreated plants.

Fig. 4.4.2.2a: Total serine protease activity (mU min<sup>-1</sup> mg<sup>-1</sup> protein) of *Spodoptera litura* larvae fed on jasmonic acid and salicylic acid treated groundnut plants.



Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

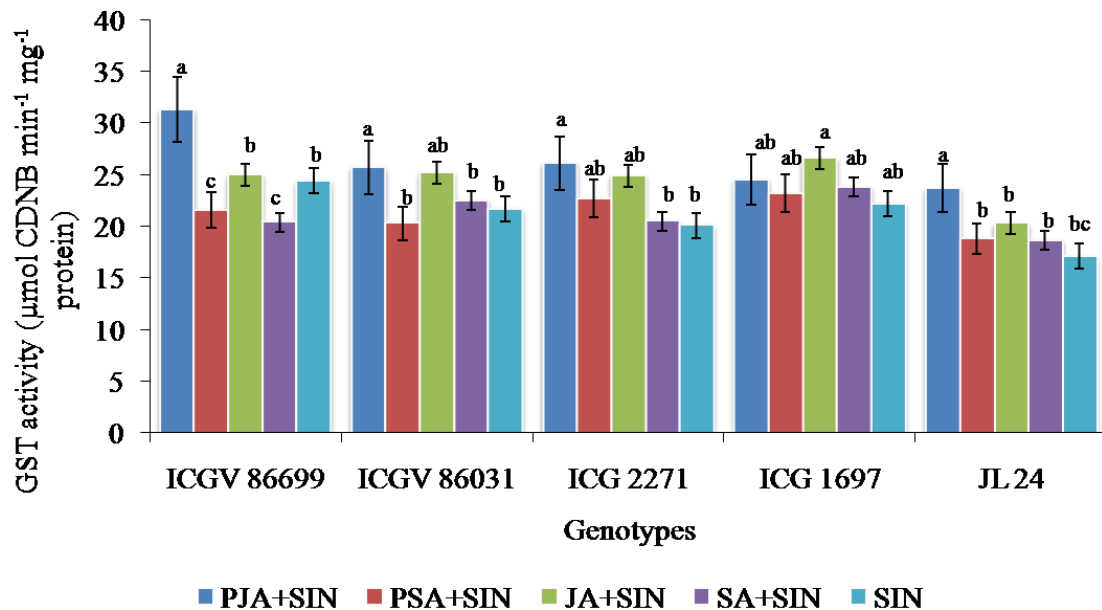
Fig. 4.4.2.2b: Trypsin activity ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$  protein) of *Spodoptera litura* larvae fed on jasmonic acid and salicylic acid treated groundnut plants.



Bars (Mean ± SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

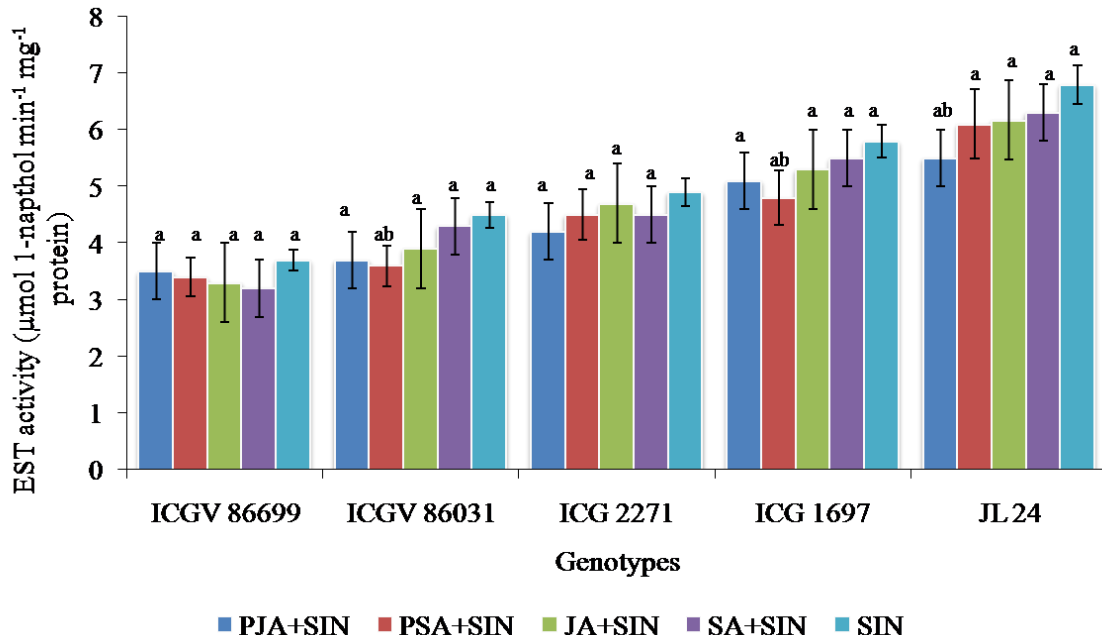
PJA+SIN = Pretreatment with JA one day prior to *S. litura* infestation; PSA+SIN = Pretreatment with SA one day prior to *S. litura* infestation; JA+SIN: Simultaneous application of JA and *S. litura* infestation; SA+SIN = Simultaneous application of SA and *S. litura* infestation; SIN = *S. litura* infested plants.

Fig. 4.4.2.2c: Glutathione-S-transferase (GST) activity ( $\mu\text{mol CDNB min}^{-1} \text{mg}^{-1}$  protein) of the *Spodoptera litura* larvae fed on jasmonic acid and salicylic acid treated groundnut plants.



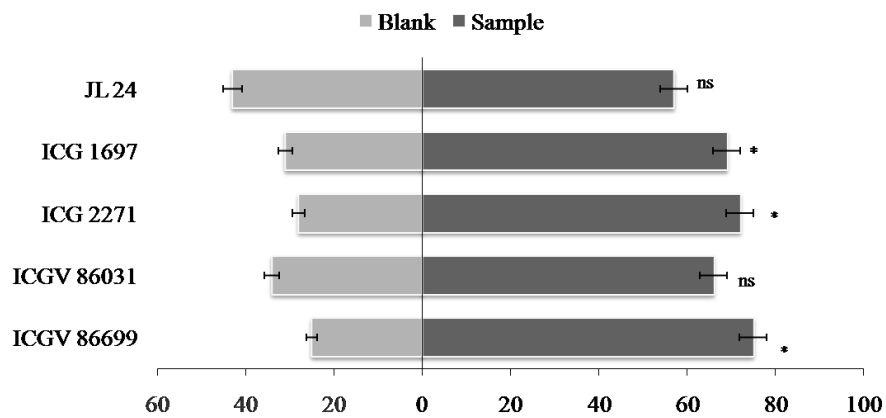
Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

Fig. 4.4.2.2d: Esterase (EST) activity ( $\mu\text{mol 1-naphthol min}^{-1} \text{mg}^{-1}$  protein) of the *Spodoptera litura* larvae fed on jasmonic acid and salicylic acid treated groundnut plants.

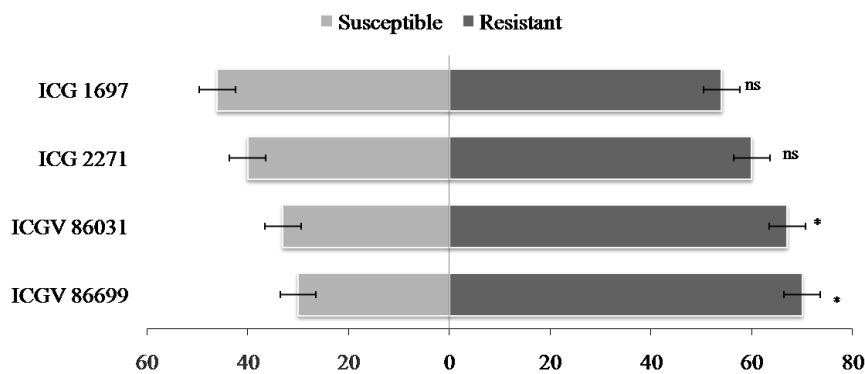


Bars (Mean  $\pm$  SD) of same color with similar letters within a genotype are not statistically different at  $P \leq 0.05$ .

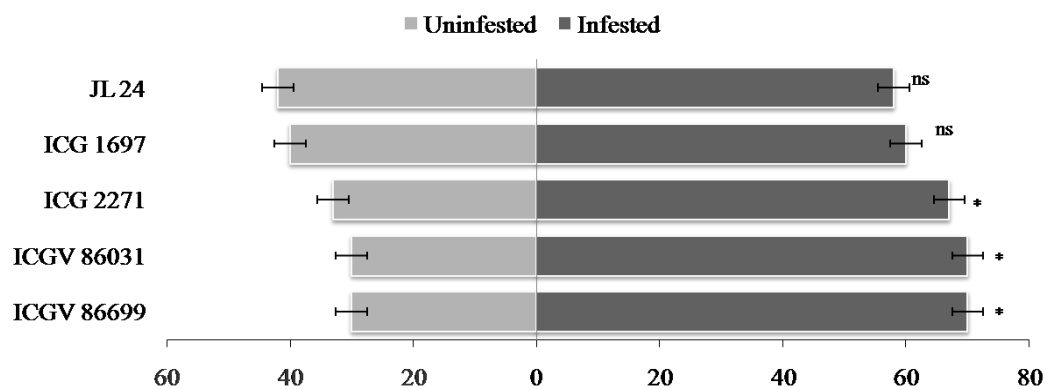
PJA+SIN = Pretreatment with JA one day prior to *S. litura* infestation; PSA+SIN = Pretreatment with SA one day prior to *S. litura* infestation; JA+SIN: Simultaneous application of JA and *S. litura* infestation; SA+SIN = Simultaneous application of SA and *S. litura* infestation; SIN = *S. litura* infested plants; CDNB = 1-chloro-2, 4-dinitrobenzene.



**b**

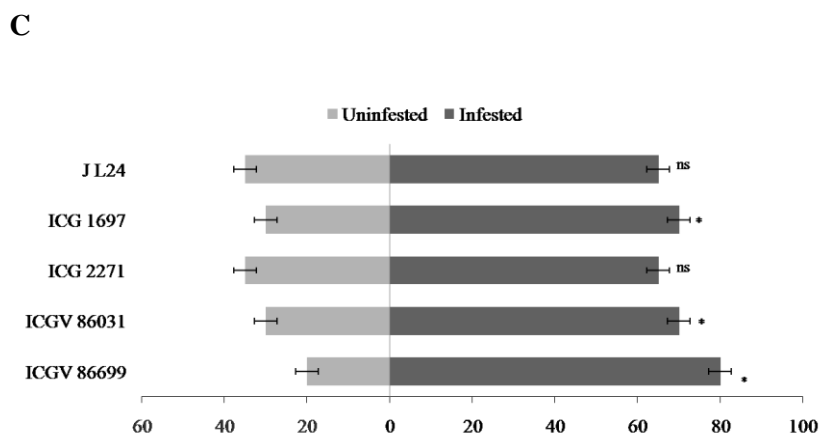
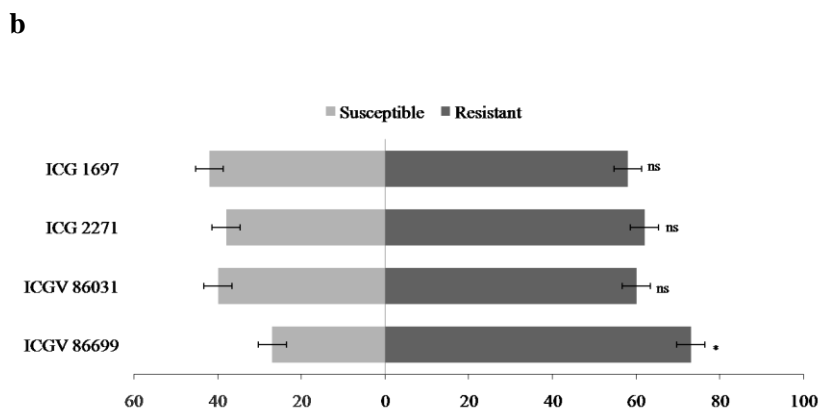
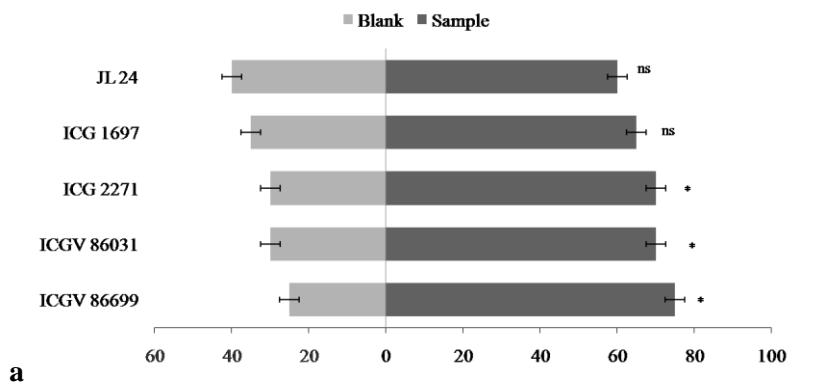


**c**



**Fig 4.8.1a,b,c:** Orientation of *Campolepis chlorideae* towards different groundnut genotypes through Y-tube olfactometer; (a) Orientation between blank and sample (groundnut genotype); (b) Orientation between susceptible genotype (JL 24) and insect resistant genotypes; (c) Orientation between uninfested plants and insect infested plants.

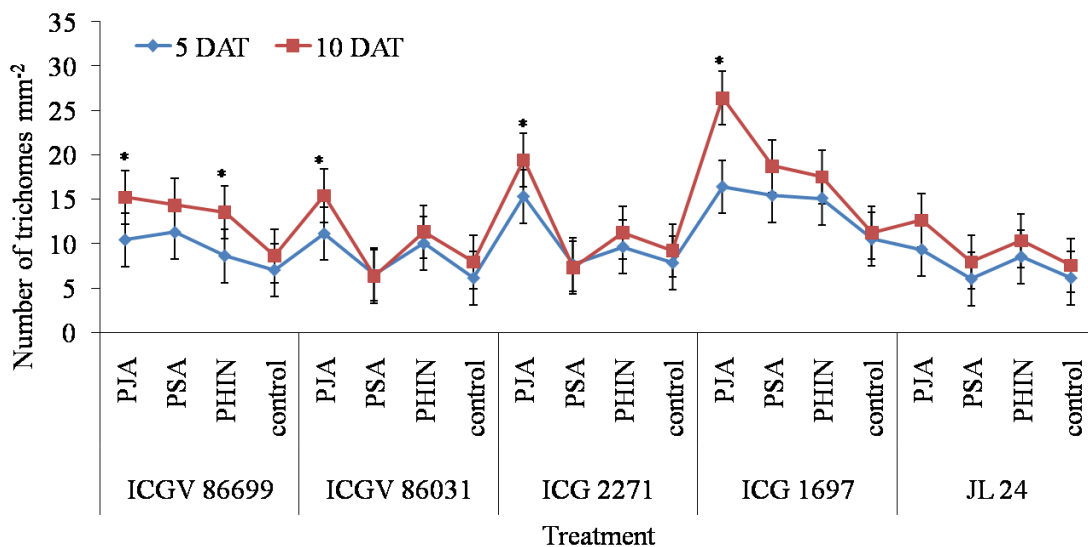
\*= significant difference at  $P < 0.05$ , ns = non significant (Chi- Square test).



**Fig 4.8.2a,b,c:** Orientation of *Trichogramma chilonis* towards different groundnut genotypes through Y-tube olfactometer; (a) Orientation between blank and sample (groundnut genotype); (b) Orientation between susceptible genotype (JL 24) and insect resistant genotypes; (c) Orientation between uninfested plants and insect infested plants.

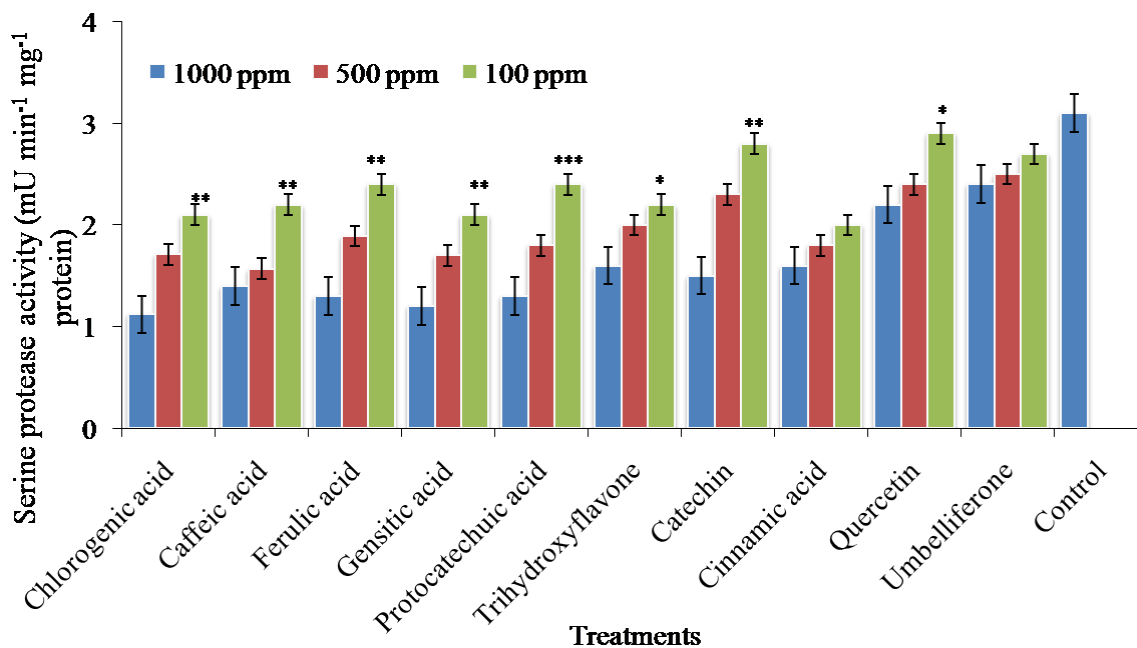
\*= significant difference at  $P < 0.05$ , ns = non significant (Chi- Square test).

**Fig. 4.6: Number of trichomes (per mm<sup>2</sup>) of groundnut leaves pretreated with jasmonic acid and salicylic acid and infested with insects.**



Lines of same color with \* are significantly different at  $P \leq 0.05$ ; PJA = pretreatment with jasmonic acid; PSA = Pretreatment with salicylic acid; PHIN = Preinfested with *H. armigera*

**Fig. 4.9.1a: Serine protease activity (mU min<sup>-1</sup> mg<sup>-1</sup> protein) of *Helicoverpa armigera* larvae fed on flavonoid treated diet.**

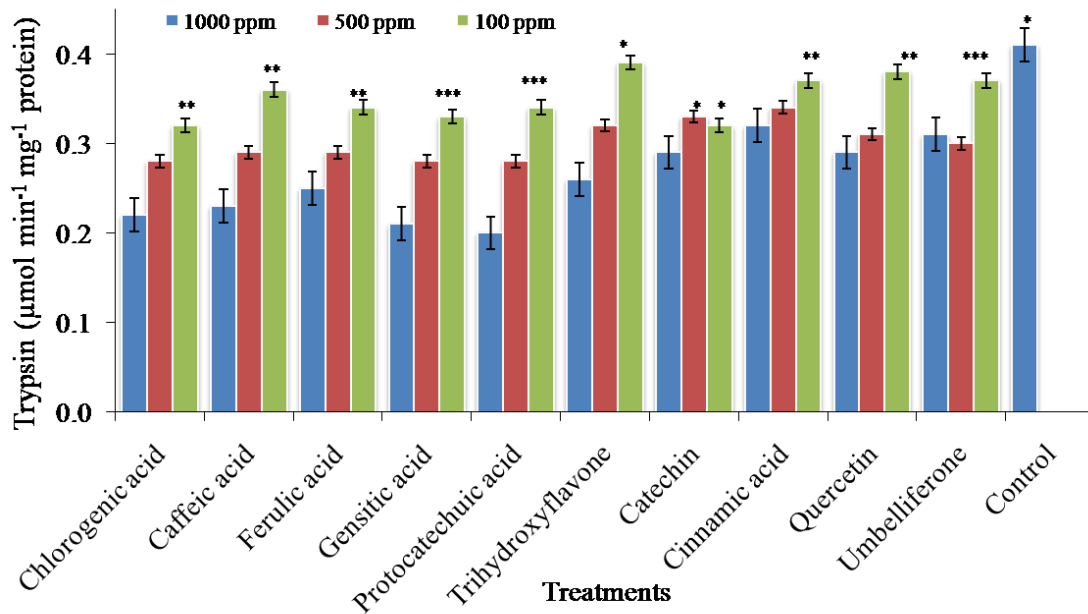


Bars (Mean  $\pm$  SD) of same color with similar asterisks within a genotype are not statistically different. \*, \*\*, \*\*\* on bars within a treatment shows the significant difference in enzyme activity among the different concentrations at  $P \leq 0.05$ , 0.01, and 0.001, respectively. \* on



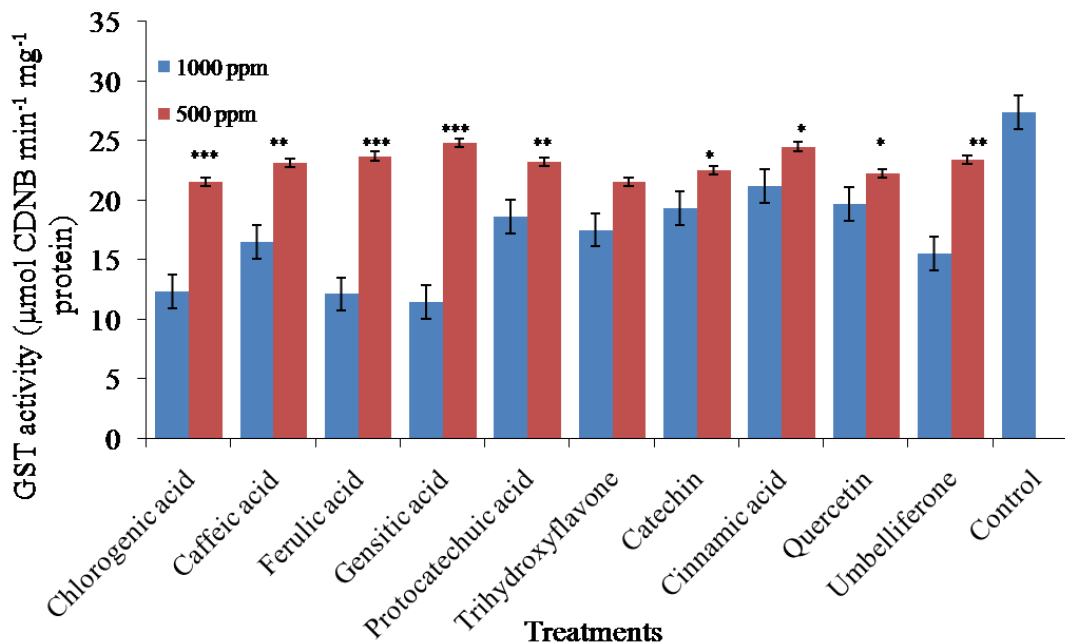
**control shows the greater activity of the larvae fed on control diet as compared to the larvae fed on treated diet.**

**Fig. 4.9.1b: Trypsin activity ( $\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$ ) of *Helicoverpa armigera* larvae fed on flavonoid treated diet.**



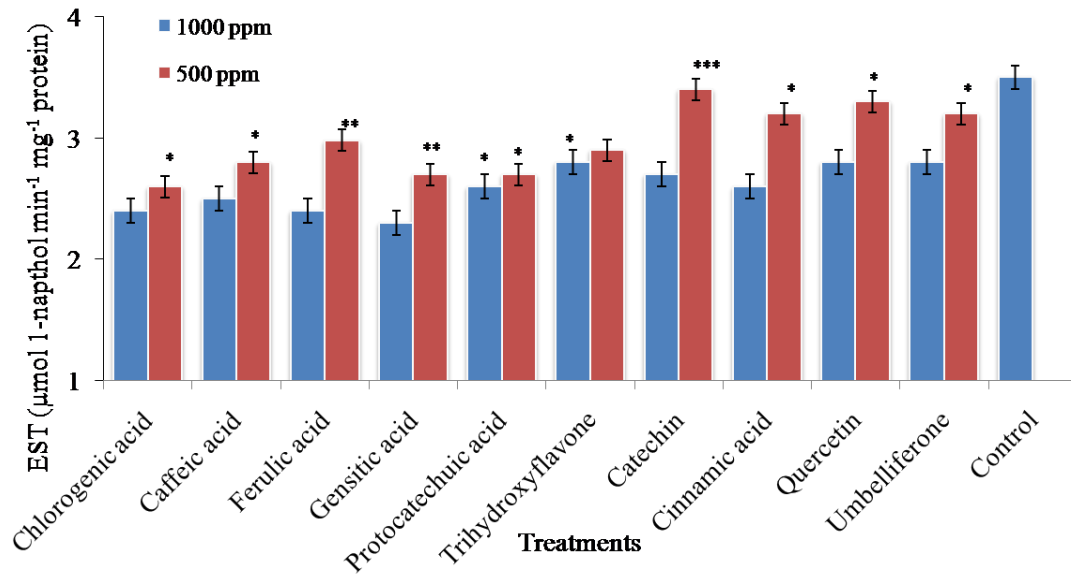
Bars (Mean  $\pm$  SD) of same color with similar asterisks within a genotype are not statistically different. \*, \*\*, \*\*\* on bars within a treatment shows the significant difference in enzyme activity among the different concentrations at  $P \leq 0.05$ , 0.01, and 0.001, respectively.

**Fig. 4.9.1c: Glutathione-S-transferase (GST) activity ( $\mu\text{mol CDNB min}^{-1} \text{mg}^{-1} \text{protein}$ ) of the *Helicoverpa armigera* larvae fed on flavonoid treated diet.**



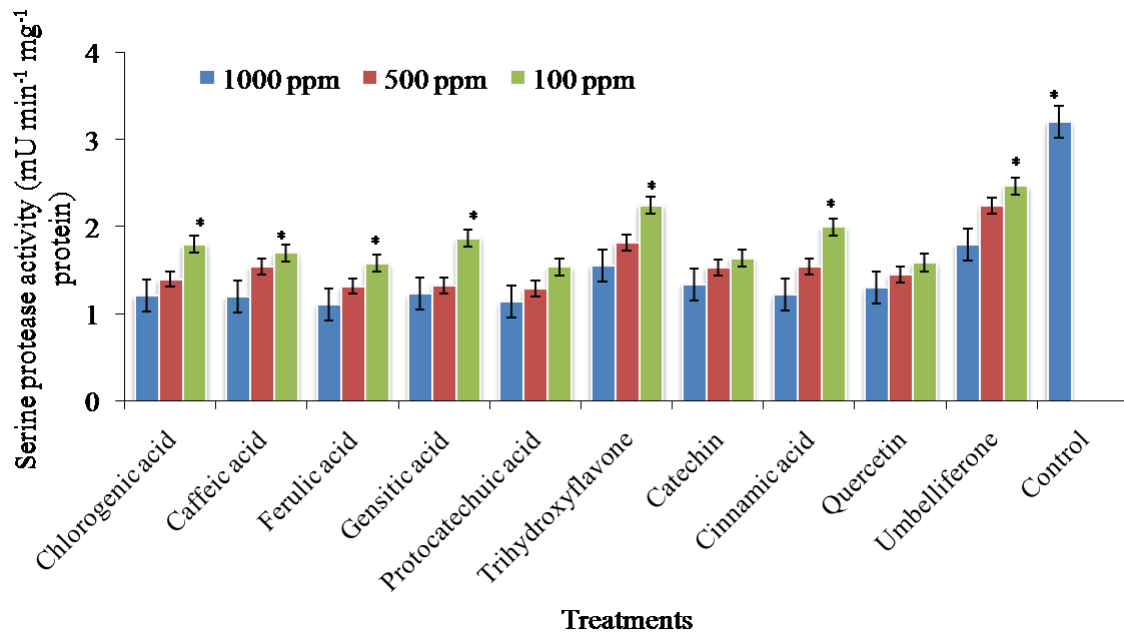
Bars (Mean  $\pm$  SD) of same color with similar asterisks within a genotype are not statistically different. \*, \*\* on bars within a treatment shows the significant difference in enzyme activity among the different concentrations at  $P \leq 0.05$ , and 0.01, respectively. GST activity at 100 ppm was not shown, since there was no significant difference.

**Fig. 4.9.1d: Esterase (EST) activity ( $\mu\text{mol 1-naphthol min}^{-1} \text{mg}^{-1} \text{protein}$ ) of the *Helicoverpa armigera* larvae fed on flavonoid treated diet.**



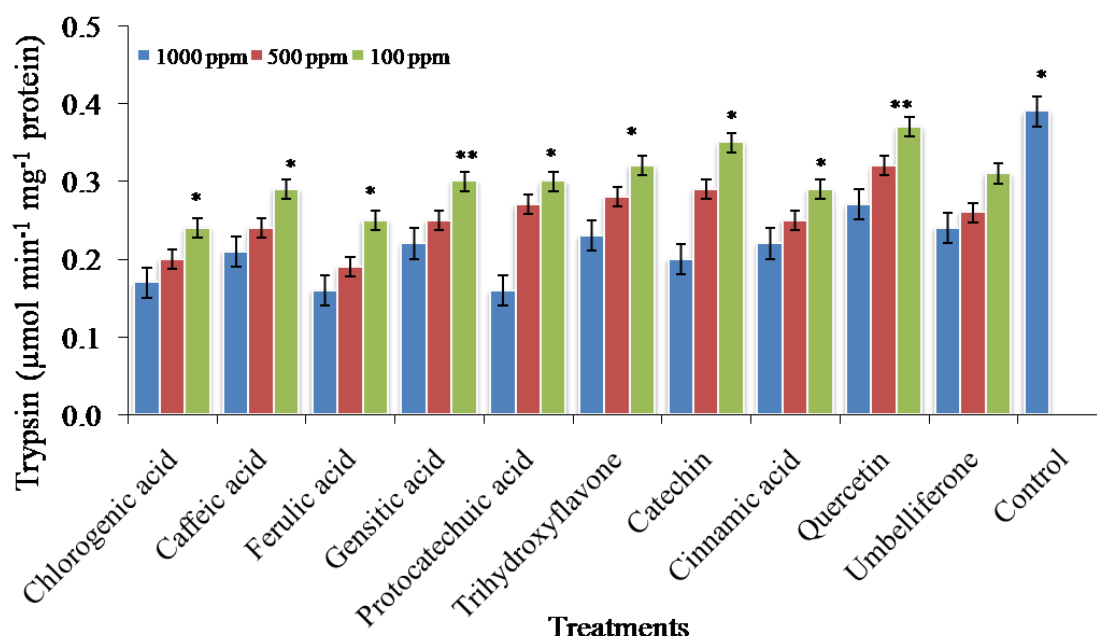
Bars (Mean  $\pm$  SD) of same color with similar asterisks within a genotype are not statistically different. \*, \*\*, \*\*\* on bars within a treatment shows the significant difference in enzyme activity among the different concentrations at  $P \leq 0.05$ , 0.01 and 0.001, respectively. EST activity at 100 ppm was not shown, since there was no significant difference.

**Fig. 4.9.2a: Serine protease activity ( $\text{mU min}^{-1} \text{mg}^{-1} \text{protein}$ ) of *Spodoptera litura* larvae fed on flavonoid treated diet.**



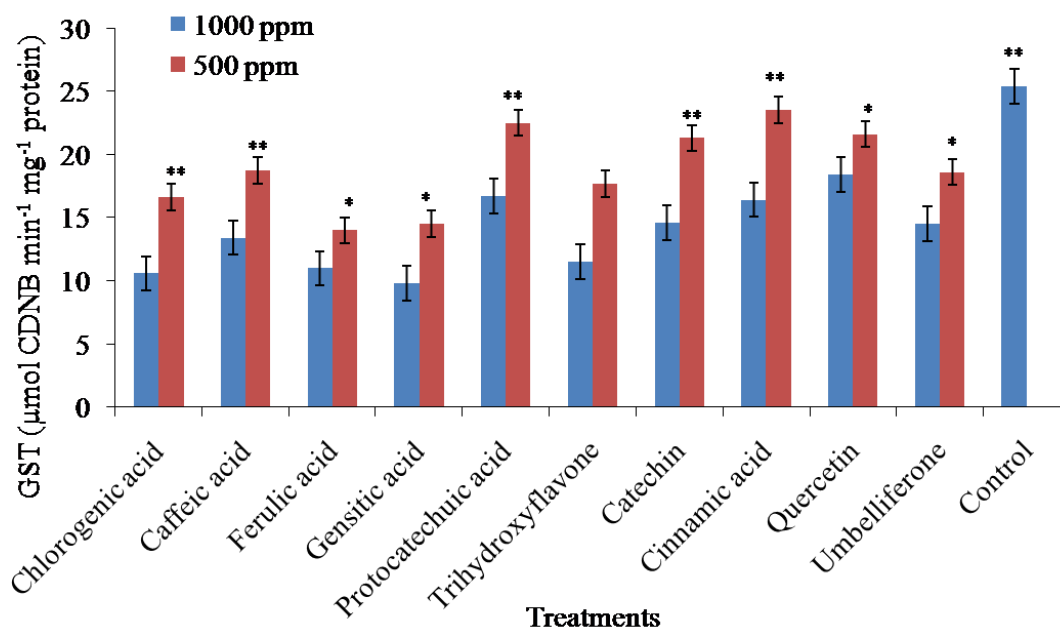
Bars (Mean  $\pm$  SD) of same color with similar asterisks within a genotype are not statistically different. \*, \*\* on bars within a treatment shows the significant difference in enzyme activity among the different concentrations at  $P \leq 0.05$ , and 0.01, respectively.

**Fig. 4.9.2b: Trypsin activity ( $\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$ ) of *Spodoptera litura* larvae fed on flavonoid treated diet.**



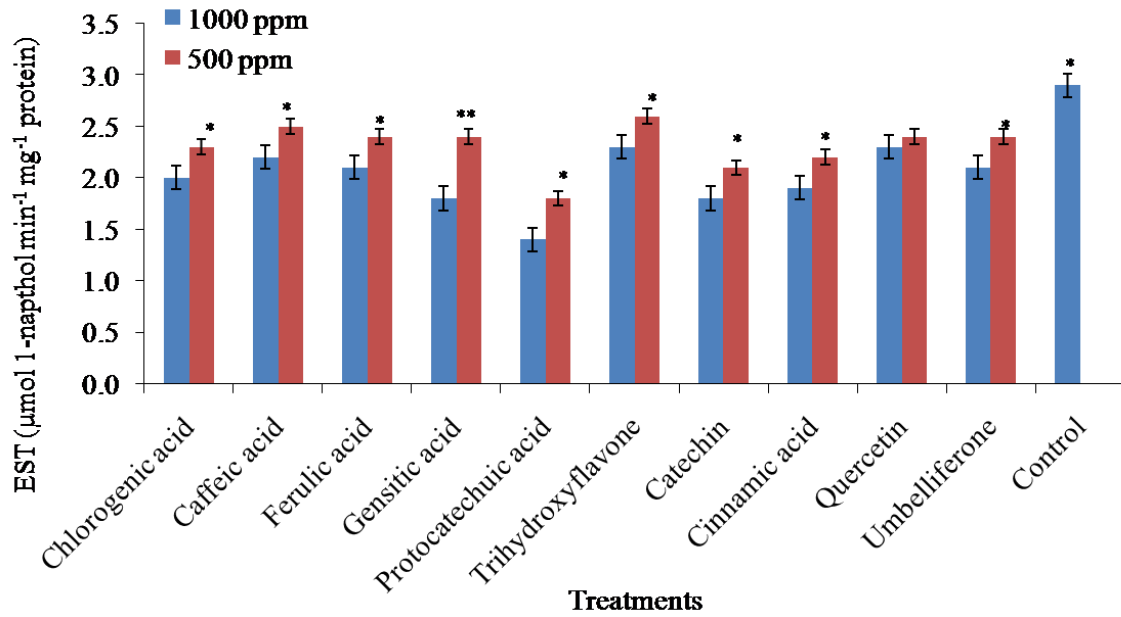
Bars (Mean  $\pm$  SD) of same color with similar asterisks within a genotype are not statistically different. \*,\*\* on bars within a treatment shows the significant difference in enzyme activity among the different concentrations at  $P \leq 0.05$  and  $0.01$ , respectively.

Fig. 4.9.2c: Glutathione-S-transferase (GST) activity ( $\mu\text{mol CDNB min}^{-1} \text{mg}^{-1} \text{protein}$ ) of the *Spodoptera litura* larvae fed on flavonoid treated diet.



Bars (Mean  $\pm$  SD) of same color with similar asterisks within a genotype are not statistically different. \*,\*\* on bars within a treatment shows the significant difference in enzyme activity among the different concentrations at  $P \leq 0.05$ , and  $0.01$ , respectively. GST activity at 100 ppm was not shown.

**Fig. 4.9.2d: Esterase (EST) activity ( $\mu\text{mol 1-naphthol min}^{-1} \text{mg}^{-1} \text{protein}$ ) of the *Spodoptera litura* larvae fed on flavonoid treated diet.**



Bars (Mean  $\pm$  SD) of same color with similar asterisks within a genotype are not statistically different. \*,\*\* on bars within a treatment shows the significant difference in enzyme activity among the different concentrations at  $P \leq 0.05$ , and  $0.01$  respectively. EST activity at 100 ppm was not shown.



**Table 4.1.1: Screening of various groundnut genotypes against *Helicoverpa armigera*, *Spodoptera litura* and leaf hoppers under field conditions.**

Genotypes	Damage rating <sup>x</sup>	
	<i>H. armigera/ S. litura</i>	Leaf hoppers
<b>ICGV 86699</b>	2.6 ± 0.09 <sup>b</sup>	2.0 ± 0.04 <sup>c</sup>
<b>ICGV 86031</b>	3.1 ± 0.06 <sup>b</sup>	3.2 ± 0.06 <sup>b</sup>
<b>ICG 2271</b>	2.9 ± 0.06 <sup>b</sup>	3.1 ± 0.08 <sup>b</sup>
<b>ICG 1697</b>	3.2 ± 0.09 <sup>b</sup>	2.9 ± 0.05 <sup>bc</sup>
<b>JL 24</b>	7.0 ± 0.09 <sup>a</sup>	6.0 ± 0.09 <sup>a</sup>

Values (Mean ± SD) carrying same alphabet(s) within a column are not significantly different at  $P \leq 0.05$  (Tukey's HSD test).

<sup>x</sup>Plants damage rating visually to a scale 1-9, with 1 being no or slight damage, i.e.,  $\leq 10\%$  and 9 being  $\geq 80\%$  damage.

**Table 4.1.2a: Defensive enzyme activities of groundnut plants under field conditions.**

<b>Genotypes</b>	<b>POD (IU g<sup>-1</sup> FW)</b>	<b>PPO (IU g<sup>-1</sup> FW)</b>	<b>PAL ( μmol cinnamic acid mg<sup>-1</sup> protein)</b>	<b>LOX ( IU g<sup>-1</sup> FW)</b>	<b>SOD (IU g<sup>-1</sup> FW)</b>	<b>APX ( IU g<sup>-1</sup> FW)</b>	<b>CAT ( μmol min<sup>-1</sup> mg<sup>-1</sup> protein)</b>
<b>ICGV 86699</b>	0.24 ± 0.03 <sup>a</sup>	0.044 ± 0.001 <sup>a</sup>	6.4 ± 0.9 <sup>a</sup>	4.1 ± 0.1 <sup>a</sup>	7.6 ± 1.03 <sup>a</sup>	0.54 ± 0.02 <sup>a</sup>	6.8 ± 0.7 <sup>a</sup>
<b>ICGV 86031</b>	0.21 ± 0.06 <sup>a</sup>	0.034 ± 0.004 <sup>a</sup>	6.8 ± 0.9 <sup>a</sup>	3.4 ± 0.7 <sup>ab</sup>	6.3 ± 0.08 <sup>ab</sup>	0.46 ± 0.04 <sup>b</sup>	7.3 ± 0.3 <sup>a</sup>
<b>ICG 2271</b>	0.19 ± 0.02 <sup>a</sup>	0.038 ± 0.004 <sup>a</sup>	5.5 ± 0.7 <sup>a</sup>	2.7 ± 0.3 <sup>b</sup>	5.9 ± 0.05 <sup>b</sup>	0.34 ± 0.01 <sup>c</sup>	5.2 ± 0.4 <sup>b</sup>
<b>ICG 1697</b>	0.18 ± 0.07 <sup>a</sup>	0.035 ± 0.003 <sup>a</sup>	5.3 ± 0.3 <sup>a</sup>	3.1 ± 0.4 <sup>b</sup>	5.4 ± 0.05 <sup>b</sup>	0.37 ± 0.07 <sup>c</sup>	5.8 ± 1.0 <sup>b</sup>
<b>JL 24</b>	0.09 ± 0.01 <sup>b</sup>	0.021 ± 0.001 <sup>b</sup>	3.2 ± 0.1 <sup>b</sup>	1.7 ± 0.5 <sup>c</sup>	4.1 ± 1.01 <sup>c</sup>	0.26 ± 0.04 <sup>d</sup>	4.1 ± 0.5 <sup>c</sup>

Values (Mean ± SD) with same letter (s) in a column are not significantly different at  $P \leq 0.05$  (Tukey's HSD test).

SOD = Superoxide dismutase; POD = Peroxidase, PPO = Polyphenol oxidase; LOX = Lipoxigenase; APX = Ascorbate peroxidase; PAL = Phenylalanine ammonia lyase; CAT = Catalase; FW = fresh weight.



**Table 4.1.2b: Plant defensive compounds of groundnut under field conditions.**

<b>Genotype</b>	<b>Phenols (<math>\mu\text{g GAE g}^{-1}\text{ FW}</math>)</b>	<b>CondensD tannins (<math>\mu\text{g TAE g}^{-1}\text{ FW}</math>)</b>	<b>H<sub>2</sub>O<sub>2</sub> (<math>\mu\text{mol g}^{-1}\text{ FW}</math>)</b>	<b>MDA (<math>\mu\text{mol g}^{-1}\text{ FW}</math>)</b>	<b>Protein (<math>\text{mg g}^{-1}\text{FW}</math>)</b>
<b>ICGV 86699</b>	87.4 $\pm$ 2.6 <sup>a</sup>	9.5 $\pm$ 1.3 <sup>a</sup>	60.7 $\pm$ 2.3 <sup>a</sup>	8.9 $\pm$ 0.9 <sup>a</sup>	10.2 $\pm$ 1.02 <sup>a</sup>
<b>ICGV 86031</b>	79.4 $\pm$ 2.4 <sup>a</sup>	8.8 $\pm$ 0.9 <sup>ab</sup>	42.9 $\pm$ 3.7 <sup>b</sup>	8.6 $\pm$ 1.0 <sup>a</sup>	7.9 $\pm$ 0.09 <sup>ab</sup>
<b>ICG 2271</b>	73.7 $\pm$ 3.5 <sup>a</sup>	8.3 $\pm$ 1.3 <sup>b</sup>	38.6 $\pm$ 2.0 <sup>b</sup>	6.5 $\pm$ 0.9 <sup>b</sup>	8.5 $\pm$ 0.1 <sup>ab</sup>
<b>ICG 1697</b>	65.2 $\pm$ 2.6 <sup>b</sup>	8.6 $\pm$ 1.1 <sup>ab</sup>	31.4 $\pm$ 1.9 <sup>bc</sup>	5.3 $\pm$ 0.1 <sup>bc</sup>	6.2 $\pm$ 0.07 <sup>bc</sup>
<b>JL 24</b>	45.3 $\pm$ 1.9 <sup>c</sup>	3.9 $\pm$ 0.2 <sup>c</sup>	15.9 $\pm$ 0.9 <sup>d</sup>	7.7 $\pm$ 0.7 <sup>ab</sup>	4.4 $\pm$ 0.01 <sup>c</sup>

Values (Mean  $\pm$  SD) with the same letter (s) in a column are not significantly different at  $P \leq 0.05$  (Tukey's HSD test).

H<sub>2</sub>O<sub>2</sub> = Hydrogen peroxide; MDA = Malondialdehyde; FW = Fresh weight; TAE = Tannic acid equivalents; GAE = Gallic acid equivalents.

**Table 4.2.1: Nutritional indices of *Helicoverpa armigera* larvae fed on groundnut genotypes.**

Genotype	Nutritional indices			
	CI (mg/mg/day)	AD (%)	ECI (%)	ECD (%)
<b>ICGV 86699</b>	2.3 ± 0.01 <sup>bc</sup>	36.5 ± 3.8 <sup>c</sup>	21.3 ± 1.5 <sup>b</sup>	27.1 ± 1.3 <sup>bc</sup>
<b>ICGV 86031</b>	2.6 ± 0.03 <sup>bc</sup>	41.2 ± 2.3 <sup>b</sup>	25.5 ± 1.2 <sup>b</sup>	23.6 ± 1.4 <sup>bc</sup>
<b>ICG 2271</b>	3.5 ± 0.02 <sup>b</sup>	44.3 ± 2.9 <sup>b</sup>	28.2 ± 1.8 <sup>b</sup>	30.2 ± 2.5 <sup>b</sup>
<b>ICG 1697</b>	2.9 ± 0.01 <sup>b</sup>	45.4 ± 3.0 <sup>b</sup>	24.7 ± 1.9 <sup>b</sup>	29.3 ± 2.2 <sup>b</sup>
<b>JL 24</b>	4.1 ± 0.04 <sup>a</sup>	67.5 ± 3.7 <sup>a</sup>	54.1 ± 2.3 <sup>a</sup>	45.7 ± 2.7 <sup>a</sup>

Within columns, (means ± SD) followed by same letter(s) do not differ significantly (Tukey's HSD test, P < 0.05).

CI = consumption index, AD = Approximate digestibility, ECI = Efficiency of conversion of ingested food and

ECD = Efficiency of conversion of digested food.

**Table 4.2.2: Nutritional indices of *Spodoptera litura* larvae fed on groundnut genotypes.**

Genotype	Nutritional indices			
	CI (mg/mg/day)	AD (%)	ECI (%)	ECD (%)
<b>ICGV 86699</b>	2.1 ± 0.09 <sup>bc</sup>	41.7 ± 2.1 <sup>b</sup>	24.6 ± 1.1 <sup>b</sup>	25.8 ± 1.5 <sup>c</sup>
<b>ICGV 86031</b>	3.1 ± 0.08 <sup>b</sup>	47.3 ± 2.8 <sup>b</sup>	27.2 ± 2.3 <sup>b</sup>	30.5 ± 3.2 <sup>bc</sup>
<b>ICG 2271</b>	2.7 ± 0.08 <sup>b</sup>	50.6 ± 3.9 <sup>b</sup>	25.7 ± 1.1 <sup>b</sup>	37.8 ± 1.3 <sup>b</sup>
<b>ICG 1697</b>	1.9 ± 0.04 <sup>c</sup>	48.1 ± 1.2 <sup>b</sup>	29.1 ± 1.4 <sup>b</sup>	30.2 ± 2.4 <sup>bc</sup>
<b>JL 24</b>	5.2 ± 0.83 <sup>a</sup>	70.3 ± 5.1 <sup>a</sup>	52.1 ± 1.1 <sup>a</sup>	50.2 ± 4.2 <sup>a</sup>

Within columns, values (means ± SE) followed by a same letter do not differ significantly (Tukey's HSD test, P < 0.05)

CI = consumption index, AD = Approximate digestibility, ECI = Efficiency of conversion of ingested food and

ECD = Efficiency of conversion of digested food.

**Table 4.3.1: The *in vitro* proteinase inhibitor (PI) activity (%) of groundnut genotypes infested with *Helicoverpa armigera*, *Spodoptera litura* and *Aphis craccivora*.**

Genotypes	PI activity (%)			
	<i>H. armigera</i>	<i>S. litura</i>	<i>A. craccivora</i>	Control
<b>ICGV 86699</b>	33.5 ± 2.3 <sup>a</sup>	30.9 ± 3.3 <sup>a</sup>	25.5 ± 1.2 <sup>a</sup>	21.6 ± 1.2 <sup>a</sup>
<b>ICGV 86031</b>	30.6 ± 2.9 <sup>a</sup>	28.2 ± 2.9 <sup>a</sup>	23.6 ± 1.1 <sup>a</sup>	19.4 ± 2.1 <sup>a</sup>
<b>ICG 2271</b>	23.4 ± 1.2 <sup>b</sup>	25.6 ± 2.7 <sup>b</sup>	17.2 ± 1.3 <sup>b</sup>	20.1 ± 1.7 <sup>a</sup>
<b>ICG 1697</b>	21.2 ± 2.9 <sup>b</sup>	21.5 ± 1.1 <sup>b</sup>	16.9 ± 1.5 <sup>a</sup>	19.7 ± 1.2 <sup>a</sup>
<b>JL 24</b>	19.8 ± 1.1 <sup>bc</sup>	19.6 ± 1.3 <sup>bc</sup>	17.5 ± 1.5 <sup>ab</sup>	14.5 ± 0.9 <sup>b</sup>

Values (Mean ± SD) with same letter within a column do not differ significantly at  $P \leq 0.05$  (Tukey's HSD test).

**Table 4.3.2: Plant damage, larval survival and weight of *Helicoverpa armigera*, *Spodoptera litura* and *Aphis craccivora* fed on groundnut genotypes.**

Genotypes	DR <sup>x</sup>		Larval survival (%)		Larval weight (mg)*		Aphid DR <sup>y</sup>	No. of aphids
	<i>H. armigera</i>	<i>S. litura</i>	<i>H. armigera</i>	<i>S. litura</i>	<i>H. armigera</i>	<i>S. litura</i>		
<b>ICGV 86699</b>	2.8 <sup>c</sup>	3.3 <sup>c</sup>	33.5 ± 2.4 <sup>c</sup>	41.2 ± 2.2 <sup>bc</sup>	55.5 ± 3.9 <sup>bc</sup>	65.4 ± 3.5 <sup>bc</sup>	2.5 <sup>b</sup>	31.5 ± 2.2 <sup>b</sup>
<b>ICGV 86031</b>	3.5 <sup>bc</sup>	3.5 <sup>c</sup>	39.4 ± 3.8 <sup>bc</sup>	48.7 ± 3.5 <sup>b</sup>	68.9 ± 3.9 <sup>b</sup>	73.5 ± 5.9 <sup>b</sup>	2.6 <sup>b</sup>	27.8 ± 3.1 <sup>b</sup>
<b>ICG 2271</b>	4.2 <sup>b</sup>	4.4 <sup>b</sup>	45.6 ± 3.6 <sup>b</sup>	52.3 ± 2.2 <sup>b</sup>	65.6 ± 2.2 <sup>b</sup>	79.2 ± 4.1 <sup>b</sup>	2.3 <sup>b</sup>	37.8 ± 2.2 <sup>b</sup>
<b>ICG 1697</b>	3.8 <sup>b</sup>	3.4 <sup>c</sup>	48.3 ± 4.4 <sup>b</sup>	50.6 ± 4.7 <sup>b</sup>	67.4 ± 4.0 <sup>b</sup>	68.2 ± 3.0 <sup>bc</sup>	2.0 <sup>b</sup>	19.0 ± 2.1 <sup>c</sup>
<b>JL 24</b>	7.5 <sup>a</sup>	7.9 <sup>a</sup>	77.5 ± 6.6 <sup>a</sup>	80.3 ± 5.4 <sup>a</sup>	95.5 ± 7.8 <sup>a</sup>	120.3 ± 7.8 <sup>a</sup>	4.2 <sup>a</sup>	56.5 ± 4.2 <sup>a</sup>

Values (Mean ± SD) carrying same alphabet(s) within a column are not significantly different at  $P \leq 0.05$  (Tukey's HSD test).

<sup>x</sup> DR = *Helicoverpa* damage rating to a scale 1-9 (1 ≤ 10 % and 9 ≥ 90 %) 6 days after infestation

\* Weight per larva at the time of recovery.

<sup>y</sup> = Aphid damage rating to a scale 1-5 (1 = highly resistant, and 5 = highly susceptible)

**Table 4.4.1.2.1a: Plant damage and *Helicoverpa armigera* larval survival on plants treated with jasmonic acid and salicylic acid.**

Genotypes	Plant damage rating (DR) <sup>x</sup>					Survival (%)				
	PJA+HIN	PSA+HIN	JA+HIN	SA+HIN	HIN	PJA+HIN	PSA+HIN	JA+HIN	SA+HIN	HIN
<b>ICGV 86699</b>	2.0 ± 0.9 <sup>c</sup>	2.6 ± 0.5 <sup>b</sup>	2.4 ± 0.9 <sup>bc</sup>	2.7 ± 0.4 <sup>b</sup>	3.1 ± 0.7 <sup>b</sup>	20.4 ± 2.1 <sup>c</sup>	32.3 ± 2.3 <sup>bc</sup>	30.2 ± 4.6 <sup>c</sup>	36.5 ± 3.4 <sup>bc</sup>	41.2 ± 3.1 <sup>c</sup>
<b>ICGV 86031</b>	2.5 ± 0.8 <sup>bc</sup>	3.0 ± 0.3 <sup>b</sup>	2.6 ± 0.8 <sup>b</sup>	3.2 ± 0.6 <sup>b</sup>	3.5 ± 0.3 <sup>b</sup>	26.6 ± 2.1 <sup>bc</sup>	34.3 ± 2.2 <sup>bc</sup>	35.5 ± 3.3 <sup>c</sup>	39.6 ± 4.4 <sup>bc</sup>	47.4 ± 2.1 <sup>b</sup>
<b>ICG 2271</b>	3.2 ± 0.9 <sup>b</sup>	3.5 ± 0.3 <sup>b</sup>	3.1 ± 0.6 <sup>b</sup>	3.5 ± 0.7 <sup>b</sup>	4.0 ± 0.6 <sup>b</sup>	32.4 ± 1.4 <sup>b</sup>	40.5 ± 3.8 <sup>b</sup>	40.4 ± 2.1 <sup>b</sup>	44.5 ± 2.1 <sup>b</sup>	48.9 ± 3.1 <sup>b</sup>
<b>ICG 1697</b>	3.0 ± 0.7 <sup>b</sup>	3.4 ± 0.6 <sup>b</sup>	3.0 ± 0.4 <sup>b</sup>	3.6 ± 0.9 <sup>b</sup>	3.9 ± 0.7 <sup>b</sup>	35.7 ± 3.2 <sup>b</sup>	44.8 ± 2.6 <sup>b</sup>	48.2 ± 3.2 <sup>b</sup>	50.5 ± 3.6 <sup>b</sup>	54.4 ± 4.7 <sup>b</sup>
<b>JL 24</b>	5.5 ± 1.1 <sup>a</sup>	6.4 ± 1.1 <sup>a</sup>	6.2 ± 1.2 <sup>a</sup>	7.0 ± 0.6 <sup>a</sup>	7.5 ± 1.3 <sup>a</sup>	58.3 ± 2.1 <sup>a</sup>	69.4 ± 3.8 <sup>a</sup>	75.9 ± 2.3 <sup>a</sup>	79.6 ± 4.1 <sup>a</sup>	81.4 ± 6.6 <sup>a</sup>

Values (Mean ± SD) carrying same letter(s) within a column are not significantly different at  $P \leq 0.05$  (Tukey's HSD test).

<sup>x</sup> DR = *Helicoverpa* damage rating to a scale 1-9 (1 ≤ 10 % and 9 ≥ 90 %) 6 days after infestation.

PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN: Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants.

**Table 4.4.1.2.1b: Weight (mg)\* of *H. armigera* larvae fed on jasmonic acid and salicylic acid treated groundnut plants.**

Genotypes	Treatments				
	PJA+HIN	PSA+HIN	JA+HIN	SA+HIN	HIN
<b>ICGV 86699</b>	37.5 ± 3.1 <sup>d</sup>	48.6 ± 5.3 <sup>d</sup>	47.5 ± 5.6 <sup>d</sup>	59.7 ± 3.5 <sup>c</sup>	69.6 ± 3.6 <sup>c</sup>
<b>ICGV 86031</b>	44.5 ± 2.8 <sup>bc</sup>	60.6 ± 3.7 <sup>c</sup>	75.5 ± 7.7 <sup>bc</sup>	74.4 ± 3.7 <sup>c</sup>	97.7 ± 5.3 <sup>bc</sup>
<b>ICG 2271</b>	55.4 ± 3.2 <sup>b</sup>	65.6 ± 5.3 <sup>bc</sup>	87.6 ± 3.4 <sup>b</sup>	98.8 ± 4.7 <sup>bc</sup>	110.3 ± 8.8 <sup>b</sup>
<b>ICG 1697</b>	59.6 ± 2.7 <sup>a</sup>	80.6 ± 6.4 <sup>b</sup>	95.5 ± 4.3 <sup>b</sup>	114.4 ± 6.3 <sup>ab</sup>	127.5 ± 7.3 <sup>b</sup>
<b>JL 24</b>	73.6 ± 4.3 <sup>a</sup>	102.4 ± 7.6 <sup>a</sup>	120.3 ± 8.7 <sup>a</sup>	129.5 ± 9.5 <sup>a</sup>	159.5 ± 10.0 <sup>a</sup>

Values (Mean ± SD) carrying same letter(s) within a column are not significantly different at  $P \leq 0.05$  (Tukey's HSD test).  
PJA+HIN = Pretreatment with JA one day prior to *H. armigera* infestation; PSA+HIN = Pretreatment with SA one day prior to *H. armigera* infestation; JA+HIN: Simultaneous application of JA and *H. armigera* infestation; SA+HIN = Simultaneous application of SA and *H. armigera* infestation; HIN = *H. armigera* infested plants.

**Table 4.4.1.2.2: Protein concentration (mg mL<sup>-1</sup> tissue) of *Helicoverpa armigera* larvae fed on jasmonic acid and salicylic acid treated groundnut plants.**

Genotypes	Treatments				
	PJA+HIN	PSA+HIN	JA+HIN	SA+HIN	HIN
<b>ICGV 86699</b>	5.5 ± 0.1 <sup>b*</sup>	7.7 ± 0.8 <sup>b</sup>	6.1 ± 0.6 <sup>b*</sup>	7.9 ± 0.5 <sup>b</sup>	8.2 ± 0.6 <sup>b</sup>
<b>ICGV 86031</b>	6.2 ± 0.8 <sup>b*</sup>	7.3 ± 0.7 <sup>b</sup>	6.8 ± 0.7 <sup>b</sup>	7.7 ± 0.7 <sup>b</sup>	7.9 ± 0.3 <sup>b</sup>
<b>ICG 2271</b>	6.4 ± 0.7 <sup>b*</sup>	6.9 ± 1.3 <sup>b</sup>	6.6 ± 0.4 <sup>b*</sup>	8.1 ± 0.7 <sup>b</sup>	8.4 ± 0.8 <sup>b</sup>
<b>ICG 1697</b>	5.9 ± 0.7 <sup>b*</sup>	7.0 ± 1.4 <sup>b</sup>	6.8 ± 1.3 <sup>b</sup>	7.8 ± 0.3 <sup>b</sup>	8.0 ± 0.3 <sup>b</sup>
<b>JL 24</b>	10.7 ± 1.3 <sup>a*</sup>	13.3 ± 1.6 <sup>a</sup>	12.6 ± 1.7 <sup>a</sup>	14.0 ± 1.5 <sup>a</sup>	17.4 ± 1.6 <sup>a</sup>

Values (Mean ± SD) carrying same letter(s) within a column are not significantly different at P ≤ 0.05 (Tukey's HSD test).

In control, the values in all the columns have been mentioned to facilitate the comparison with other treatments.



**Table 4.4.2.2.1a: Plant damage and *Spodoptera litura* larval survival on plants treated with jasmonic acid and salicylic acid.**

Genotypes	Plant damage rating (DR) <sup>x</sup>					Survival (%)				
	PJA+SIN	PSA+SIN	JA+SIN	SA+SIN	HIN	PJA+SIN	PSA+SIN	JA+SIN	SA+SIN	SIN
<b>ICGV 86699</b>	2.4 ± 0.4 <sup>c</sup>	2.6 ± 0.2 <sup>bc</sup>	2.5 ± 0.1 <sup>bc</sup>	2.8 ± 0.2 <sup>b</sup>	3.0 ± 0.4 <sup>bc</sup>	25.8 ± 4.1 <sup>c</sup>	46.7 ± 2.4 <sup>bc</sup>	37.6 ± 1.6 <sup>c</sup>	43.5 ± 1.4 <sup>bc</sup>	45.9 ± 6.1 <sup>c</sup>
<b>ICGV 86031</b>	2.3 ± 0.3 <sup>bc</sup>	3.1 ± 0.5 <sup>b</sup>	2.8 ± 0.4 <sup>b</sup>	3.5 ± 0.4 <sup>b</sup>	3.5 ± 0.7 <sup>b</sup>	30.9 ± 2.5 <sup>bc</sup>	50.3 ± 3.5 <sup>b</sup>	43.5 ± 2.4 <sup>b</sup>	45.6 ± 2.4 <sup>bc</sup>	51.6 ± 2.6 <sup>b</sup>
<b>ICG 2271</b>	2.8 ± 0.6 <sup>b</sup>	3.2 ± 0.9 <sup>b</sup>	3.0 ± 0.6 <sup>b</sup>	3.6 ± 0.3 <sup>b</sup>	3.9 ± 0.8 <sup>b</sup>	37.5 ± 2.7 <sup>b</sup>	53.5 ± 2.3 <sup>b</sup>	47.6 ± 2.1 <sup>b</sup>	50.7 ± 1.5 <sup>b</sup>	50.3 ± 3.3 <sup>b</sup>
<b>ICG 1697</b>	3.3 ± 0.5 <sup>b</sup>	3.7 ± 0.1 <sup>b</sup>	3.5 ± 0.3 <sup>b</sup>	3.5 ± 0.8 <sup>b</sup>	4.3 ± 0.6 <sup>b</sup>	45.6 ± 4.5 <sup>b</sup>	56.6 ± 4.6 <sup>b</sup>	48.8 ± 3.2 <sup>b</sup>	54.9 ± 2.8 <sup>b</sup>	57.8 ± 2.7 <sup>b</sup>
<b>JL 24</b>	5.7 ± 1.4 <sup>a</sup>	5.9 ± 0.9 <sup>a</sup>	5.7 ± 0.4 <sup>a</sup>	6.9 ± 0.9 <sup>a</sup>	7.6 ± 1.3 <sup>a</sup>	60.6 ± 3.1 <sup>a</sup>	67.7 ± 2.6 <sup>a</sup>	74.4 ± 3.6 <sup>a</sup>	75.8 ± 3.9 <sup>a</sup>	77.7 ± 4.6 <sup>a</sup>

Values (Mean ± SD) carrying same letter(s) within a column are not significantly different at  $P \leq 0.05$  (Tukey's HSD test).

<sup>x</sup> DR = *Spodoptera* damage rating to a scale 1-9 (1 ≤ 10 % and 9 ≥ 90 %) 6 days after infestation.

PJA+SIN = Pretreatment with JA one day prior to *S. litura* infestation; PSA+SIN = Pretreatment with SA one day prior to *S. litura* infestation; JA+SIN: Simultaneous application of JA and *S. litura* infestation; SA+SIN = Simultaneous application of SA and *S. litura* infestation; SIN = *S. litura* infested plants.

**Table 4.4.2.2.1b: Weight (mg)\* of *Spodoptera litura* larvae fed on treated groundnut plants.**

Genotypes	Treatments				
	PJA+SIN	PSA+SIN	JA+SIN	SA+SIN	SIN
<b>ICGV 86699</b>	47.4 ± 5.1 <sup>bc</sup>	55.6 ± 4.4 <sup>bc</sup>	50.8 ± 4.3 <sup>bc</sup>	65.6 ± 2.5 <sup>c</sup>	68.7 ± 2.6 <sup>d</sup>
<b>ICGV 86031</b>	54.0 ± 4.5 <sup>b</sup>	65.4 ± 3.7 <sup>b</sup>	60.6 ± 2.2 <sup>b</sup>	70.5 ± 3.0 <sup>c</sup>	76.6 ± 3.4 <sup>c</sup>
<b>ICG 2271</b>	57.9 ± 4.3 <sup>b</sup>	60.8 ± 2.9 <sup>b</sup>	58.5 ± 3.3 <sup>b</sup>	79.6 ± 2.7 <sup>c</sup>	85.6 ± 2.7 <sup>c</sup>
<b>ICG 1697</b>	52.5 ± 3.6 <sup>b</sup>	63.6 ± 3.8 <sup>b</sup>	60.4 ± 3.2 <sup>b</sup>	107.1 ± 3.9 <sup>ab</sup>	116.5 ± 3.4 <sup>b</sup>
<b>JL 24</b>	77.7 ± 4.8 <sup>a</sup>	98.8 ± 2.8 <sup>a</sup>	118.5 ± 4.8 <sup>a</sup>	117.6 ± 5.8 <sup>a</sup>	133.6 ± 4.7 <sup>a</sup>

Values (Mean ± SD) carrying same letter(s) within a column are not significantly different at  $P \leq 0.05$  (Tukey's HSD test).

PJA+SIN = Pretreatment with JA one day prior to *S. litura* infestation; PSA+SIN = Pretreatment with SA one day prior to *S. litura* infestation; JA+SIN: Simultaneous application of JA and *S. litura* infestation; SA+SIN = Simultaneous application of SA and *S. litura* infestation; SIN = *S. litura* infested plants.

\*weight (mg) per larva

**Table 4.4.2.2.2: Protein concentration of *Spodoptera litura* larvae fed on JA and SA treated groundnut plants.**

Genotypes	Treatments				
	PJA+SIN	PSA+SIN	JA+SIN	SA+SIN	SIN
<b>ICGV 86699</b>	6.2 ± 1.6 <sup>bc*</sup>	8.0 ± 1.1 <sup>b</sup>	7.0 ± 1.5 <sup>b*</sup>	8.8 ± 1.3 <sup>b</sup>	10.2 ± 1.2 <sup>b</sup>
<b>ICGV 86031</b>	6.9 ± 1.3 <sup>b*</sup>	7.8 ± 1.4 <sup>b*</sup>	7.5 ± 1.8 <sup>b*</sup>	8.6 ± 0.9 <sup>b</sup>	9.9 ± 1.1 <sup>b</sup>
<b>ICG 2271</b>	7.1 ± 1.2 <sup>b*</sup>	8.9 ± 1.9 <sup>b</sup>	7.7 ± 1.1 <sup>b*</sup>	8.7 ± 0.8 <sup>b</sup>	8.9 ± 1.8 <sup>b</sup>
<b>ICG 1697</b>	6.0 ± 1.2 <sup>bc*</sup>	8.3 ± 0.4 <sup>b</sup>	6.9 ± 0.9 <sup>b*</sup>	9.0 ± 1.3 <sup>b</sup>	9.5 ± 0.6 <sup>b</sup>
<b>JL 24</b>	12.9 ± 1.7 <sup>a*</sup>	14.6 ± 0.1 <sup>a</sup>	13.5 ± 1.8 <sup>a*</sup>	14.8 ± 1.3 <sup>a</sup>	16.4 ± 0.5 <sup>a</sup>

Values (Mean ± SD) carrying same letter(s) within a column are not significantly different at  $P \leq 0.05$  (Tukey's HSD test).

In control, the values in all the columns have been mentioned to facilitate the comparison with other treatments.

**Table 4.5.1.1: Weight gain<sup>x</sup> of *Helicoverpa armigera* and *Spodoptera litura* third instar larvae fed on control and preinfested groundnut plants.**

Genotypes	Weight gain (mg/larvae)			
	<i>H. armigera</i>		<i>S. litura</i>	
	Control	Preinfested	Control	Preinfested
<b>ICGV 86699</b>	25.5 ± 1.8 <sup>b</sup>	17.9 ± 1.8 <sup>c**</sup>	23.8 ± 2.1 <sup>c</sup>	15.6 ± 1.4 <sup>c*</sup>
<b>ICGV 86031</b>	27.3 ± 1.3 <sup>b</sup>	20.4 ± 2.4 <sup>c*</sup>	29.7 ± 1.6 <sup>c</sup>	22.4 ± 1.7 <sup>c*</sup>
<b>ICG 2271</b>	32.6 ± 2.3 <sup>bc</sup>	24.5 ± 1.6 <sup>bc**</sup>	36.6 ± 3.0 <sup>bc</sup>	34.0 ± 2.9 <sup>bc</sup>
<b>ICG 1697</b>	38.4 ± 1.8 <sup>b</sup>	32.4 ± 3.1 <sup>b</sup>	43.7 ± 1.8 <sup>b</sup>	39.8 ± 1.4 <sup>b</sup>
<b>JL 24</b>	49.6 ± 2.7 <sup>a</sup>	43.5 ± 2.3 <sup>a*</sup>	53.5 ± 3.9 <sup>a</sup>	49.5 ± 3.9 <sup>a</sup>

Values (Mean ± SD) with same letter within a column do not differ significantly at  $P \leq 0.05$  (Tukey's HSD test).

<sup>x</sup>Weight gain = weight of the larvae after infestation – weight of larvae before infestation. \* In a row within a trait shows significant difference between the larvae fed on the control and preinfested plants; \*, \*\* show the significant difference at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively.

**Table 4.5.1.2: Enzyme activities of *Helicoverpa armigera* larvae fed on control and preinfested groundnut plants.**

Genotypes	Serine protease (mU min <sup>-1</sup> mg <sup>-1</sup> protein)		Trypsin (μmol min <sup>-1</sup> mg <sup>-1</sup> protein)		GST (μmol CDNB min <sup>-1</sup> mg <sup>-1</sup> protein)		EST (μmol 1-naphthol min <sup>-1</sup> mg <sup>-1</sup> protein)	
	Control	Preinfested	Control	Preinfested	Control	Preinfested	Control	Preinfested
<b>ICGV 86699</b>	1.40 ± 0.05 <sup>bc</sup>	0.63 ± 0.004 <sup>e**</sup>	0.48 ± 0.004 <sup>c</sup>	0.34 ± 0.002 <sup>b*</sup>	30.5 ± 3.1 <sup>ab</sup>	40.4 ± 1.2 <sup>a*</sup>	4.9 ± 0.09 <sup>b</sup>	3.8 ± 0.09 <sup>b*</sup>
<b>ICGV 86031</b>	1.50 ± 0.03 <sup>b</sup>	1.01 ± 0.001 <sup>d**</sup>	0.50 ± 0.002 <sup>bc</sup>	0.34 ± 0.005 <sup>b*</sup>	33.7 ± 2.5 <sup>a</sup>	44.3 ± 2.4 <sup>a*</sup>	4.5 ± 0.05 <sup>b</sup>	4.1 ± 0.04 <sup>ab</sup>
<b>ICG 2271</b>	1.25 ± 0.001 <sup>b</sup>	1.19 ± 0.003 <sup>c</sup>	0.56 ± 0.003 <sup>b</sup>	0.41 ± 0.006 <sup>ab*</sup>	35.3 ± 2.3 <sup>a</sup>	42.7 ± 1.7 <sup>a*</sup>	5.3 ± 0.12 <sup>b</sup>	4.3 ± 0.02 <sup>ab*</sup>
<b>ICG 1697</b>	1.39 ± 0.02 <sup>b</sup>	1.34 ± 0.005 <sup>b</sup>	0.50 ± 0.005 <sup>b</sup>	0.37 ± 0.001 <sup>b*</sup>	32.6 ± 1.4 <sup>a</sup>	39.5 ± 2.0 <sup>a*</sup>	4.9 ± 0.15 <sup>b</sup>	4.0 ± 0.05 <sup>ab</sup>
<b>JL 24</b>	1.76 ± 0.05 <sup>a</sup>	1.71 ± 0.03 <sup>a</sup>	0.65 ± 0.005 <sup>a</sup>	0.49 ± 0.001 <sup>a*</sup>	30.3 ± 2.7 <sup>a</sup>	35.5 ± 3.0 <sup>a</sup>	6.7 ± 0.15 <sup>a</sup>	5.0 ± 0.05 <sup>a</sup>

Values (Mean ± SD) with same letter within a column do not differ significantly at  $P \leq 0.05$  (Tukey's HSD test).

\* In a row within a trait shows significant difference between the larvae fed on the control and preinfested plants; \*, \*\* show the significant difference at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively. CDNB = 1-chloro-2, 4-dinitrobenzene; GST = Glutathione-S-transferase; EST = Esterase.

Control = Plants not infested earlier; Preinfested = plants infested with *H. armigera* larvae.

**Table 4.5.1.3: Protein content (mg mL<sup>-1</sup> tissue) of *Helicoverpa armigera* and *Spodoptera litura* third instar larvae fed on control and preinfested groundnut plants.**

Genotypes	Protein (mg mL <sup>-1</sup> tissue)			
	<i>H. armigera</i>		<i>S. litura</i>	
	Control	Preinfested	Control	Preinfested
<b>ICGV 86699</b>	18.2 ± 0.9 <sup>b</sup>	12.0 ± 1.0 <sup>b*</sup>	19.9 ± 0.9 <sup>b</sup>	12.6 ± 0.9 <sup>c*</sup>
<b>ICGV 86031</b>	17.9 ± 1.0 <sup>b</sup>	12.2 ± 0.8 <sup>b*</sup>	19.6 ± 0.9 <sup>b</sup>	13.4 ± 0.9 <sup>c*</sup>
<b>ICG 2271</b>	18.4 ± 0.9 <sup>b</sup>	13.1 ± 1.0 <sup>b*</sup>	18.5 ± 0.7 <sup>b</sup>	18.0 ± 0.6 <sup>bc</sup>
<b>ICG 1697</b>	18.0 ± 1.3 <sup>b</sup>	16.3 ± 0.9 <sup>b</sup>	19.2 ± 0.8 <sup>b</sup>	18.4 ± 0.7 <sup>b</sup>
<b>JL 24</b>	27.4 ± 1.2 <sup>a</sup>	25.4 ± 1.1 <sup>a</sup>	24.0 ± 1.0 <sup>a</sup>	22.2 ± 1.5 <sup>a</sup>

Values (Mean ± SD) with same letter within a column do not differ significantly at P ≤ 0.05 (Tukey's HSD test).

\* In a row shows significant difference in protein content between the larvae fed on the control and preinfested plants;

\* shows the significant difference at P ≤ 0.05.

**Table 4.5.2.1: Enzyme activities of *Spodoptera litura* larvae fed on control and preinfested groundnut plants.**

Genotypes	Serine protease (mU min <sup>-1</sup> mg <sup>-1</sup> protein)		Trypsin (μmol min <sup>-1</sup> mg <sup>-1</sup> protein)		GST (μmol CDNB min <sup>-1</sup> mg <sup>-1</sup> protein)		EST (μmol 1-naphthol min <sup>-1</sup> mg <sup>-1</sup> protein)	
	Control	Preinfested	Control	Preinfested	Control	Preinfested	Control	Preinfested
<b>ICGV 86699</b>	1.32 ± 0.03 <sup>b</sup>	1.20 ± 0.002 <sup>b*</sup>	0.32 ± 0.002 <sup>c</sup>	0.20 ± 0.001 <sup>c*</sup>	25.8 ± 4.1 <sup>a</sup>	36.4 ± 1.2 <sup>ab*</sup>	3.2 ± 0.02 <sup>bc</sup>	2.0 ± 0.09 <sup>c*</sup>
<b>ICGV 86031</b>	1.24 ± 0.01 <sup>b</sup>	1.13 ± 0.001 <sup>b*</sup>	0.44 ± 0.003 <sup>bc</sup>	0.29 ± 0.003 <sup>b*</sup>	20.13 ± 2.5 <sup>a</sup>	32.3 ± 2.4 <sup>a*</sup>	2.5 ± 0.01 <sup>c</sup>	1.9 ± 0.03 <sup>c</sup>
<b>ICG 2271</b>	1.31 ± 0.005 <sup>b</sup>	1.14 ± 0.001 <sup>b*</sup>	0.22 ± 0.001 <sup>d</sup>	0.18 ± 0.001 <sup>c</sup>	21.5 ± 2.3 <sup>a</sup>	30.7 ± 1.7 <sup>a*</sup>	4.6 ± 0.09 <sup>b</sup>	2.4 ± 0.06 <sup>c**</sup>
<b>ICG 1697</b>	1.26 ± 0.06 <sup>b</sup>	1.17 ± 0.004 <sup>b</sup>	0.43 ± 0.003 <sup>b</sup>	0.33 ± 0.001 <sup>b</sup>	25.0 ± 1.4 <sup>a</sup>	28.4 ± 3.0 <sup>a</sup>	3.7 ± 0.05 <sup>b</sup>	3.1 ± 0.05 <sup>b</sup>
<b>JL 24</b>	1.64 ± 0.09 <sup>a</sup>	1.58 ± 0.06 <sup>a</sup>	0.59 ± 0.005 <sup>a</sup>	0.43 ± 0.003 <sup>a*</sup>	23.3 ± 2.7 <sup>a</sup>	27.5 ± 2.0 <sup>a</sup>	6.3 ± 0.21 <sup>a</sup>	5.8 ± 0.05 <sup>a</sup>

Values (Mean ± SD) with same letter within a column do not differ significantly at P ≤ 0.05 (Tukey's HSD test).

\* In a row within a trait shows significant difference between the larvae fed on the control and preinfested plants;

\*, \*\* show the significant difference at P ≤ 0.05 and P ≤ 0.01, respectively.

**Table 4.7: Eggs laid by *Helicoverpa armigera* on groundnut plants treated with jasmonic and salicylic acids.**

Genotypes	Treatments					
	PJA+HA	PSA+HA	PHI+HA	JA+HA	SA+HA	HA
<b>ICGV 86699</b>	50.4 ± 3.5 <sup>c</sup>	79.9 ± 1.8 <sup>b</sup>	69.7 ± 3.8 <sup>bc</sup>	66.5 ± 2.6 <sup>bc</sup>	94.8 ± 5.7 <sup>b</sup>	103.5 ± 5.4 <sup>b</sup>
<b>ICGV 86031</b>	65.7 ± 2.3 <sup>b</sup>	82.5 ± 2.6 <sup>b</sup>	65.5 ± 3.5 <sup>b</sup>	72.0 ± 4.4 <sup>b</sup>	89.3 ± 4.9 <sup>b</sup>	131.2 ± 6.9 <sup>b</sup>
<b>ICG 2271</b>	69.0 ± 5.9 <sup>b</sup>	85.9 ± 4.3 <sup>b</sup>	73.8 ± 2.7 <sup>b</sup>	79.5 ± 3.4 <sup>b</sup>	92.8 ± 4.5 <sup>b</sup>	137.1 ± 3.4 <sup>b</sup>
<b>ICG 1697</b>	45.4 ± 2.5 <sup>c</sup>	63.4 ± 4.8 <sup>c</sup>	54.3 ± 4.5 <sup>c</sup>	57.7 ± 3.7 <sup>c</sup>	89.5 ± 3.8 <sup>b</sup>	98.8 ± 5.7 <sup>c</sup>
<b>JL 24</b>	111.5 ± 3.3 <sup>a</sup>	144.0 ± 5.4 <sup>a</sup>	119 ± 3.7 <sup>a</sup>	126.9 ± 5.6 <sup>a</sup>	174.0 ± 5.2 <sup>a</sup>	231.6 ± 6.5 <sup>a</sup>

Values (Mean ± SD) carrying same alphabet(s) within a column are not significantly different ( $P \leq 0.05$ ).

Students ‘t’ test was used to compare the data between treatments.

PJA + HA = Pretreatment with JA for one day and an adult pair of *H. armigera* released; PSA+HA = Pretreatment with SA for one day and an adult pair of *H. armigera* released; PHI+HA = Preinfested with *H. armigera* for one day and an adult pair of *H. armigera* released; JA+HA = Jasmonic acid sprayed + an adult pair of *H. armigera* released; SA+HA = Salicylic acid sprayed + an adult pair of *H. armigera* released; HA = An adult pair of *H. armigera* released.



**Table 4.8.1: Average time taken (min) by *Camponotus chlorideae* to choose groundnut plants**

Genotype	Time (min)					
	No choice		Choice		Choice	
	Test genotype	Blank	Resistant genotype	JL 24	Infested	Control
<b>ICGV 86699 (R)</b>	0.5 ± 0.02 <sup>ab</sup>	1.3 ± 0.01 <sup>ab</sup>	1.3 ± 0.02 <sup>ab</sup>	1.3 ± 0.06 <sup>b</sup>	0.5 ± 0.05 <sup>ab</sup>	1.1 ± 0.05 <sup>a</sup>
<b>ICGV 86031 (R)</b>	1.1 ± 0.07 <sup>ab</sup>	1.8 ± 0.04 <sup>a</sup>	1.0 ± 0.03 <sup>a</sup>	1.5 ± 0.602 <sup>b</sup>	0.5 ± 0.03 <sup>a</sup>	1.2 ± 0.01 <sup>a</sup>
<b>ICG 2271 (R)</b>	1.0 ± 0.02 <sup>ab</sup>	1.4 ± 0.09 <sup>ab</sup>	1.3 ± 0.05 <sup>a</sup>	2.0 ± 0.02 <sup>a</sup>	1.0 ± 0.04 <sup>a</sup>	1.2 ± 0.04 <sup>a</sup>
<b>ICG 1697 (R)</b>	1.3 ± 0.04 <sup>a</sup>	1.8 ± 0.03 <sup>a</sup>	1.4 ± 0.7 <sup>a</sup>	1.5 ± 0.04 <sup>ab</sup>	1.3 ± 0.02 <sup>a</sup>	1.4 ± 0.02 <sup>a</sup>
<b>JL 24 (S)</b>	1.5 ± 0.09 <sup>a</sup>	2.2 ± 0.08 <sup>a</sup>	-	-	1.1 ± 0.01 <sup>a</sup>	1.6 ± 0.03 <sup>a</sup>

Values (Mean ± SD) with similar letters within a column do not differ significantly at  $P \leq 0.05$  (Tukey's HSD test).

In no choice, test genotype vs. blank, one arm of olfactometer was filled with groundnut leaves and the other was left blank.

In choice, resistant vs. JL 24, one arm of olfactometer was filled with insect-resistant groundnut genotypes and the other with JL 24.

In choice, infested vs. control, one arm of olfactometer was filled with insect-infested leaves and the other with uninfested control

leaves. \* Shows the significant difference between the treatments at  $P \leq 0.05$ ; R = Resistant; S = Susceptible.

**Table 4.8.2: Average time taken (min) by *Trichogramma chilonis* to choose groundnut plants**

Genotype	Time (min)					
	No choice		Choice		Choice	
	Test genotype	Blank	Resistant genotype	Susceptible (JL 24)	Infested	Control
<b>ICGV 86699 (R)</b>	3.4 ± 0.1 <sup>c</sup>	5.7 ± 0.7 <sup>c*</sup>	5.3 ± 0.7 <sup>b</sup>	6.7 ± 0.5 <sup>b</sup>	6.8 ± 0.5 <sup>c</sup>	8.8 ± 1.3 <sup>b*</sup>
<b>ICGV 86031 (R)</b>	5.7 ± 0.2 <sup>b</sup>	6.2 ± 0.6 <sup>bc</sup>	5.4 ± 0.6 <sup>b</sup>	7.6 ± 0.6 <sup>a*</sup>	8.4 ± 0.5 <sup>ab</sup>	9.0 ± 1.1 <sup>b</sup>
<b>ICG 2271 (R)</b>	7.4 ± 0.9 <sup>ab</sup>	7.7 ± 0.9 <sup>b</sup>	7.6 ± 0.5 <sup>a</sup>	8.3 ± 0.5 <sup>a</sup>	8.1 ± 1.0 <sup>ab</sup>	11.2 ± 1.4 <sup>a*</sup>
<b>ICG 1697 (R)</b>	7.8 ± 0.2 <sup>a</sup>	10.2 ± 1.0 <sup>a*</sup>	5.4 ± 0.3 <sup>b</sup>	5.7 ± 0.4 <sup>c</sup>	9.7 ± 1.2 <sup>a</sup>	12.4 ± 1.5 <sup>a*</sup>
<b>JL 24 (S)</b>	8.6 ± 0.7 <sup>a</sup>	9.7 ± 0.9 <sup>a</sup>	-	-	9.4 ± 1.1 <sup>a</sup>	11.8 ± 1.8 <sup>a*</sup>

Values (Mean ± SD) with similar letters within a column do not differ significantly at  $P \leq 0.05$  (Tukey's HSD test).

In no choice, test genotype vs. blank, one arm of olfactometer was filled with groundnut leaves and the other was left blank.

In choice, resistant vs. JL 24, one arm of olfactometer was filled with insect-resistant groundnut genotypes and the other with JL 24.

In choice, infested vs. control, one arm of olfactometer was filled with insect-infested leaves and the other with uninfested control leaves.

\* Shows the significant difference between the treatments at  $P \leq 0.05$ ; R = Resistant; S = Susceptible.

**Table 4.9.1a: Mortality (%) of *Helicoverpa armigera* larvae fed on flavonoids incorporated diet at 10 DAT.**

Treatment	Concentration (ppm)		
	100	500	1000
Quercetin	17.5 ± 2.5 <sup>a</sup>	20.3 ± 6.4 <sup>ab</sup>	27.5 ± 4.7 <sup>b</sup>
Cinnamic acid	12.5 ± 1.4 <sup>a</sup>	22.0 ± 4.5 <sup>ab</sup>	25.8 ± 4.7 <sup>b</sup>
Caffeic acid	15.0 ± 2.9 <sup>a</sup>	26.0 ± 3.1 <sup>a</sup>	37.2 ± 5.5 <sup>a</sup>
Chlorogenic acid	15.0 ± 4.2 <sup>a</sup>	22.5 ± 2.1 <sup>ab</sup>	42.5 ± 6.2 <sup>b</sup>
Catechin	15.8 ± 4.6 <sup>a</sup>	20.5 ± 4.5 <sup>ab</sup>	25.0 ± 3.5 <sup>b</sup>
Trihydroxyflavone	12.5 ± 2.2 <sup>a</sup>	12.5 ± 3.3 <sup>bc</sup>	15.5 ± 2.7 <sup>c</sup>
Gensitic acid	13.5 ± 2.1 <sup>a</sup>	15.5 ± 2.4 <sup>b</sup>	20.5 ± 3.3 <sup>c</sup>
Ferulic acid	5.5 ± 1.4 <sup>b</sup>	17.4 ± 3.1 <sup>b</sup>	23.3 ± 2.8 <sup>b</sup>
Protocatechuic acid	17.5 ± 1.8 <sup>a</sup>	25.5 ± 2.3 <sup>a</sup>	34.5 ± 3.6 <sup>a</sup>
Umbelliferone	7.5 ± 1.1 <sup>b</sup>	12.5 ± 1.9 <sup>bc</sup>	17.0 ± 2.5 <sup>c</sup>
Control	2.5 ± 0.9 <sup>c</sup>	2.5 ± 0.9 <sup>d</sup>	2.5 ± 0.9 <sup>d</sup>

Values (Mean ± SD) with similar letters within a column do not differ significantly at  $P \leq 0.05$  (Tukey's HSD test)

DAT = Days after treatment.

**Table 4.9.1b: Larval weight (mg per five larvae) of *Helicoverpa armigera* fed on flavonoids incorporated diet.**

Treatment	5 DAT			10 DAT		
	Concentration (ppm)					
	100	500	1000	100	500	1000
<b>Quercetin</b>	133.7 ± 10.5 <sup>b</sup>	72.3 ± 7.7 <sup>bc</sup>	54.5 ± 2.3 <sup>b</sup>	512.1 ± 17.7 <sup>c</sup>	371.4 ± 10.2 <sup>c</sup>	261.5 ± 8.8 <sup>cd</sup>
<b>Cinnamic acid</b>	156.0 ± 13.3 <sup>b</sup>	91.5 ± 6.9 <sup>b</sup>	71.2 ± 2.9 <sup>b</sup>	621.2 ± 9.9 <sup>b</sup>	492.2 ± 9.3 <sup>b</sup>	321.2 ± 7.4 <sup>b</sup>
<b>Caffeic acid</b>	134.8 ± 9.3 <sup>b</sup>	74.8 ± 6.5 <sup>bc</sup>	44.9 ± 1.6 <sup>bc</sup>	483.1 ± 10.4 <sup>c</sup>	250.4 ± 8.0 <sup>d</sup>	129.9 ± 6.8 <sup>cd</sup>
<b>Chlorogenic acid</b>	101.5 ± 12.4 <sup>c</sup>	76.7 ± 4.91 <sup>bc</sup>	30.8 ± 1.8 <sup>c</sup>	451.2 ± 13.2 <sup>cd</sup>	314.3 ± 5.6 <sup>c</sup>	190.4 ± 5.7 <sup>cd</sup>
<b>Catechin</b>	110.7 ± 10.3 <sup>bc</sup>	81.4 ± 5.9 <sup>b</sup>	62.6 ± 2.5 <sup>b</sup>	551.7 ± 10.1 <sup>bc</sup>	402.7 ± 10.5 <sup>b</sup>	242.5 ± 8.9 <sup>c</sup>
<b>Trihydroxyflavone</b>	109.5 ± 9.2 <sup>bc</sup>	89.0 ± 6.3 <sup>b</sup>	34.7 ± 1.2 <sup>c</sup>	470.3 ± 9.8 <sup>c</sup>	382.3 ± 9.9 <sup>c</sup>	223.4 ± 9.1 <sup>c</sup>
<b>Gensitic acid</b>	103.2 ± 8.6 <sup>c</sup>	59.9 ± 2.4 <sup>c</sup>	27.6 ± 1.5 <sup>cd</sup>	412.3 ± 10.3 <sup>d</sup>	295.6 ± 10.1 <sup>cd</sup>	195.7 ± 5.3 <sup>cd</sup>
<b>Ferulic acid</b>	132.9 ± 11.2 <sup>b</sup>	77.5 ± 2.9 <sup>bc</sup>	33.8 ± 1.8 <sup>c</sup>	521.9 ± 11.2 <sup>c</sup>	322.3 ± 11.7 <sup>c</sup>	205.7 ± 7.7 <sup>c</sup>
<b>Protocatechuic acid</b>	135.7 ± 8.3 <sup>b</sup>	73.3 ± 3.2 <sup>bc</sup>	24.4 ± 1.6 <sup>cd</sup>	491.2 ± 8.7 <sup>c</sup>	273.6 ± 10.9 <sup>d</sup>	181.4 ± 5.3 <sup>cd</sup>
<b>Umbelliferone</b>	105.8 ± 9.8 <sup>bc</sup>	111.2 ± 7.3 <sup>b</sup>	55.5 ± 1.5 <sup>b</sup>	432.5 ± 7.3 <sup>cd</sup>	250.5 ± 11.3 <sup>d</sup>	194.5 ± 7.8 <sup>cd</sup>
<b>Control</b>	177.8 ± 12.3 <sup>a</sup>	177.8 ± 12.3 <sup>a</sup>	177.8 ± 12.3 <sup>a</sup>	701.7 ± 12.2 <sup>a</sup>	701.7 ± 12.2 <sup>a</sup>	701.7 ± 12.2 <sup>a</sup>

Values (Mean ± SD) with similar letters within a column do not differ significantly at P ≤ 0.05 (Tukey's HSD test).

**Table 4.9.2a: Mortality (%) of *Spodoptera litura* larvae fed on flavonoids incorporated diet at 10 DAT.**

Treatment	Concentration (ppm)		
	100	500	1000
Quercetin	10.3 ± 2.3 <sup>a</sup>	20.6 ± 2.9 <sup>a</sup>	24.5 ± 3.7 <sup>b</sup>
Cinnamic acid	9.5 ± 0.8 <sup>b</sup>	16.5 ± 3.4 <sup>b</sup>	21.3 ± 2.3 <sup>b</sup>
Caffeic acid	20.5 ± 2.2 <sup>b</sup>	30.0 ± 5.2 <sup>a</sup>	33.1 ± 5.5 <sup>a</sup>
Chlorogenic acid	11.3 ± 1.2 <sup>b</sup>	17.9 ± 2.5 <sup>b</sup>	40.3 ± 6.2 <sup>b</sup>
Catechin	16.6 ± 1.7 <sup>a</sup>	21.9 ± 1.5 <sup>a</sup>	25.4 ± 4.7 <sup>b</sup>
Trihydroxyflavone	5.3 ± 0.5 <sup>bc</sup>	7.4 ± 0.9	10.2 ± 1.3 <sup>c</sup>
Gensitic acid	4.3 ± 0.4 <sup>bc</sup>	7.7 ± 0.4 <sup>c</sup>	12.5 ± 1.5 <sup>c</sup>
Ferulic acid	6.7 ± 0.9 <sup>c</sup>	10.4 ± 1.1 <sup>bc</sup>	17.8 ± 1.5 <sup>bc</sup>
Protocatechuic acid	10.3 ± 1.0 <sup>c</sup>	24.6 ± 3.5 <sup>bc</sup>	37.2 ± 3.6 <sup>ab</sup>
Umbelliferone	3.9 ± 0.5 <sup>c</sup>	6.3 ± 0.9 <sup>c</sup>	10.5 ± 1.3 <sup>c</sup>
Control	2.5 ± 0.5 <sup>d</sup>	2.0 ± 0.5 <sup>d</sup>	2.0 ± 0.5 <sup>d</sup>

Values (Mean ± SD) with similar letters within a column do not differ significantly at  $P \leq 0.05$  (Tukey's HSD test).

DAT = Days after treatment.

**Table 4.9.1b: Larval weight (mg per five larvae) of *Spodoptera litura* fed on flavonoids incorporated diet.**

Treatment	5 DAT			10 DAT		
	Concentration (ppm)					
	100	500	1000	100	500	1000
<b>Quercetin</b>	101.7 ± 5.5 <sup>cd</sup>	97.6 ± 3.4 <sup>b</sup>	69.9 ± 6.73 <sup>b</sup>	470.8 ± 12.7 <sup>bc</sup>	356.6 ± 7.1 <sup>c</sup>	280.8 ± 12.8 <sup>cd</sup>
<b>Cinnamic acid</b>	119.5 ± 10.3 <sup>c</sup>	99.5 ± 2.9 <sup>b</sup>	54.2 ± 5.5 <sup>bc</sup>	465.8 ± 9.9 <sup>bc</sup>	376.6 ± 5.7 <sup>c</sup>	471.2 ± 9.8 <sup>b</sup>
<b>Caffeic acid</b>	111.9 ± 9.7 <sup>bc</sup>	76.6 ± 7.7 <sup>b</sup>	35.8 ± 4.6 <sup>c</sup>	383.6 ± 7.4 <sup>c</sup>	302.6 ± 4.0 <sup>cd</sup>	226.7 ± 11.3 <sup>d</sup>
<b>Chlorogenic acid</b>	110.8 ± 7.0 <sup>c</sup>	69.6 ± 8.3 <sup>c</sup>	37.6 ± 3.4 <sup>c</sup>	451.2 ± 5.7 <sup>bc</sup>	273.8 ± 3.3 <sup>d</sup>	204.8 ± 9.8 <sup>d</sup>
<b>Catechin</b>	124.3 ± 6.6 <sup>bc</sup>	91.4 ± 5.9 <sup>b</sup>	42.4 ± 2.5 <sup>cd</sup>	498.9 ± 4.6 <sup>b</sup>	321.8 ± 4.9 <sup>cd</sup>	279.8 ± 10.7 <sup>cd</sup>
<b>Trihydroxyflavone</b>	132.3 ± 4.6 <sup>b</sup>	67.8 ± 7.9 <sup>c</sup>	47.7 ± 4.7 <sup>b</sup>	401.3 ± 7.2 <sup>c</sup>	375.3 ± 10.7 <sup>c</sup>	321.9 ± 8.5 <sup>c</sup>
<b>Gensitic acid</b>	111.3 ± 8.1 <sup>c</sup>	90.9 ± 6.1 <sup>b</sup>	70.3 ± 4.4 <sup>b</sup>	521.3 ± 6.2 <sup>b</sup>	332.6 ± 6.7 <sup>cd</sup>	286.7 ± 9.9 <sup>cd</sup>
<b>Ferulic acid</b>	123.5 ± 10.7 <sup>bc</sup>	97.5 ± 5.6 <sup>b</sup>	45.4 ± 5.5 <sup>c</sup>	397.8 ± 5.2 <sup>c</sup>	342.4 ± 13.6 <sup>cd</sup>	219.9 ± 10.9 <sup>d</sup>
<b>Protocatechuic acid</b>	141.7 ± 6.6 <sup>b</sup>	59.8 ± 4.2 <sup>c</sup>	34.5 ± 4.2 <sup>cd</sup>	453.8 ± 6.5 <sup>bc</sup>	302.6 ± 6.9 <sup>cd</sup>	231.8 ± 11.7 <sup>d</sup>
<b>Umbelliferone</b>	121.4 ± 9.1 <sup>bc</sup>	97.2 ± 8.2 <sup>b</sup>	51.9 ± 3.4 <sup>bc</sup>	502.7 ± 4.6 <sup>b</sup>	503.7 ± 6.8 <sup>b</sup>	276.8 ± 9.9 <sup>cd</sup>
<b>Control</b>	164.5 ± 10.7 <sup>a</sup>	164.5 ± 10.7 <sup>a</sup>	164.5 ± 10.7 <sup>a</sup>	645.8 ± 9.8 <sup>a</sup>	645.8 ± 9.8 <sup>a</sup>	645.8 ± 9.8 <sup>a</sup>

Values (Mean ± SD) with similar letters within a column do not differ significantly at  $P \leq 0.05$  (Tukey's HSD test).

In control, the values in all the columns have been mentioned to facilitate the comparison with other treatments.

**Table 4.10.1.1: Weight (mg per five larvae) of *Helicoverpa armigera* larvae fed on lectin and phenyl  $\beta$ - glucoside treated diet.**

Treatments	5 DAT			10 DAT		
	Concentration (mg ml <sup>-1</sup> )					
	1.25	2.5	5	1.25	2.5	5
<b>Groundnut leaf lectin</b>	72.4 ± 5.8 <sup>b</sup>	54.4 ± 2.3 <sup>c</sup>	39.8 ± 3.5 <sup>bc</sup>	378.4 ± 8.5 <sup>c</sup>	265.8 ± 9.8 <sup>c</sup>	169.6 ± 5.4 <sup>c</sup>
<b>Concavalin</b>	79.7 ± 7.4 <sup>b</sup>	63.9 ± 4.8 <sup>c</sup>	43.5 ± 2.7 <sup>bc</sup>	403.6 ± 7.8 <sup>c</sup>	332.7 ± 11.1 <sup>b</sup>	276.8 ± 7.9 <sup>b</sup>
<b>Phenyl <math>\beta</math>- glucoside</b>	134.2 ± 6.3 <sup>ab</sup>	106.3 ± 7.52 <sup>b</sup>	67.6 ± 5.5 <sup>b</sup>	478.8 ± 6.1 <sup>b</sup>	368.8 ± 9.7 <sup>b</sup>	309.9 ± 9.6 <sup>b</sup>
<b>Control</b>	166.9 ± 9.7 <sup>a</sup>	166.9 ± 9.7 <sup>a</sup>	166.9 ± 9.7 <sup>a</sup>	678.9 ± 11.8 <sup>a</sup>	678.9 ± 11.8 <sup>a</sup>	678.9 ± 11.8 <sup>a</sup>

Values (Mean ± SD) with similar letters within a column do not differ significantly at  $P \leq 0.05$  (Tukey's HSD test).

In control, the values in all the columns have been mentioned to facilitate the comparison with other treatments.

DAT = Days after treatment.

**Table 4.10.1.2: Total serine protease and trypsin activities of *Helicoverpa armigera* larvae fed on lectin and phenyl  $\beta$ -glucoside treated diet at 10 DAT.**

Treatments	Serine protease (mU min <sup>-1</sup> mg <sup>-1</sup> protein)			Trypsin ( $\mu$ mol min <sup>-1</sup> mg <sup>-1</sup> protein)		
	Concentration ( $\mu$ g mL <sup>-1</sup> )					
	1.25	2.5	5	1.25	2.5	5
Groundnut leaf lectin	1.45 $\pm$ 0.02 <sup>a</sup>	1.17 $\pm$ 0.01 <sup>b</sup>	1.09 $\pm$ 0.02 <sup>b</sup>	0.32 $\pm$ 0.001 <sup>a</sup>	0.17 $\pm$ 0.001 <sup>b</sup>	0.15 $\pm$ 0.001 <sup>b</sup>
Concavalin	1.56 $\pm$ 0.04 <sup>a</sup>	1.27 $\pm$ 0.02 <sup>b</sup>	1.10 $\pm$ 0.04 <sup>b</sup>	0.33 $\pm$ 0.006 <sup>a</sup>	0.25 $\pm$ 0.004 <sup>ab</sup>	0.23 $\pm$ 0.003 <sup>ab</sup>
Phenyl $\beta$ -glucoside	1.68 $\pm$ 0.04 <sup>a</sup>	1.32 $\pm$ 0.09 <sup>b</sup>	1.21 $\pm$ 0.03 <sup>b</sup>	0.29 $\pm$ 0.007 <sup>ab</sup>	0.27 $\pm$ 0.001 <sup>ab</sup>	0.25 $\pm$ 0.000 <sup>ab</sup>
Control	1.63 $\pm$ 0.05 <sup>a</sup>	1.63 $\pm$ 0.05 <sup>a</sup>	1.63 $\pm$ 0.05 <sup>a</sup>	0.35 $\pm$ 0.009 <sup>a</sup>	0.35 $\pm$ 0.009 <sup>a</sup>	0.35 $\pm$ 0.009 <sup>a</sup>

Values (Mean  $\pm$  SD) with similar letters within a column do not differ significantly at  $P \leq 0.05$  (Tukey's HSD test).

In control, the values in all the columns have been mentioned to facilitate the comparison with other treatments.

DAT = Days after treatment.



**Table 4.10.1.3: GST and EST activities of *Helicoverpa armigera* larvae fed on lectin and phenyl  $\beta$ -glucoside treated diet at 10 DAT.**

Treatments	GST activity ( $\mu\text{mol CDNB min}^{-1} \text{mg}^{-1} \text{protein}$ )			EST ( $\mu\text{mol 1-naphthol min}^{-1} \text{mg}^{-1} \text{protein}$ )		
	Concentration ( $\mu\text{g mL}^{-1}$ )					
	1.25	2.5	5	1.25	2.5	5
<b>Groundnut leaf lectin</b>	19.5 $\pm$ 1.5 <sup>ab</sup>	25.0 $\pm$ 2.6 <sup>ab</sup>	24.9 $\pm$ 2.2 <sup>a</sup>	3.7 $\pm$ 0.08 <sup>b</sup>	3.8 $\pm$ 0.07 <sup>b</sup>	2.8 $\pm$ 0.08 <sup>ab</sup>
<b>Concavalin</b>	20.1 $\pm$ 4.1 <sup>a</sup>	23.3 $\pm$ 1.9 <sup>a</sup>	23.6 $\pm$ 2.7 <sup>a</sup>	4.3 $\pm$ 0.07 <sup>b</sup>	3.9 $\pm$ 0.03 <sup>b</sup>	3.2 $\pm$ 0.06 <sup>b</sup>
<b>Phenyl <math>\beta</math>-glucoside</b>	25.5 $\pm$ 2.1 <sup>a</sup>	26.7 $\pm$ 3.0 <sup>a</sup>	24.6 $\pm$ 1.2 <sup>a</sup>	5.0 $\pm$ 0.04 <sup>a</sup>	4.6 $\pm$ 0.09 <sup>ab</sup>	4.3 $\pm$ 0.04 <sup>a</sup>
<b>Control</b>	20.0 $\pm$ 2.3 <sup>a</sup>	20.0 $\pm$ 2.3 <sup>a</sup>	20.0 $\pm$ 2.3 <sup>a</sup>	5.8 $\pm$ 0.02 <sup>a</sup>	5.8 $\pm$ 0.02 <sup>a</sup>	5.8 $\pm$ 0.02 <sup>a</sup>

Values (Mean  $\pm$  SD) carrying same letter(s) within a column are not significantly different at  $P \leq 0.05$  (Tukey's HSD test).

In control, the values in all the columns have been mentioned to facilitate the comparison with other treatments.

DAT = Days after treatment.

**Table 4.10.1.4: Total protein content (mg mL<sup>-1</sup>) of *Helicoverpa armigera* and *Spodoptera litura* larvae fed on lectin and phenyl  $\beta$ -glucoside treated diet at 10 DAT.**

Treatments	<i>H. armigera</i>			<i>S. litura</i>		
	Concentration ( $\mu\text{g mL}^{-1}$ )					
	1.25	2.5	5	1.25	2.5	5
<b>Groundnut leaf lectin</b>	16.8 $\pm$ 0.3 <sup>a</sup>	12.4 $\pm$ 0.4 <sup>b</sup>	8.8 $\pm$ 0.2 <sup>b*</sup>	16.8 $\pm$ 0.2 <sup>a</sup>	13.0 $\pm$ 0.1 <sup>b</sup>	9.8 $\pm$ 0.3 <sup>c*</sup>
<b>Concavalin</b>	18.7 $\pm$ 0.4 <sup>a</sup>	14.7 $\pm$ 0.6 <sup>ab</sup>	12.3 $\pm$ 0.7 <sup>ab</sup>	17.9 $\pm$ 0.5 <sup>a</sup>	14.2 $\pm$ 0.2 <sup>ab</sup>	10.9 $\pm$ 0.2 <sup>bc*</sup>
<b>Phenyl <math>\beta</math>-glucoside</b>	16.9 $\pm$ 0.1 <sup>a</sup>	16.7 $\pm$ 0.6 <sup>a</sup>	13.2 $\pm$ 0.5 <sup>ab</sup>	17.4 $\pm$ 0.3 <sup>a</sup>	16.9 $\pm$ 0.5 <sup>a</sup>	13.7 $\pm$ 0.4 <sup>b</sup>
<b>Control</b>	17.6 $\pm$ 0.9 <sup>a</sup>	17.6 $\pm$ 0.9 <sup>a</sup>	17.6 $\pm$ 0.9 <sup>a</sup>	19.6 $\pm$ 0.7 <sup>a</sup>	19.6 $\pm$ 0.7 <sup>a</sup>	19.6 $\pm$ 0.7 <sup>a</sup>

Values (Mean  $\pm$  SD) carrying same letter(s) within a column are not significantly different at  $P \leq 0.05$  (Tukey's HSD test).

In control, the values in all the columns have been mentioned to facilitate the comparison with other treatments.

DAT = Days after treatment.

**Table 4.10.2.1: Weight (mg per five larvae) of the *Spodoptera litura* larvae fed on lectin and phenyl  $\beta$ - glucoside treated diet.**

Treatments	5 DAT			10 DAT		
	Concentration ( $\mu\text{g ml}^{-1}$ )					
	1.25	2.5	5	1.25	2.5	5
<b>Groundnut leaf lectin</b>	$87.7 \pm 7.7^b$	$63.9 \pm 4.8^{bc}$	$47.8 \pm 2.9^c$	$343.9 \pm 7.9^c$	$302.4 \pm 10.4^c$	$206.8 \pm 6.8^d$
<b>Concavalin</b>	$103.3 \pm 5.4^b$	$82.4 \pm 6.3^c$	$59.8 \pm 4.1^{bc}$	$378.4 \pm 8.2^{bc}$	$329.7 \pm 9.1^c$	$269.6 \pm 9.7^c$
<b>Phenyl <math>\beta</math>- glucoside</b>	$142.7 \pm 7.1^{ab}$	$89.5 \pm 4.8^b$	$73.3 \pm 4.5^b$	$421.6 \pm 4.9^b$	$377.6 \pm 6.8^b$	$323.9 \pm 10.9^b$
<b>Control</b>	$171.7 \pm 6.9^a$	$171.7 \pm 6.9^a$	$171.7 \pm 6.9^a$	$560.9 \pm 9.6^a$	$560.9 \pm 9.6^a$	$560.9 \pm 9.6^a$

Values (Mean  $\pm$  SD) with similar letters within a column do not differ significantly at  $P \leq 0.05$  (Tukey's HSD test).

In control, the values in all the columns have been mentioned to facilitate the comparison with other treatments.

DAT = Days after treatment.

**Table 4.10.2.2: Total serine protease and trypsin activities of *Spodoptera litura* larvae fed on lectin and phenyl  $\beta$ -glucoside treated diet at 10 DAT**

Treatments	Serine protease (mU min <sup>-1</sup> mg <sup>-1</sup> protein)			Trypsin ( $\mu$ mol min <sup>-1</sup> mg <sup>-1</sup> protein)		
	Concentration ( $\mu$ g mL <sup>-1</sup> )					
	1.25	2.5	5	1.25	2.5	5
<b>Groundnut leaf lectin</b>	1.32 $\pm$ 0.02 <sup>b</sup>	1.25 $\pm$ 0.05 <sup>bc</sup>	1.18 $\pm$ 0.03 <sup>c</sup>	0.25 $\pm$ 0.003 <sup>a</sup>	0.19 $\pm$ 0.003 <sup>b</sup>	0.17 $\pm$ 0.004 <sup>b</sup>
<b>Concavalin</b>	1.34 $\pm$ 0.03 <sup>b</sup>	1.32 $\pm$ 0.02 <sup>b</sup>	1.22 $\pm$ 0.05 <sup>bc</sup>	0.27 $\pm$ 0.005 <sup>a</sup>	0.22 $\pm$ 0.002 <sup>ab</sup>	0.19 $\pm$ 0.001 <sup>b</sup>
<b>Phenyl <math>\beta</math>-glucoside</b>	1.47 $\pm$ 0.01 <sup>ab</sup>	1.35 $\pm$ 0.09 <sup>b</sup>	1.30 $\pm$ 0.03 <sup>b</sup>	0.26 $\pm$ 0.007 <sup>a</sup>	0.23 $\pm$ 0.002 <sup>ab</sup>	0.20 $\pm$ 0.002 <sup>b</sup>
<b>Control</b>	1.56 $\pm$ 0.07 <sup>a</sup>	1.56 $\pm$ 0.07 <sup>a</sup>	1.56 $\pm$ 0.07 <sup>a</sup>	0.30 $\pm$ 0.008 <sup>a</sup>	0.30 $\pm$ 0.008 <sup>a</sup>	0.30 $\pm$ 0.008 <sup>a</sup>

Values (Mean  $\pm$  SD) with similar letters within a column do not differ significantly at  $P \leq 0.05$  (Tukey's HSD test).

In control, the values in all the columns have been mentioned to facilitate the comparison with other treatments.

DAT = Days after treatment.

**Table 4.10.2.3: GST and EST activities of *Spodoptera litura* larvae fed on lectin and phenyl  $\beta$ -glucoside treated diet at 10 DAT.**

Treatments	GST activity ( $\mu\text{mol CDNB min}^{-1} \text{mg}^{-1} \text{protein}$ )			EST ( $\mu\text{mol 1-naphthol min}^{-1} \text{mg}^{-1} \text{protein}$ )		
	Concentration ( $\mu\text{g mL}^{-1}$ )					
	1.25	2.5	5	1.25	2.5	5
<b>Groundnut leaf lectin</b>	14.8 $\pm$ 1.3 <sup>a</sup>	17.5 $\pm$ 1.4 <sup>a</sup>	21.2 $\pm$ 1.2 <sup>ab</sup>	3.8 $\pm$ 0.02 <sup>b</sup>	3.0 $\pm$ 0.07 <sup>b</sup>	2.3 $\pm$ 0.03 <sup>c</sup>
<b>Concavalin</b>	15.7 $\pm$ 1.4 <sup>a</sup>	16.8 $\pm$ 1.6 <sup>a</sup>	17.4 $\pm$ 1.7 <sup>a</sup>	3.9 $\pm$ 0.03 <sup>b</sup>	3.2 $\pm$ 0.02 <sup>b</sup>	2.8 $\pm$ 0.05 <sup>bc</sup>
<b>Phenyl <math>\beta</math>-glucoside</b>	16.9 $\pm$ 1.1 <sup>a</sup>	17.0 $\pm$ 1.6 <sup>a</sup>	17.6 $\pm$ 1.5 <sup>a</sup>	4.5 $\pm$ 0.06 <sup>b</sup>	3.9 $\pm$ 0.04 <sup>b</sup>	3.8 $\pm$ 0.07 <sup>b</sup>
<b>Control</b>	14.9 $\pm$ 1.9 <sup>a</sup>	14.9 $\pm$ 1.9 <sup>a</sup>	14.9 $\pm$ 1.9 <sup>a</sup>	6.4 $\pm$ 0.03 <sup>a</sup>	6.4 $\pm$ 0.03 <sup>a</sup>	6.4 $\pm$ 0.03 <sup>a</sup>

Values (Mean  $\pm$  SD) with similar letters within a column do not differ significantly at  $P \leq 0.05$  (Tukey's HSD test).

In control, the values in all the columns have been mentioned to facilitate the comparison with other treatments.

DAT = Days after treatment.

*Chapter 5*

*Discussion*

## DISCUSSION

Insects are one of the major constraints in crop production and often cause heavy economic loss to plants. Host plant resistance, natural enemies, cropping practices and pesticides are the important strategies adopted to prevent pest infestation. Amongst these, host plant resistance is the simplest, most economic and eco-friendly method of controlling insect pests (Sharma and Ortiz 2002). Host plant resistance to pests is the result of co-evolution between plants and insects for millions of years. Plant defense against herbivory can be constitutive or induced. The constitutive resistance is always expressed in the plants irrespective of the external stimuli, whereas induced resistance is activated in response to the damage/wounding by the insect pests and/or by the elicitor application. The host plant resistance against herbivores is initially constitutive. Constitutive and induced resistances are utilized by the plants against insects either individually or in combination with each other. Plant traits interfering with host plant selection, feeding and oviposition by the insects are the potent factors contributing to plant resistance. Most of the surface components are involved in constitutive resistance. These include thorns, spines, hairs, sclerophylly and surface wax (Dwivedi et al. 1986; Baur et al. 1991; Sharma et al. 2009; Chamarthi et al. 2010; He et al. 2011). However, trichome density at times may be influenced by insect attack and/or elicitor application, and thus, may constitute an inducible trait.

Feeding by insect pests result in the production of an array of plant defensive compounds in host plants in order to avoid further feeding by herbivorous insects and the subsequent infestation. Insect herbivory increases the amounts of glucosinolates (Mewis et al. 2006), proteinase inhibitors (PIs; Green and Ryan 1972), trichomes (Baur et al. 1991; Dalin and Bjorkman 2003), phenols (Walling 2000; Arnold et al. 2004; Usha Rani and Jyothsna 2010), H<sub>2</sub>O<sub>2</sub> (Orozco-Cardenas et al. 2001; Foreman et al. 2003; Maffei et al.

2007), and plant volatiles (Karban et al. 2006; Kost and Heil 2006; Arimura et al. 2009). Deterrence of insects by the plant volatiles in the absence of insect damage is very effective and a potent form of constitutive resistance.

In the present study different groundnut genotypes showed different levels of damage by insect pests under field conditions. Leaf damage due to *H. armigera* and *S. litura* was relatively low in ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 compared to JL 24. However, ICGV 86699 showed the lowest damage among all the tested genotypes. Similar trend was observed for leafhopper damage. These results confirm the earlier reports of Sharma et al. (2003), who reported the diversity of resistance in various groundnut genotypes against insect pests. The genotypes such as ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697 exhibited moderate levels of resistance against insect pests. The distinct resistant levels of the tested genotypes were further confirmed by differential levels of defensive enzymes and secondary metabolites. The enzymes such as POD, PPO and PAL showed greater activities in ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697 than that of JL 24. However, SOD, LOX, APX and CAT activities were significantly higher in ICGV 86699 and ICGV 86031 than rest of the genotypes. Phenols, condensed tannins, H<sub>2</sub>O<sub>2</sub>, and total proteins were also significantly higher in insect resistant genotypes than JL 24. Insect-resistant genotypes have been reported to possess higher levels of antioxidative enzymes and secondary metabolites, and responded strongly to different stresses (Heng-Moss et al. 2004; Chen et al. 2009; Rangasamy et al. 2009; Gulsen et al. 2010). The differential levels of resistance in groundnut genotypes might be due to the differential activities of enzymes such as POD, PPO, PAL, LOX, SOD, CAT and APX, and total amounts of phenols, tannins, H<sub>2</sub>O<sub>2</sub> and proteins. These are important biochemical markers that allow plants to withstand various biotic and abiotic stresses (Bi et al. 1997; Chaman et al. 2001; Apel and Hirt 2004; Sankar et al. 2007; Idrees et al. 2011).



Consumption, digestion and utilization are the important parameters to measure the antibiosis and antixenosis mechanism of defense in plants against insect pests. Insect-plant interaction is important for successful colonization and survival of the insect herbivores. Insects mostly scout for healthy plants that can serve as a source of food, site for oviposition, and also provide food for the offsprings. Any imbalance in food obtained by insects will have drastic effects on their growth and development. Among the nutrients, plant nitrogen content is regarded as the most important limiting factor for herbivores (Zhong-xian et al. 2007). Nutrient availability for insect growth and development over a period of time depends on the amount of food available and the efficiency of conversion of ingested food into body matter. In the present study, we observed a reduction in approximate digestibility (AD), consumption index (CI), efficiency of conversion of ingested food (ECI), and efficiency of conversion of digested food (ECD) in *H. armigera* and *S. litura* larvae fed on insect-resistant groundnut genotypes. In general, larvae fed on insect-resistant genotypes showed considerably lower AD, CI, ECI and ECD as compared to those fed on JL 24. This might be because of the constitutive resistance by secondary metabolites such as phenols, flavonoids, tannins and defensive proteins (Grayer et al. 1992; Stevenson et al. 1993; Senguttuvan and Sujatha 2000; Rao 2003). Although the consumption index of *H. armigera* was more in ICG 1697, ICG 2271 and ICGV 86031, the ECI and ECD were significantly less. This clearly showed the strong antibiosis mechanism of these genotypes. Once ingested, plant allelochemicals affect the postingestive nutrient utilization through physiological and biochemical mechanisms (Sharma and Norris 1991; Hasan and Ansari 2011; Ansari et al. 2011). The insect also tries to excrete the toxic chemicals, resulting in reduced efficiency of food utilization. Antibiosis has been suggested as a potent mechanism of host plant resistance against insects, and affects survival, growth, and fecundity of the target pests (Sharma and Norris 1991; Sharma et al. 2005; Sujana et al.

2008; Ansari et al. 2011). Differential responses of insects in terms of efficiency of digestion and conversion of food into body matter can be used for assessing the nature of host plant resistance to insect pests (Sharma and Norris 1991; Sharma and Franzman 2000). The information derived from studies on consumption and utilization of food is also useful to understand adaptation of insect pests to various genotypes/host plants and the co-evolution between the insect pests and their host plants.

Plants respond differentially to insects with different modes of feeding, and hence, we studied the response of groundnut genotypes to feeding by two chewing insects (*H. armigera*, *S. litura*) and a sucking type (*A. craccivora*) of insect pest under greenhouse conditions. Insect damage activates several oxidative enzymes including POD, PPO, LOX, SOD, PAL, and CAT in plants (Felton et al. 1994a,b; Zhao et al. 2009; He et al. 2011). Damage to groundnut plants by *H. armigera*, *S. litura* and *A. craccivora* resulted in greater induction of defensive enzymes such as POD, PPO, PAL, CAT, SOD, APX, and LOX. However, the level of induction varied between the insects and across the genotypes. There were no significant differences in the activities of POD, PAL and CAT in groundnut genotypes infested by *H. armigera*, *S. litura* and *A. craccivora*, except in ICGV 86699 and ICGV 86031, where *H. armigera* and *S. litura* infested plants exhibited greater POD and PPO activities, respectively, than the *A. craccivora* infested plants. In the susceptible check, JL 24, the *H. armigera*, *S. litura* and *A. craccivora* infested plants showed greater induction of POD and PPO, but levels of induced response was lower than the other genotypes tested. The role of POD in production of semiquinone free radicals and the subsequent formation of quinines have been attributed to their direct post ingestive toxicity against insects (Zhu-Salzman et al. 2008; Barbehenn et al. 2010). In addition, it also mediates the oxidation of hydroxycinnamyl alcohols into free radical intermediates, oxidation of phenols, cross-linking of polysaccharides and monomers, lignification, and suberization (Zhang et al.

2008; Chen et al. 2009), and the production of anti-nutritive compounds (Gulsen et al. 2010; He et al. 2011). The PPO is an antinutritional enzyme involved in plant defense as it reduces the food quality of plant tissues (Mahanil et al. 2008; Bhonwong et al. 2009). PPO also mediates the oxidation of phenols to highly reactive and toxic quinines that interact with the nucleophilic side chain of amino acids, leading to cross-linking of proteins, and thereby, reducing their availability to insect pests (Zhang et al. 2008; Bhonwong et al. 2009). In addition to their role in reducing digestibility and palatability of plant tissues, melanin formation by PPOs increases the cell wall resistance to insects and infection by the pathogens (Zhao et al. 2009).

The PAL activity increased in plants infested with *H. armigera*, *S. litura* and *A. craccivora*; however, the level of induction varied across the genotypes and the insect species. Constitutive levels of PAL activity of insect-resistant genotypes were significantly higher than that of JL 24. The *de novo* synthesis and increased activity of PAL is an initial plant defensive response to insect damage (Campos-Vargas and Saltveit 2002), and leads to the accumulation of phenolic compounds in plants that are sequestered in cell vacuole (Zhao et al. 2009), which form toxic compounds upon oxidation (Bhonwong et al. 2009). It is an important and primary enzyme of the phenylpropanoid pathway that leads to the production of many toxic secondary metabolites involved in plant defense. A number of reports have suggested the induction of PAL activity in plants infested with insects (Zhang et al. 2008; Zhao et al. 2009; Chen et al. 2009). Furthermore, a negative correlation has been observed between PAL activity and growth and development of insect pests (Sethi et al. 2009).

The LOX activity increased significantly in all the treatments and in all the genotypes, and there were no significant differences in LOX activity between the plants infested with different insect species, and across genotypes. Overall, the insect-resistant genotypes exhibited greater induction of LOX activity than the susceptible check, JL 24.

Greater induction of plant defensive enzymes in groundnut plants in response to *H. armigera* and *S. litura* infestation could be attributed to the extensive tissue damage caused by the chewing insects and strong response of the host plant to it. The LOX catalyzes hydroperoxidation of polyunsaturated fatty acids resulting in the formation of fatty acid hydroperoxides, which then form highly reactive aldehydes,  $\gamma$ -ketols, epoxides (Bruinsma et al. 2009). These interact with proteins, and form protein-protein cross linking (Maffei et al. 2007). *N. attenuata* plants deficient in LOX have been found to be susceptible to *M. sexta* (Rayapuram and Baldwin 2007). Induction of LOX in plants after insect infestation has been well documented (Bi et al. 1997; Fidantsef et al. 1999; Tschardt et al. 2001; Voelckel et al. 2004). Furthermore, higher LOX activity has been reported to cause midgut toxicity in *H. zea* and the epithelial cells are damaged that leads to the reduced growth and development of the larvae (Felton et al. 1994b).

In general, greater SOD activity was observed in insect infested plants than the uninfested control plants across the genotypes. However, ICGV 86699 and ICG 2271 showed significantly greater SOD activity in *H. armigera* and *S. litura* infested plants than the *A. craccivora* infested and the uninfested control plants. The increase in SOD activity of plants infested with *H. armigera* and *S. litura* might be due to the production of more free radicals by the large tissue damage and the subsequent scavenging of these radicals. SOD is a potent antioxidative enzyme that plays an important role in plant defense against many biotic and abiotic stresses (Khattab and Khattab 2005; Sankar et al. 2007; Usha Rani and Jyothsna 2010). It catalyzes the dismutation of superoxide into oxygen and  $H_2O_2$  (Raychaudhuri and Deng 2000). Apel and Hirt (2004) observed that SOD levels were induced within 6 hours of aphid infestation in lima bean.

Overall, the insect resistant genotypes exhibited greater CAT activity in *H. armigera*, *S. litura* and *A. craccivora* infested plants than the uninfested control plants. CAT

is an important component of the oxygen-scavenging systems. It scavenges the toxic and unstable ROS and converts them into less toxic and more stable components such as H<sub>2</sub>O<sub>2</sub> and water (Khattab and Khattab 2005). Higher induction of CAT in *H. armigera* infested plants than that of *A. craccivora* could be attributed to the high stress because of tissue damage caused by chewing insects that leads to the formation of free radicals. Increased CAT activity in plants increases cell wall resistance, and also mediates signaling for the induction of defensive genes (Chen et al. 1993). An upregulation of genes for CAT has been found in several plants (Khattab and Khattab 2005; Boyko et al. 2006; Divol et al. 2007). In contrast, Heng-Moss et al. (2004) and Rangasamy et al. (2009) did not find any alteration in CAT activity in plants infested with insects.

Greater APX activity was observed in *H. armigera* and *S. litura* infested plants than *A. craccivora* infested plants, and the uninfested control plants, except in ICG 2271 and JL 24. Higher levels of APX reduce the ascorbate content in plant tissues, thus limiting the availability of ascorbic acid in them and thereby decreasing the insect growth and development (Barbehenn et al. 2005). Furthermore, non-availability of ascorbate in insect midgut increases the oxidative stress and leads to the generation of highly unstable free radicals, including semiquinone, peroxides, and hydroxyl radicals and the toxicity on the gut lining (Barbehenn et al. 2005). In addition, APX also reduces excessive H<sub>2</sub>O<sub>2</sub> to water, and oxidizes phenolic compounds to quinines, which inhibit insect feeding (Felton et al. 1994a,b; Barbehenn et al. 2005).

Amounts of total phenols and condensed tannins were greater in *H. armigera* and *S. litura* infested plants than those infested by *A. craccivora* in different genotypes. However, increase was stronger in insect-resistant genotypes than in the susceptible check, JL 24. Phenolic compounds induced in plants are either directly toxic to insects (Walling 2000; Bhonwong et al. 2009) or mediate the signaling of various transduction pathways, which in

turn, produce toxic secondary metabolites and activate the defensive enzymes (Walling 2000; Maffei et al. 2007; Bhonwong et al. 2009). Tannins have been reported to reduce the growth and survivorship in many insect pests (Grayer et al. 1992; Bernards and Bastrup-Spohr 2008; Sharma et al. 2009). Sharma et al. (2009) reported higher quantity of polyphenols and condensed tannins in *H. armigera* resistant genotypes of pigeonpea. Higher tannin levels have been suggested to confer resistance in groundnut against *A. craccivora* (Grayer et al. 1992). However, there are some reports where no induction of tannins was recorded in plants in response to insect attack (Keinanen et al. 1999; Hikosaka et al. 2005).

Greater amounts of H<sub>2</sub>O<sub>2</sub> were recorded in insect infested plants, and insect-resistant genotypes responded more strongly than the susceptible check, JL 24. H<sub>2</sub>O<sub>2</sub> acts as a toxicant to the insects or as a secondary messenger, whereby it serves as an important component of intra- and intercellular signal transduction pathways, which in turn result in the production of various defensive proteins (Walling 2000; Orozco-Cardenas et al. 2001; Maffei et al. 2007; Howe and Jander 2008; Torres 2010).

The *H. armigera* and *S. litura* infestation showed greater induction in MDA content than *A. craccivora*. Higher accumulation of MDA was observed in JL 24 than in other genotypes tested. This might be due to the greater insect damage in this genotype. An important lipid oxidation product, MDA, is involved in signaling the plant defense against variety of stresses (Huang et al. 2007). Lipid peroxidation also stimulates green leaf volatile emission in plants in response to herbivory that attract the natural enemies of the herbivores (Arimura et al. 2009). Induction of MDA in plants infested with insect pests has been reported in many plants (Huang et al. 2007; Boka et al. 2007).

Insects with different modes of action showed differential induction of PIs. The present study revealed the chewing insects, *H. armigera* and *S. litura* induced greater PI

activity in groundnut plants than the sucking insect, *A. craccivora*. This might be due to the strong induction of defensive responses by chewing insects, because of more damage to the plant tissues. PIs inhibit digestion of proteins, and thus, deprive the insects of basic metabolites such as amino acids, nitrogen and other constituents, and thereby, affecting their growth and development. A considerable number of studies have shown the induction of PIs in plants in response to insect herbivory, and the counter effects on insect pests (Ritonja et al. 1990; Tschardt et al. 2001; Miranda et al. 2007; Zhu-Salzman et al. 2008).

The present findings revealed increase in protein content in groundnut plants infested with *H. armigera*, *S. litura* and *A. craccivora*. Increase in protein concentration due to insect infestation might be partly because of the increased activities of antioxidative enzymes after herbivory. Protein based compounds mediate a wide ranging defense responses in plants. Greater production of defensive protein based compounds following insect infestation is one the important strategies of host plant defense against herbivory (Ni et al. 2001; Chen et al. 2009). The observed differences in protein content in *H. armigera*, *S. litura* and *A. craccivora* infested plants might be due to the differential stress experienced by plants due to damage by insects with different modes of feeding. A significant elevation in protein content has been reported in plants after insect infestation (Ni et al. 2001; Zhao et al. 2009).

Genotypes with insect resistance affect growth and development of herbivores (Sharma et al. 2003). Insect-resistant genotypes suffered lower leaf damage by *H. armigera* and *S. litura*. The *H. armigera* and *S. litura* larvae fed on resistant genotypes exhibited lower larval survival and weights than those fed on the susceptible check, JL 24. Rate of increase of *A. craccivora* population was also significantly lower on the insect-resistant genotypes than that on the susceptible check, JL 24. Reduced plant damage and high larval mortality on insect-resistant genotypes could be due to the increased enzyme activities

(Mahanil et al. 2008; Bhonwong et al. 2009; Usha Rani and Jyothisna 2010; Gulsen et al. 2010; He et al. 2011), and greater amounts of secondary metabolites (Sharma et al. 2009; Bhonwong et al. 2009; Chen et al. 2009; Usha Rani and Jyothisna 2010) either constitutively present or induced by herbivory.

The HPLC fingerprinting showed the presence or absence of peaks in *H. armigera* and *A. craccivora* infested and uninfested groundnut genotypes. More numbers of peaks were observed in insect infested plants, especially in the insect-resistant genotypes (ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697) than in the susceptible check, JL 24. Peak areas also differed across treatments and the genotypes (data not shown). The most common compounds observed in insect-resistant genotypes were chlorogenic, syringic, quercetin and ferulic acids. ICGV 86699 plants infested with *H. armigera* showed larger peaks corresponding to chlorogenic acid, syringic acid, ferulic acid, ginsitin, umbelliferone and quercetin. Infestation by *A. craccivora* also induced the production of more number of phenolic compounds. The results showed that depending on the mode of feeding, flavonoids are induced differentially. The toxicity of these compounds has been studied in detail. Chlorogenic acid is considered as an important component of host plant resistance to insects in groundnut (Mallikarjuna et al. 2004). The toxicity of chlorogenic acid against insect pests is ascribed to the production of the highly reactive chlorogenoquinone that reacts with nucleophilic –SH and –NH<sub>2</sub> groups in proteins, and thus, reducing their availability to insect pests (Felton et al. 1992). Chlorogenic acid plays important role in constitutive defense; however, it also gets induced in response to insect or pathogen attack (Felton et al. 1992; Mallikarjuna et al. 2004; Erb et al. 2009). Furthermore, differences in the number of peaks in control plants in different genotypes showed the variation of constitutive levels of resistance among these genotypes. Sharma and Norris (1991) observed the negative effect of flavonoids from soybean on *T. ni*.



The native PAGE showed differences in isozymes of POD and PPO in *A. craccivora* and *H. armigera* infested groundnut plants. The bands were dense in resistant genotypes as compared to the susceptible check, JL 24. This confirmed the differential induction of activity of these enzymes in response to damage by insects with different modes of feeding.

Phytohormones play an active role in plant defense against various biotic and abiotic stresses. Although various phytohormones are involved in host plant defense against various stresses, JA and SA are very important in modulating plant defense against insect herbivory. The JA and SA mediated induced resistance operates through octadecanoid pathway and phenylpropanoid pathways, respectively, that leads to the production of JA and SA, and the secondary metabolites and plant volatiles (Cipollini et al. 2004; Stout et al. 2009; Shivaji et al. 2010; Scott et al. 2010). JA also regulates the activity of CDPKs, which are involved in plant defense against a variety of biotic and abiotic stresses through signal transduction (Ludwig et al. 2004). Increase in the level of host plant resistance against herbivores has been observed through exogenous application of JA or MeJA (Farmer and Ryan 1990; Steppuhn and Baldwin 2007; Shivaji et al. 2010). JA induced on account of insect damage activates the expression of various plant defensive proteins including PIs, which reduce insect growth and development (Howe et al. 1996; Parra-Lobato et al. 2009; Scott et al. 2010).

Increase in POD activity is regarded as the initial response of plants to the insect attack (Moloi and van der Westhuizen 2006; He et al. 2011). Our results revealed that pretreatment with JA and SA, followed by infestation with *H. armigera* and *S. litura* resulted in greater POD activity in groundnut. However, a strong response was observed in plants pretreated with JA and infested with *H. armigera* (PJA+HIN) and *S. litura* (PJA+SIN) than those pretreated with SA and infested with *H. armigera* (PSA+HIN) and *S. litura* (PSA+SIN), respectively. This might be due to the greater accumulation of JA after

insect infestation and exogenous application of JA, and the subsequent activation of plant defensive pathways, which resulted in the production of defensive enzymes including POD. Induction of POD activity in response to JA and SA application and/or insect attack enhances the cell lignifications, wound healing, and the production of secondary metabolites, besides detoxifying the peroxides, and thus, defending the plants against insects, pathogens and other stresses (Thaler et al. 1996; Cipollini and Redman 1999; Heng-Moss et al. 2004; Han et al. 2009; Gulsen et al. 2010). Similar response was observed in the present studies. Production of phenoxy and other oxidative radicals by PODs in association with phenols directly deters the feeding by insects and/or produces toxins that reduce the plant digestibility, resulting in nutrient deficiency in insects, and drastic effects on their growth and development (Zhang et al. 2008; Zhu-Salzman et al. 2008; Chen et al. 2009; Barbehenn et al. 2010). Rangasamy et al. (2009) recorded two fold increases in POD activity in chinch bug resistant *S. secundatum* at 5 and 8 days after infestation as compared to uninfested control plants. Wheat resistance to *D. noxia* has been reported to be positively correlated to POD activity, since greater induction of POD was observed in *D. noxia* infested resistant Halt than susceptible Arapahoe (Ni et al. 2001). Resistant cultivars have been reported to respond to defensive elicitors strongly and showed greater elevation in plant defensive enzymes including POD as compared to the susceptible genotypes (Heng-Moss et al. 2004; Gulsen et al. 2010). High levels of POD in insect resistant genotypes can reduce the plant tissue damage by detoxifying the peroxides than in the susceptible genotypes (Hildebrand et al. 1986).

The PPO activity was elevated in plants on treatment with JA and SA. However, plants pretreated with JA and infested with insects exhibited greater PPO activity than pretreated with SA, and the plants simultaneously sprayed with JA and SA, and infested with *H. armigera* and *S. litura* across the genotypes. The insect resistant genotypes showed

greater response to JA pretreatment. This might be due to the faster induction of plant defensive pathways that resulted in the higher levels of PPO activity after insect infestation in JA pretreated plants. PPO is an important component of plant defense against insect herbivory, because it reduces the nutritional quality of plant tissues rendering them less digestible and/or unpalatable to herbivores by catalyzing the oxidation of phenols leading to the production of toxic quinines (Zhao et al. 2009; Gould et al. 2009). These highly reactive and toxic quinines interact with nucleophilic side chain of amino acids cross-link the proteins in plant tissues, which lead to reduction in their digestibility (Felton et al. 1992; Zhang et al. 2008; Mahanil et al. 2008; Bhonwong et al. 2009).

PAL activity is induced by various biotic and abiotic stresses including wounding, insect herbivory, and pathogen infection (Hahlbrock and Scheel 1989; Dixon and Paiva 1995; Hu et al. 2009). Our results showed that PAL activity of groundnut plants was greater when treated with JA and SA than the insect-infested and uninfested control plants. In *H. armigera* infested plants, there were no significant differences in PJA + HIN, PJA + SIN and JA + HIN treated plants. However, PJA + SIN plants showed greater activity than PSA + SIN and JA + SIN treated plants. This could be due to the differences in the activation of phenylpropanoid pathways in response to damage by *S. litura* and *H. armigera*. The increased PAL activity also leads to the accumulation of phenolic compounds in the plants, which on oxidation produce various defensive compounds (Zhao et al. 2009). Johnson and Felton (2001) showed that over-expression of PAL in *N. tabacum* is associated with reduced digestibility of leaves by the larvae of *H. virescens*. PAL mediates the expression of lignin synthesis through phenylpropanoid pathway (Ritter and Schulz 2004). Induction of PAL by herbivory has also been recorded at the transcript, protein and enzyme levels (Bi and Felton 1995; Bernardis and Bastrup-Spohr 2008; Zhao et al. 2009; Kiselev et al. 2010).

Lipoxygenase (LOXs) constitutes a large family of plant defensive enzymes. LOX catalyzes the JA production from linolenic acid in octadecanoid pathway that induces the expression of various defensive genes, which in turn signals various transduction pathways (Farmer and Ryan 1990). It has been well established that LOX is a key enzyme in JA synthesis from linolenic acid, and elicits a wide range of plant defense responses (Felton et al. 1994b; Blee 1998; Fidantsef et al. 1999; Feussner and Wasternack 2002; Mao et al. 2007). It also elicits the production of various plant defensive secondary metabolites and plant volatiles. Aphid feeding has been found to induce the LOX transcripts by 1.52-fold in *Arabidopsis* (Moran and Thompson 2001). The present study revealed that plants pretreated with JA and infested with *H. armigera* and *S. litura* (PJA + HIN and PJA + SIN, respectively), and the plants treated with JA + HIN and JA + SIN showed significantly greater levels of LOX activity. This increase in LOX in JA treated plants might be due to the signaling of octadecanoid pathway by exogenous application of JA. It has been proposed that increase in LOX activity in plants in response to insect attack and/or application of elicitors activates the JA-signaling pathway, which leads to the synthesis of JA, and JA in turn mediates the transcription of multiple defense genes (Zhao et al. 2009). LOX also activates the oxidation of fatty acids producing oxylipins (acyclic or cyclic compounds). These oxylipins are active compounds in plant cells, and play a wide array of functions in plant growth and development, senescence, and defense against biotic and abiotic stresses including insect herbivory (Felton et al. 1994b; Feussner and Wasternack 2002; Porta and Rocha-Sosa 2002; Kessler et al. 2004; Bruinsma et al. 2009). Compounds formed from the LOX mediated reactions are either directly deterrent to insect pests and or produce post-ingestive toxicity in insects (Ongena et al. 2004; Mao et al. 2007). Increased LOX activity has been correlated to the oxidative damage in midgut epithelial cells of *H. zea* larvae, which resulted in reduced growth and development of the larvae (Felton et al.

1994b). Lipoxygenase gene expression is regulated by JA (Creelman and Mullet 1997; Maserti et al. 2011) and different stresses, including insect herbivory (Moran and Thompson 2001; Maserti et al. 2011).

Ascorbate is an important nutrient for the insect herbivores, and imbalance in its availability in insect diet leads to severe consequences. The availability of ascorbate mainly depends on APX. The present study revealed greater increase in APX activity in plants pretreated with JA and SA, and then infested with *H. armigera* and *S. litura*, and in plants with simultaneous treatment of JA and insect infestation across the genotypes. Insect resistant genotypes exhibited significantly higher APX activity than the susceptible check, JL 24. Increase in APX activity in plants in response to insect damage and elicitor application decreases the availability of ascorbate in plant tissues by utilizing ascorbic acid as the electron donor in ASC–GSH recycling while catalyzing the reduction of H<sub>2</sub>O<sub>2</sub> to water, which in turn reduces the insect growth and development (Felton and Summers 1993). APX induced in soybean leaves removed ascorbate from *H. zea* caterpillar's midgut and reduced the growth and development (Felton and Summers 1993). However, no correlation between higher APX activities of transgenic poplar and ascorbate content in midgut of *L. dispar* and *M. sanguinipes* was observed by Barbehenn et al. (2008).

Pretreatment with JA, followed by insect infestation and simultaneous application of JA and insect infestation caused greater increase in CAT activity across genotypes against both *H. armigera* and *S. litura*. Insect resistant genotypes showed greater increase as compared to the susceptible check, JL 24. Constitutive levels of CAT were also higher in insect-resistant genotypes than in JL 24. The greater increase in CAT following JA treatment could be due to the signaling of transduction pathways and production of antioxidative enzymes to scavenge the toxic free radicals produced by herbivory. Catalase is also induced in plants in response to herbivory. For example, *S. incertulas* and *C. medinalis*

damage induced higher levels of CAT in rice (Usha Rani and Jyothsna 2010). In soybean, CAT has been found to resist the oxidative plant damage by *H. zea* (Bi and Felton 1995). Furthermore, the higher constitutive levels of CAT in insect-resistant genotypes might protect them from the initial oxidative damage before the induced resistance is activated. The CAT reduces the toxic free radicals in mitochondria and peroxisomes into water and O<sub>2</sub>. However, some reports have suggested that there is no alteration in CAT activity upon insect infestation (Heng-Moss et al. 2004; Rangasamy et al. 2009), while Zhu-Salzman (2004) reported down regulation of CAT genes on insect infestation.

Plants treated with JA showed significantly greater levels of SOD activity in groundnut genotypes. Although PJA + HIN and JA + HIN treated plants exhibited increased SOD activity in many genotypes, pretreatment with PJA + HIN alone increased the activity of SOD in ICGV 86699 and ICG 1697. Surprisingly, there were no significant differences in SOD activity in plants pretreated with JA and SA, and infested with *S. litura* and JA + SIN treated plants. The differential response across the genotypes might be due to differential ability of the genotypes to perceive insect damage and/or the ability to withstand the stress and then to mount the defensive response. The SOD converts the toxic free radicals, especially of oxygen, into less toxic and relatively stable H<sub>2</sub>O<sub>2</sub> (Raychaudhuri and Deng 2000). It also influences the production of plant defensive secondary metabolites under stress. Induction of SOD activity by SA has been found to reduce plant oxidative damage in maize (Saruhan 2012). *H. zea* infestation increased the SOD activity in tomato (Felton et al. 1994a) and soybean (Bi and Felton 1995). Apel and Hirt (2004) observed that SOD levels were induced within 6 hours of aphid infestation in Lima bean. Infestation by the spotted clover aphid significantly increased SOD and POD activity in alfalfa.

Plants produce many defensive proteins against insect pests. However, PIs are the most exploited plant defensive proteins for host plant resistance to insect herbivory (De Leo

et al. 2001; Azzouz et al. 2005; Parde et al. 2010, 2012). The *in vitro* PI activity of groundnut plants pretreated with JA and infested with *H. armigera* and *S. litura* was significantly greater than the uninfested control plants, and rest of the treatments. PI activity in plants treated with JA + HIN and JA + SIN was similar to the PJA + HIN and PSA + SIN treated plants, suggesting strong involvement of JA in induction of PIs and the faster signaling of defensive pathways and secondary metabolites. SA did not influence the PI activity in groundnut genotypes. The reduction in protein digestibility by PIs and deprivation of insects of essential amino acids leads to retarded growth and development in insects (Koiwa et al. 1997; Lawrence and Koundal 2002; Azzouz et al. 2005; Browse and Howe 2008; Dunse et al. 2010). The exogenous application of MeJA in *N. attenuata* results in the quick accumulation of JA, and induces the production of trypsin proteinase inhibitors against *M. sexta* (Wu et al. 2008). PIs are strongly up-regulated in plants in response to wounding or herbivore damage and/or elicitor application (Ryan 2000; Koiwa et al. 1997; Tschardt et al. 2001; Miranda et al. 2007).

Phenols constitute one of the most important and extensively studied groups of secondary metabolites against insect pests (Stevenson et al. 1993; Johnson and Felton 2001; Sharma et al. 2009; Usha Rani and Jyothsna 2010). An abrupt increase in phenolic content occurs in plants damaged by insects and/or treated with elicitors including JA or SA (Housti et al. 2002; War et al. 2011a,b). Plants pretreated with JA and SA and infested with insects, and the plants treated with JA followed by insect infestation exhibited greater phenolic content than the untreated plants. Further, insect-resistant genotypes showed a greater increase as compared to the susceptible check, JL 24. This might be due to the strong induction of the octadecanoid and phenylpropanoid signaling pathways by JA and SA, respectively. Phenols defend plants not only against insect pests, but also against microorganisms and competing plants (Harborne 1993; Matsuki 1996; Ballhorn et al. 2011).

Antifeedant activity of some phenols including salicylates have been reported against *O. brumata* in *Salix* leaves with a strong, but negative correlation between the salicylate levels and the larval growth (Simmonds 2003). In the present studies, we recorded induction of phenols in response to herbivory, wounding, and application of JA and SA, as has been observed by Arnold et al. (2004), Naz (2006), Usha Rani and Jyothsna (2010) and War et al. (2011a,b). JA has been found to elevate the phenolic biosynthesis gene expression and/or the activity of defensive enzymes (Karban and Baldwin 1997; Arnold et al. 2004; Naz 2006). Cell suspension culture of *H. perforatum* showed 6-fold increase in phenolic content after JA application (Gadzovska et al. 2007). The defensive function of phenols against insect pests is mainly by oxidation to polymers, which reduces digestibility, palatability and nutritional value of the plant tissue (Ananthakrishnan 1997). Flavonoids play an important role in plant defense against insect herbivores. They act as feeding inhibitors, and reduce the growth and development in insects (Sharma and Norris 1991; Stevenson et al. 1993; Widstrom and Snook 2001; Simmonds 2003; Treutter 2006). These are the key components involved in plant interaction with other organisms. Our results showed that plants treated with PJA + HIN, PJA + SIN, JA + HIN and JA + SIN exhibited greater levels of total flavonoids than the plants treated with PSA + HIN, PSA + SIN, SA + HIN and JA + SIN, and then infested with insects. Flavonoids have been reported to confer resistance against *S. frugiperda* in *A. thaliana* (Johnson and Dowd 2004). Higher levels of flavonoids such as, daidzin and genistin have been observed in soybean plants infested with *N. viridula* (Piubelli et al. 2003).

Role of tannins in plant defense has been studied in many plant species (Barbehenn and Constabel 2011). Like proteinase inhibitors and oxidative enzymes, tannins have been reported to be systemically induced in the neighboring leaves of the damaged plant (Peters and Constabel 2002). Tannins have a strong deleterious effect on phytophagous insects and



affect the insect growth and development by causing midgut lesions (Barbehenn and Constabel 2011). Antibiosis effect of tannins against insect pests' especially, lepidopteran larvae has been observed in many plants, which reduces growth and survivorship in many insect pests (Nomura and Itioka 2002; Kranthi et al. 2003). In *N. attenuata*, application of MeJA induced greater accumulation of JA, which in turn activated the production of phenols, flavonoids, nicotine and trypsin proteinase inhibitors and plant resistance against *M. sexta* (Wu et al. 2008).

Oxidative state of the host plants is associated with plant resistance to insects (Maffei et al. 2007; Zhao et al. 2009; He et al. 2011), which results in the production of ROS, that are toxic to the herbivores. The production of ROS is a preliminary response of plants to biotic stress that provides signal in insect–plant interaction (Maffei et al. 2007). Among them, H<sub>2</sub>O<sub>2</sub> is the most important component of plant defense, because of its relatively higher stability and it diffuses easily through the membranes (Maffei et al. 2007). Our results showed that both JA and SA induced higher levels of H<sub>2</sub>O<sub>2</sub> in all the genotypes infested with *H. armigera* and *S. litura*. However, the induction was greater in plants pretreated with JA and SA, and in plants provided with simultaneous treatments of JA and infestation with *H. armigera* and *S. litura*. Insect-resistant genotypes showed strong response in terms of accumulation of H<sub>2</sub>O<sub>2</sub>. The higher induction of H<sub>2</sub>O<sub>2</sub> by pretreatment with JA and SA could be attributed to the elevation of the antioxidative enzymes in the treated plants and their conversion of the toxic free radicals into H<sub>2</sub>O<sub>2</sub>. Transduction pathways signaled by H<sub>2</sub>O<sub>2</sub> produce many defensive compounds, which result in oxidation of phenols and other compounds producing many defensive compounds (Maffei et al. 2006; Vicent and Plasencia 2011). Orozco-Cardenas et al. (2001) reported that oxidative damage in midgut of the insects feeding on pre-wounded plants was due to the accumulation of H<sub>2</sub>O<sub>2</sub> through JA mediated pathways.

Malondialdehyde is an important lipid peroxidation product, which indicates the extent of plant defensive response to the stress. Our results showed that PSA + HIN, SA + HIN and HIN in *H. armigera* and *S. litura* infested plants had higher MDA content than the rest of the treatments. Overall, JL 24 showed higher amounts of MDA among all the genotypes. This could be due to more stress experienced by this genotype and higher levels of lipid peroxidation. Lipid peroxidation and hydroxyl ion formation ( $\text{OH}^\cdot$ ) have been proposed to play an important role in plant defense by increasing the activity of oxidative enzymes (Bi and Felton 1995). Bi and Felton (1995) reported greater increase in enzyme activities and lipid peroxidation in soybean (*Glycine max* (L.) Merr.) in response to caterpillar feeding. It has been reported that MDA is also involved in volatile emission, thus having role in indirect plant defense (Arimura et al. 2009). The free radicals produced during stress could lead to the production of MDA by lipid peroxidation. Hao et al. (2011) reported the higher amounts of MDA in rice plants in response to rice stripe virus and small brown planthopper, *N. lugens*.

Induction of proteins and their role in induced resistance against insect pests has been well established (Zavala et al. 2004; Chen et al. 2009; Usha Rani and Jyothisna 2010; He et al. 2011). The present studies indicated that there was a significant increase in proteins in PJA + HIN and PSA + SIN followed by JA + HIN and JA + SIN treated plants. Increase in protein concentration may be due to the increase in antioxidative enzymes and other non-enzymatic defensive proteins. Defense related enzymes and other protein based defensive compounds accumulate in plants in response to oxidative stress (Chen et al. 2009; Gulsen et al. 2010), and on application of elicitors (Shivaji et al 2010; Scott et al. 2010; War et al. 2011a,b; Idrees et al. 2011), which defend them from various stresses.

Plant resistance and insect growth and development are closely related (Green and Ryan 1972; Scott et al. 2010; Sharma et al. 2005; Chen et al. 2009). The PJA + HIN and

PJA + SIN treated plants suffered relatively lower damage due to insect pests across genotypes. The insect-resistant genotypes experienced greater reduction in plant damage than the susceptible check, JL 24. Similar results were observed in terms of larval survival and larval weights in both the insect species. Reduced damage, lower larval survival and larval weights might be because of greater induction of toxic secondary metabolites in the insect resistant genotypes by insect damage and JA application (Felton et al. 1994a,b; Mao et al. 2007; Bhonwong et al. 2009; Chen et al. 2009). Reduced damage and lower larval growth and development have been correlated with the increased activity of POD, PPO and other defensive enzymes induced after insect attack and/or elicitor application. Larvae of *M. sexta* and *S. exigua* fed on JA deficient mutant (*def1*) tomato plants exhibited higher survival and weight gain as compared to those fed on wild-type tomato (Howe et al. 1996; Thaler et al. 2002). Increased levels of POD activity in tomato, barley, lettuce, and buffalo grass (Stout et al. 1999; Chaman et al. 2001; Sethi et al. 2009; Gulsen et al. 2010), PPO in tomato, poplar, barley and lettuce (Wang and Constabel 2004; Chaman et al. 2001; Sethi et al. 2009; Bhonwong et al. 2009), and LOX in tomato (Felton et al. 1994b) have been correlated with reduction of insect growth and development. Plant defensive compounds induced in insect resistant genotype reduced the survival and development of *S. frugiperda* larvae (Chen et al. 2009). Reduced larval weights due to antibiosis and antixenosis against *H. armigera* have also been observed in chickpea (Sharma et al. 2005) and pigeonpea (Sujana et al. 2008). In tomato, alkaloids, phenolics, PIs, and the oxidative enzymes when ingested separately result in a reduced effect, but act together in a synergistic manner, affecting the insect during ingestion, digestion and metabolism (Duffey and Stout 1996). In *N. attenuata*, trypsin proteinase inhibitors and nicotine expression, contributed synergistically to the defensive response against *S. exigua* (Steppuhn and Baldwin 2007).

The post-ingestive interaction, where plant defensive compounds affect the insect physiology, plays a great role in determining the consumption and utilization of plant tissues by insects, and the effectiveness of the plant defense. Induced resistance is the key component in this, because it produces vagueness in the plant tissues and makes them unpredictable to the insects. Activity of serine proteases and trypsin in *H. armigera* and *S. litura* larvae fed on plants pretreated with JA, and plants simultaneously treated with JA and infested with insects was significantly lower as compared to those fed on SA pretreated, and simultaneously SA treated and insect infested plants. Both *H. armigera* and *S. litura* larvae fed on JA and SA pretreated plants showed reduced levels of serine protease and trypsin activities. This may be due to the toxicity of various oxidative products of phenols produced in insect gut, PIs, lectins and other plant defensive traits that result in inhibition of activity of digestive enzymes produced by JA, SA and/or insect infestation (Howe and Jander 2008; Barbehenn et al. 2010). The inhibition of the activities of insect digestive enzymes makes them more prone to plant defense, and ultimately decreases insect growth and development. Serine proteases are important digestive endopeptidase in insects. It has been reported that JA induces arginase and Thr deaminase (TD2) in plants, which degrade the amino acids necessary for insect growth (Chen et al. 2005). Plant defensive enzymes including peroxidase also result in direct toxicity to insect gut (Zhu-Salzman et al. 2008; Barbehenn et al. 2010).

Polyphagous insects express a wide range of defensive enzymes to counteract plant defensive compounds produced in plants. The GST and esterase enzymes are the most important defensive enzymes produced in insects on account of toxicity by various chemicals. In the present study significant alterations of GST activities in *H. armigera* and *S. litura* fed on groundnut plants were recorded in different treatments. Substantial increase in GST activity was observed in larvae fed on plants pretreated with JA, followed by insect

infestation and/or plants simultaneously treated with JA and infested with insects. Larvae fed on insect-resistant genotypes exhibited greater activity than those fed on the susceptible check, JL 24. This indicates the higher stress induced by toxic plant defensive compounds on insects. *S. avenae* showed higher GST activity when fed on insect-resistant wheat cultivars having higher levels of phenols (Leszczynski and Dixon 1992). Plant allelochemicals increased GST activity in *S. frugiperda* by nearly 5- to 26-fold (Yu and Hsu 1985).

In general, both *H. armigera* and *S. litura* fed on groundnut plants with different treatments did not show any significant differences in esterase activity across the treatments and groundnut genotypes. However, the larvae fed on the insect-resistant genotypes showed some reduction in esterase activity as compared to JL 24 and may be due to the severe toxicity and/or the inhibition of esterase production by the secondary metabolites in plants. Esterases hydrolyze the ester bonds from various substrates. Esterases have been reported to metabolize the toxic plant xenobiotics into less toxic compounds (Yang et al. 2005). The alteration of insect midgut enzymes confers a direct influence of induced plant compounds on the insect metabolism.

Both *H. armigera* and *S. litura* larvae fed on the preinfested plants exhibited reduced weights. Significant difference in larval weights were observed in *H. armigera* larvae fed on preinfested ICGV 86699, ICGV 86031 and ICG 2271 plants and in *S. litura* larvae fed on preinfested plants of ICGV 86699 and ICGV 86031. The midgut digestive enzymes, total serine protease and trypsin showed reduced activity in both *H. armigera* and *S. litura* larvae fed on preinfested plants of groundnut genotypes; however, the levels varied depending on the insect and the genotype. The GST activity showed significant alteration in *H. armigera* larvae fed on insect-resistant preinfested plants of insect-resistant genotypes. Similarly, *S.*

*litura* larvae fed on ICGV 86699, ICGV 86031 and ICG 2271 preinfested plants showed increased GST activity. Esterase activity was significantly different between *H. armigera* and *S. litura* larvae fed on control and preinfested plants of ICGV 86699 and ICG 2271. The reduction in growth and development on the preinfested plants might be due to the alteration in the nutritive quality and the induction of toxic secondary metabolites (Bi and Felton 1995). The alteration in various enzyme activities in insects fed on preinfested plants shows induction of various plant defensive compounds in preinfested plants by preinfestation. A reduction of about 61% larval weight in *H. zea* fed on damaged leaves has been reported in comparison with the larvae fed on control foliage (Bi et al. 1997). Similar results were obtained by Kranthi et al. (2003), where *H. armigera* growth and development was significantly reduced when fed on semilooper preinfested plants. It has been suggested that prior herbivory by semilooper induced resistance resulted in alteration in secondary metabolites, defensive proteins and other defensive compounds, which in turn reduced the growth and development of *H. armigera*. Preinfestation of cotton plants by mites, *Tetranychus* sp. showed lower infestation subsequently (Karban 1986). The *H. zea* larvae fed on previously infested soybean plants suffered midgut oxidative damage (Bi and Felton 1995), which was associated with the induced levels of antioxidative enzymes such as POD, LOX, APX and NADH oxidase and of MDA and ROS in plants after infestation.

Plants respond to herbivory not only through biochemical mechanisms, but also through the induction of morphological features, such as trichome density in the leaves that grow subsequently (Baur et al. 1991; Traw 2002; Traw and Dawson 2002; Agrawal 1999). The present study revealed the increase in number of trichomes in groundnut plants in response to infestation with *H. armigera* and JA and SA application. Among the treatments, plants pretreated with JA and SA and infested with insects had more number of trichomes than the untreated control plants. At 5 DAT, significantly greater induction was recorded in

ICGV 86031 and ICG 2271 genotypes by PJA treatment. However, ICG 1697 showed greater number of trichomes in PJA, PSA and HIN treated plants than the untreated control plants. Pretreatment with JA showed greater increase in trichome numbers in all the genotypes at 10 DAT. Across the genotypes, ICG 1697 had greater number of trichomes than rest of the genotypes at 10 DAT. The types of trichomes were not studied. This increase in trichome density in response to damage was observed in leaves developing during or subsequent to insect attack and/or elicitor treatment, since the density of trichomes of existing leaves does not change (Agrawal et al. 2009). Dense covering of trichomes affects the herbivores mechanically, and interferes with the movement of insects and other arthropods on the plant surface, thereby, reducing their access to leaf epidermis. Removal of trichomes makes leaves more susceptible to insect attack (Khan et al. 1986; Lam and Pedigo 2001; Agrawal et al. 2009). Trichomes negatively affect the ovipositional behavior, feeding and larval nutrition of insect pests (Hare and Elle 2002; Handley et al. 2005). Moreover, trichome exudates also serve as extra floral nectar (EFN) for parasitoids (Olson and Nechols 1995), and the plants with dense trichomes harbor more number of predatory mites than the ones with low density of trichomes in apple (Roda et al. 2003).

Oviposition is the first encounter between insects and plants in most of the cases of insect-plant interaction and any effect on oviposition behavior of insects will have effect on the level of infestation. JA and SA application and herbivory reduced the number of eggs laid by *H. armigera* in all the groundnut genotypes tested. However, greater reduction was recorded on plants pretreated with JA than in the plants pretreated with SA, plants treated simultaneously with JA and SA, and insect infested and untreated control plants. Large numbers of eggs were laid by *H. armigera* on the susceptible check, JL 24 than on the insect resistant genotypes. However, *H. armigera* laid fewer eggs on the leaves of the JA-pretreated plants and *H. armigera* pre-infested plants as compared to the SA pretreated

plants and plants treated with JA and SA, and the untreated control plants. Although the plants pretreated with JA showed reduction in number of eggs laid by *H. armigera* than the insect infested plants, the differences were not significant. Bruinsma et al. (2007) reported that *B. oleracea* plants treated with JA caused reduction in oviposition by *P. rapae* and *P. brassicae* females.

Insect oviposition induces both direct and indirect responses in plants, which are aimed at protecting the plant from damage by future larvae from the eggs (Hilker and Meiners 2006, 2010; Hilker et al. 2002). Egg deposition has been found to induce either neoplasm formation that elevates eggs from the plant surface that in turn drops down (Doss et al. 2000) or production of ovicidal compound that kill the eggs (Seino et al. 1996; Suzuki et al. 1996; Yamasaki et al. 2003). In addition, oviposition induces necrotic tissue formation at oviposition sites by hypersensitive response of plant tissues that detaches the eggs (Petzold-Maxwell et al. 2011). In the present study, the differences in oviposition might be due to the variation in type and density of trichomes, and the volatiles emitted by plants. Infested cabbage and cotton plants have been reported to be less preferred by cabbage looper adults for oviposition as compared to the undamaged plants (Landolt 1993). Ulland et al. (2008) reported the inhibition of oviposition of cabbage moths *Mamestra brassicae* L. by MeSA released during infestation, suggesting that MeSA can also be detected by the attacking herbivores. Methyl benzoate (MeBA), which structurally resembles MeSA, has also been detected in the volatile blend of insect-infested plants (Chen et al. 2003).

Phytochemical cues play a major role in insect host selection by parasitoids (Vinson 1991; Geervliet et al. 1994; Dicke 1999). The successful parasitization of the insect host depends on the attraction of the parasitoid to the plant infested by insect pests. We investigated the behavioral response of a larval parasitoid, *C. chloridae* and an egg



parasitoid, *T. chilonis* to the cues present in groundnut genotypes under no-choice and choice conditions. Our results showed that *C. chloridae* females were attracted more towards ICGV 86699, ICG 2271 and ICG 1697 than the blank. However, the differences were significant in case of ICGV 86699 and ICGV 86031, and between infested and uninfested in case of ICGV 86699, ICGV 86031 and ICG 2271. The *T. chilonis* showed significant attraction towards ICGV 86699, ICGV 86031 and ICG 2271 as compared to blanks, towards ICGV 86699 when compared to JL 24, and towards ICGV 86699, ICGV 86031 and ICG 1697 in infested and uninfested samples. This differential response of the parasitoids towards groundnut genotypes might be due to the difference in the volatiles emitted by them. However, the time taken to reach the test sample was less in case of infested plants. Plant volatiles have been found to increase the parasitism rates by the parasitoids, including *T. chilonis* and *C. chloridae*, both under laboratory and field conditions (Altieri et al. 1981; Nordlund et al. 1985; Turlings and Wackers 2004). Host plants play a potent role in determining the parasitization by parasitoids. Host-plant-mediated differences have been found to affect the natural enemies' abundance (Pawar et al. 1986, 1989; Manjunath et al. 1989; Turlings and Wackers 2004). Average rates of parasitization of *H. armigera* eggs by *Trichogramma* spp. differs depending on the host plants. For example, on sorghum (33%), groundnut (15%), pigeonpea (0.3%), and little or no parasitism on chickpea (Pawar et al. 1986). About 98% of *H. armigera* egg parasitization by *T. chilonis* was observed on tomato, potato, and Lucerne, while as no egg parasitism was recorded on chickpea (Manjunath et al. 1989). This shows that host plant has a great role in tritrophic interactions. Here, we investigated the behavioral response of a larval parasitoid, *C. chloridae* and an egg parasitoid, *T. chilonis* to the cues present in groundnut genotypes under no-choice and choice conditions. Although, carnivorous herbivores select the insect infested plants based on cues derived from insects and the host plants, the perception of

herbivore derived cues by natural enemies is often limited, because of their low detectability (Vet and Dicke 1992). Thus, HIPVs are the main cues, which determine the attraction of parasitoids and predatory insects to the herbivore infested plants (Turlings et al. 1991; Steinberg et al. 1993; Geervliet et al. 1994; Dicke 1999; Turlings and Wackers 2004). The results suggest that groundnut plants emit volatiles that attract the parasitoids and the emission of these volatiles increases on infestation. Moreover, insect-resistant genotypes were more attractive to the parasitoids.

The present study revealed that chlorogenic acid, caffeic acid and protocatechuic acid when incorporated into artificial diet at 1000 ppm were more toxic to *H. armigera* and *S. litura* larvae at 10 DAT than quercetin, catechin, cinnamic acid, trihydroxyflavone, gentic acid, ferulic acid and umbelliferone. The weights of the larvae fed on flavonoid treated diets were significantly lower as compared to those fed on the control diet. In addition, total serine protease and trypsin activities were reduced in both *H. armigera* and *S. litura* larvae fed on diets treated with chlorogenic acid, caffeic acid, ferulic acid, trihydroxyflavone, gentic acids, cinnamic acid and umbelliferone at 1000 ppm. The GST activity was increased in larvae fed on treated diets at 1000 ppm. Esterase activity showed reduction in *H. armigera* and *S. litura* larvae fed on flavonoid treated diets at 1000 ppm concentration. However, the levels of reduction varied across the treatments. Our results are in line with earlier reports which have shown the significant increase in GST activity in larvae fed on natural host plant diet with prooxidant allelochemicals (Vanhaelen et al. 2003; Krishnan and Kodrik 2006) and/or fed on the artificial diet containing plant allelochemicals (Isman and Duffey 1982; Wadleigh and Yu 1988; Lee 1991; Morimoto et al. 2000; Ateyyat et al. 2012). Leaf discs treated with various flavonoids also reduce larval growth and development of *T. ni* (Sharma and Norris 1994). Considerable effect of flavonoids such as quercetin, chlorogenic acid and rutin from *Arachis* spp. on larval, pupal and moth

deformities of *S. litura* have been observed earlier by Mallikarjuna et al. (2004). Caffeic and chlorogenic acids are highly toxic to insect pests and have been reported to cause gut toxicity due to protein oxidation and free ion release (Summers and Felton 1994). Chlorogenic acid reduces the nutritional quality of plant tissues by decreasing their digestibility due to the binding of chlorogenoquinone, an oxidative product of chlorogenic acid, to free amino acids and proteins (Felton et al. 1992), and reduces the growth and development of many insect pests including *T. ni* (Beninger et al. 2004), *H. zea* (Isman and Duffey 1982; Felton and Duffey 1990), leaf beetles, leaf hoppers and aphids (Dowd and Vega 1996; Ikonen et al. 2002; Jassbi 2003). Sharma and Norris (1991) have reported the antifeedant and antibiotic effects of daidzein, glyceollins, sojagol and coumestrol in soybean against *T. ni*. Ananthakrishnan et al. (1992) have recorded reduction in adult longevity, fecundity and larval growth of thrips fed on castor leaves sprayed with phenols and flavonoids.

Lectins are regarded as potent plant defensive proteins that bind to soluble carbohydrates or to carbohydrate of the glycoproteins, and limit their availability to insects (Peumans and Vandamme 1995), thus depriving the insects from essential nutrients and resulting in reduced growth and development. There are number of reports that have shown the deleterious effects of lectins on insect pests (Murdock et al. 1990; Czapla and Lang 1990; Zhu-Salzman et al. 1998; Gatehouse et al. 1999). Lectins are also induced in plants in response to herbivory and/or elicitor application and play an important role in signaling the transduction pathways in plants (Van Damme et al. 2003; Lannoo et al. 2006). Our results showed that larval growth and development were significantly reduced in *H. armigera* and *S. litura* larvae fed on diet with GLL and ConA at  $5 \mu\text{g mL}^{-1}$  compared to the larvae fed at 2.5 and  $1.25 \mu\text{g mL}^{-1}$  concentrations. Larvae fed on lectin treated diets exhibited lower larval weights at 5 DAT; however, at 10 DAT, weights of the larvae fed on phenyl  $\beta$ -

glucoside treated were on par with those fed on the lectin treated diets. Moreover, there was a considerable reduction in the total serine protease and trypsin activities of *H. armigera* and *S. litura* larvae fed on GLL, ConA and phenyl  $\beta$ - glucoside treated diets at  $5 \mu\text{g mL}^{-1}$ . However, reduction in trypsin activity was greater in larvae fed on GLL treated diet than the larvae fed on the ConA and phenyl  $\beta$ - glucoside treated diets at  $5 \mu\text{g mL}^{-1}$ . The GST and esterase activities were also altered. Significant inhibitory activity on insect growth has been observed in *Callosobruchus maculatus* (F.) fed on diet treated with *Maclura pomifera* (Raf.) Schneid., derived lectin and galactose-binding peanut lectin (Murdock et al. 1990). Jacalin and *M. pomifera* lectin reduced the larval growth of *Diabrotica undecimpunctata* Barber (Czapla and Lang 1990). ConA lectin, when incorporated in the artificial diet, resulted in 90% larval mortality of tomato moth, *Lacanobia oleracea* (L.), and also reduced the size of *M. persicae* by about 30%. Expression of ConA in potato plants reduced the larval weight by 45% (Gatehouse et al. 1999). Phenolic glucosides have been reported as important defensive components against insect pests in *Populus* (Bryant et al. 1987; Boeckler et al. 2011).

*Chapter 6*

*Summary and*

*conclusion*

## SUMMARY AND CONCLUSION

The results obtained from the entire work are summarized as below:

- Under field conditions, genotypes ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 showed lower damage by insects than JL 24.
- The low levels of damage were strongly correlated with higher activities of enzymes namely POD, PPO, PAL, LOX, SOD, CAT and APX, and total amounts of phenols, tannins, H<sub>2</sub>O<sub>2</sub> and proteins.
- The above enzymes and secondary metabolites can serve as the biochemical markers for resistance in groundnut against insects.
- Food consumption and utilization of pests showed reductions in approximate digestibility (AD), consumption index (CI), efficiency of conversion of ingested food (ECI), and efficiency of conversion of digested food (ECD) in *H. armigera* and *S. litura* larvae fed on insect-resistant groundnut genotypes than on JL 24.
- Moreover, the high consumption index and lower ECI and ECD in some genotypes such as ICG 1697, ICG 2271 and ICGV 86031 showed strong antibiosis mechanism of resistance adopted against insect pests.
- Damage caused by foliage feeders namely *H. armigera* and *S. litura* induced stronger response than the sucking pest, *A. craccivora* infestation.

- Considerable effect on insect growth and development was observed by insect-resistant genotypes. Insect-resistant genotypes showed less damage and higher reduction in survival and weights of the larvae than the susceptible check, JL 24.
- HPLC fingerprinting showed more peaks in *H. armigera* infested plants as compared to *A. craccivora* and control plants. Similarly insect-resistant genotypes showed more number of peaks than that of JL 24
- Native PAGE of POD and PPO of *H. armigera* infested plants showed dense bands in insect infested plants than that of the uninfested control plants.
- Pretreatment with JA and SA elicited strong defense response in groundnut genotypes.
- Pretreatment with JA induced various enzymes and secondary metabolites in almost all the genotypes and treatments against *H. armigera* and *S. litura* than that of pretreatment with SA
- Reduced survival and weights of the larvae were observed on plants pretreated with JA and simultaneously treated with JA and infested with insects. Total serine protease and trypsin activities were significantly lower in insects fed on PJA + HIN and JA + HIN treated plants.
- A considerable effect was observed on midgut enzymes such as total serine protease and trypsin activities in insects fed on plants pretreated with JA and simultaneously treated with JA and infested with insects. The GST and esterase activities were also altered. Induced defensive proteins (oxidative enzymes, PIs, lectins) and secondary metabolites such as phenols, tannins and H<sub>2</sub>O<sub>2</sub> etc., might have produced the toxic effects on the larvae after subsequent infestation.
- Pretreatment with JA and SA and preinfestation with *H. armigera* resulted in greater number of trichomes in groundnut plants with ICG 1697 responding strongly than

rest of the genotypes. Large number of trichomes at 10 DAT shows that the induction of trichomes takes few days to weeks to be apparent. Thus the trichome based induced resistance could be effective only against the subsequent insect pests.

- The oviposition was reduced on the plants pretreated with jasmonic acid in all the genotypes; however, greater reduction in oviposition was observed in ICG 1697 genotype.
- *C. chloridae* and *T. chilonis* parasitoids were attracted more towards ICGV 86699, ICG 2271 and ICG 1697 than to blank, and to insect-resistant genotypes than the susceptible check, JL 24.
- Parasitoids preferred mostly insect-infested plants
- Flavonoid treated diets showed a considerable effect on larval growth and development, and on the insect gut enzymes. This effect was concentration dependent with higher concentration showing greater effect.
- Among the flavonoids tested, chlorogenic acid, caffeic acid, ferulic acid, trihydroxyflavone, ginsitic acids, cinnamic acid and umbelliferone at 1000 ppm were more effective against *H. armigera* and *S. litura*. The toxicity of the flavonoids leads to the reduced growth and development of insect pests and alteration in enzyme activities.
- Groundnut leaf lectin and ConA at 5  $\mu\text{g mL}^{-1}$  of diet were more effective against *H. armigera* and *S. litura* than phenyl  $\beta$ - glucoside. A considerable effect on larval weights and on total serine protease and trypsin activities was observed. Moreover, activities of GST and esterase also showed a substantial effect.



## **Conclusion**

From this study it is evident that groundnut has the potential for induced resistance against insect pests by modulating its physiology, morphology and biochemistry. The phytohormones JA and SA are involved in the groundnut plant defense. This could hold true for other related legume crops. Natural enemies were differentially attracted towards the groundnut genotypes with different levels of resistance. Groundnut leaf lectin affected the insect growth and development by altering the midgut enzyme activities. Induced plant defenses can be utilized for pest control in agricultural systems. Modulation of plant's defense will increase the effectiveness of plants to defend against herbivores, by attracting natural enemies after mild damage by the herbivores to avoid the subsequent damage and also by modifying the oviposition behavior of the herbivores. With a better understanding of mechanisms involved in induced resistance, plant breeders may be able to incorporate them into breeding programs when selecting for resistance to herbivores. An understanding of induced resistance in plants can be utilized for interpreting the ecological interactions between plants and herbivores and for exploiting in pest management in crops. Since the biochemical pathways that lead to induced resistance are highly conserved among the plants, the elicitors of these pathways could be used as inducers in many crops. The future challenge is to exploit the elicitors of induced defense in plants for pest management, and identify the genes encoding proteins that are up and/or down regulated during plant response to the herbivore attack, which can be deployed for conferring resistance to the herbivores through genetic transformation.

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