MECHANISMS OF RESISTANCE TO POD BORER, *HELICOVERPA ARMIGERA* (HUBNER), IN WILD RELATIVES OF PIGEONPEA

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To my Mother . . .

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CERTIFICATE

This is to certify that the Thesis entitled **"Mechanisms of Resistance to Pod Borer, *Helicoverpa armigera* (Hubner), in Wild Relatives of Pigeonpea"**, submitted for award of the degree of **Doctor of Philosophy** in Genetics, Osmania University, is a record of the *bona fide* research carried out by Ms. G. Sujana, under my supervision, and no part of the Thesis has been submitted for any other degree or diploma.

The assistance and help taken during the course of this investigation and the sources of literature referenced have been fully acknowledged.

D. Manohar Rao

Date: 07.06.2005 **(D. MANOHAR RAO)** 7.6.05

DECLARATION

I hereby declare that the research work presented in this Thesis entitled **"Mechanisms of Resistance to Pod Borer, *Helicoverpa armigera* (Hubner), in Wild Relatives of Pigeonpea"**, has been carried out by me at the Department of Genetics, Osmania University, Hyderabad and at ICRISAT, Patancheru, under the supervision of Prof. D. Manohar Rao, Department of Genetics, Osmania University. This work is original and no part of the Thesis has been submitted earlier for the award of any other degree or diploma of any University.

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Introduction

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) is an important pulse crop of Asia and Africa. It is largely grown between 30°N and 30°S in the semi-arid and sub-tropical regions. In India, it is mainly cultivated by the small and marginal farmers, and accounts for 85 to 90% of the world's area under pigeonpea cultivation. In India, there has been a considerable increase in the area under pigeonpea cultivation from 2.18 to 3.82 m ha, and the production from 1.72 to 2.88 m t between 1950 - 51to 1996 - 97. However, there was a significant drop in productivity from 780 to 753 kg ha^{-1} during the same period (AICPIP, 1999). Andhra Pradesh accounts for 10.2% of area and 4.26% of the pigeonpea production in the country. The exact estimates of pigeonpea production are difficult to obtain, as it is grown in minor cropping systems such as homesteads, border hedges, or as an intercrop.

Pigeonpea is a multipurpose crop. It is a major source of proteins and complements the protein deficient cereal diets in rural areas in India. Pigeonpea produces a significant amount of biomass, the dry shoots are invariably used as fuel wood, fencings and thatching, thus contributing significantly in providing relief from energy crises. It also plays a major role in enriching soil fertility through atmospheric nitrogen fixation and the leaf fall, contribute substantially to the organic matter build up in the soil, thus improving the soil texture. The acid secretions from its roots dissolve iron and phosphate, and increase the availability of phosphorus in the soil. Thus, it contributes to the sustainability of agriculture besides being used as food, fuel wood, and fodder (Nene and Sheila, 1990). Though the yield potential of pigeonpea is 2.5 to 3.0 t ha^{-1}, the average productivity is around 0.74 t ha^{-1}. Most of the differences in potential yields and the actual harvests by farmers have been attributed to biotic and abiotic stress factors, besides the low productivity potential of marginal lands, where this crop is commonly grown.

Of the several biotic and abiotic constraints limiting pigeonpea production, insect pests cause a substantial loss in grain yield. Worldwide, more than 200 species of insects

feed on pigeonpea, of which the pod borer, *Helicoverpa armigera* (Huber) (Lepidoptera: Noctuidae), is the most damaging pest. *Helicoverpa armigera* has a wide host range and hence, has become difficult to control (Fitt, 1989; Mathhews, 1989). Losses due to this pest in pigeonpea have been estimated to be US$ 317 million in the Semi-Arid Tropics (SAT), and possibly over US$ 2 billion on different crops worldwide annually (Sharma, 2001). To overcome these losses, farmers resort to excessive use of pesticides. Crop surveys have indicated that before 1975, only 20% of the pigeonpea farmers were using insecticides, but by 1993, 100% of the farmers have adopted the use of chemicals to control *H. armigera* in India. It has been estimated that over US$ 1 billion is being spent on insecticides to control this pest. Application of three to six sprays of chemicals is a common practice on pigeonpea to protect the crop from pod borers. Due to the continuous and excessive use of insecticides, the pest has developed considerable levels of resistance to most of the conventional insecticides, including the synthetic pyrethroids (Kranthi *et al.*, 2002). Natural enemy activity on *H. armigera* in pigeonpea is quite low as compared to that on other crops such as sorghum (Bhatnagar *et al.*, 1983). As a result, there is greater survival of this insect on pigeonpea causing a heavy loss in grain yield.

It has been established that *H. armigera* cannot be controlled by the use of insecticides alone and is best managed by blending various components of integrated pest management. Management strategies to control *H. armigera* require different tactics based on the relationship between population density and economic loss. Pest management strategies to control *H. armigera* include cultural management of the crop and its environment, biological control using predators, parasites and microbial pesticides, sex pheromones for population monitoring or mating disruptions, chemical control, and host plant resistance.

Host plant resistance against insect pests and pathogens is an economically and ecologically preferred alternative to other pest management strategies, particularly the synthetic pesticides. It is one of the cheapest and most effective management tools for reducing the damage by *H. armigera* as it does not require additional inputs, and does not

affect the expression of other important agronomic traits. Therefore, host plant resistance can play a central role in integrated management of *H. armigera*.

Development of crop cultivars resistant to *H. armigera* has considerable potential in integrated pest management (Fitt 1989, Sharma *et al.*, 1999), particularly under subsistence farming conditions in developing countries (Sharma, 2001). Screening of more than 14,000 accessions of pigeonpea for resistance to *H. armigera*, at ICRISAT, has revealed low to moderate levels of resistance in the cultivated genotypes (Reed and Lateef, 1990). Therefore, it is important to identify wild relatives of pigeonpea with high levels of resistance to *H. armigera* for utilization in pigeonpea improvement.

Wild species of *Cajanus* have been identified as potentially valuable source of germplasm for improving the levels of resistance in pigeonpea against insect pests (Pundir and Singh, 1987; Sharma *et al.*, 2001). High levels of resistance are available in the wild relatives of pigeonpea such as *Cajanus scarabaeoides, C. sericeus* and *C. acutifolius*, which can be used as sources of resistance in the breeding programme for the development of cultivars with resistance to *H. armigera* (Sharma *et al.*, 2001). Shanower *et al.*, (1997) reported several morphological features such as pod wall thickness, differences in the structure of pod tissue and the presence of different types of trichomes on the pod surface in wild relatives confer resistance to *H. armigera*. The distribution of trichomes in different accessions and their association with insect resistance are yet to be investigated.

Besides the morphological traits, chemical components of trichomes and pod wall surface also influence the host behavior of *H. armigera* (Green *et al.*, 2002 a, b). Damage to pods by *H. armigera* is governed by certain compounds in trichome exudates and/or on pod surface, which may stimulate or deter the feeding of larvae. Acetone extracts of *C. scarabaeoides* pod surface showed a weak, but significant feeding inhibition, which was absent in *C. cajan*. Whereas, the phagostimulants associated with the glandular trichomes of pigeonpea stimulated the larval feeding (Romeis *et al.*, 1999; Green *et al.*, 2002 a, b).

HPLC technique is gaining increasing importance in the analysis of plant extracts. The "fingerprint" chromatogram obtained, under standard conditions by the qualitative analysis of extracts can be very useful for quality control of phytochemicals. HPLC can be a useful tool in chemosystematics, for example, to characterize species on the basis of their secondary metabolite contents. Reverse-phase HPLC technique has been used for the analysis of flavonoids in plants and was used to distinguish species based on the quantitative variation of flavonoids (Harborne *et al.*, 1985). HPLC analysis of methanol pod surface extracts of ICPL 87 (*C. cajan*) and ICPW 83 (*C. scarabaeoides*) revealed five major peaks, however only four compounds were identified (Stevenson *et al.*, 2002).

The antibiosis mechanism of resistance in wild pigeonpeas to *H. armigera* has been identified in terms of slower larval growth, longer pupation time, and reduced larval and pupal weights (Lateef *et al.*, 1981; Saxena *et al.*, 1990; Shanower *et al.*, 1997). Presence of antifeedant or growth inhibiting compounds and/or poor nutritional quality of the wild species may be responsible for the antibiosis mechanism of resistance to *H. armigera* in wild relatives of pigeonpea (Yoshida and Shanower, 2000). However, most of the wild relatives of pigeonpea showing resistance to *H. armigera* have not yet been characterized for different mechanisms such as oviposition preference, antifeedant/phagostimulant effects on larvae and antibiosis. Therefore, measurement of different resistance mechanisms in wild relatives of pigeonpea to *H. armigera* is highly important to identify wild relatives with different mechanisms to develop cultivars with high and stable resistance to this pest. In view of the importance of this crop and to reduce pesticide use to minimize the losses due to *H. armigera*, the present investigations were taken up with the following objectives:

1. Evaluation of wild relatives of pigeonpea for resistance to *H. armigera.*
2. Identification of physico-chemical factors associated with resistance to *H. armigera*
3. Characterization of different resistance mechanisms such as oviposition non-preference, and antibiosis.

Review of Literature

Review of Literature

Pigeonpea (*Cajanus cajan* (L.) Millspaugh), is known by several vernacular and trade names such as red gram, tuar, Angola pea, Congo pea, no-eye pea, yellow dhal, etc. It is one of the major grain legumes in the tropics and sub tropics. Besides India, it is also grown in Kenya, Tanzania, Uganda, and Malawi in Eastern Africa, and Dominican Republic and Puerto Rico in Central America. Today, in terms of global production of legume crops, pigeonpea is ranked sixth after *Phaseolus* species (common beans), peas, chickpeas, broad beans, and lentils (Nene and Sheila, 1990).

Origin of pigeonpea

The presence of several wild relatives, including the nearest ones, larger diversity of crop gene pool, linguistic evidence, a few archeological remains and wider usage in daily cuisine are ample evidences to support the view that pigeonpea is of Indian origin (Vavilov, 1951; Vernon Royes, 1976). However, several authors considered eastern Africa as the "center of origin", since the pigeonpea occurs wild in Africa. The scarce, but often cited archeological evidence of one seed in an ancient Egyptian tomb and the wild occurrence in Africa point to African origin (Purseglove, 1968; Rachie and Roberts, 1974). However, further considerations by van der Maesen (1986) confirmed India as primary center of origin of pigeonpea.

Taxonomy

Pigeonpea belongs to the family Leguminoceae, sub family Papilionaceae, tribe Phaseola and subtribe Cajaninae. It is the only cultivated food crop of the Cajaninae subtribe. The Cajaninae subtribe consists of eleven genera, the larger ones are *Eriosema* (DC.) G. Don (200 species), *Rhyncosia* Lour (130 species), and other genera are *Dunbaria* W. and A. and *Flemingia* Roxb. ex Aiton (van der Maesen, 1986).

Till 1980's, *Cajanus* was considered to be the cultivated genus, while *Atylosia* was considered as the wild. Later, the genus *Atylosia* was merged into *Cajanus* (van der Maesen, 1986). The genus *Cajanus* has 32 species including *C. cajan*, the only cultivated species, and its close relative, the *C. cajanifolius*. The different gene pools of pigeonpea are presented in the following table:

Table-1: Different gene pools of pigeonpea.

Gene pool	Genus/species
Primary gene pool	*Cajanus cajan*
Secondary gene pool	*Cajanus acutifolius, C. albicans, C. cajanifolius, C. lanceolatus, C. latisepalus, C. lineatus, C.. reticulatus, C. scarabaeoides var scarabaeoides, C. sericeus,* and *C. trinervius*
Tertiary gene pool	*C. goensis, C. hynei, C. kerstingii, C. mollis C. platycarpus, C. rugosus, C. volubilis,* other *Cajanus* spp, other Cajaninae (e.g., *Rhyncosia, Dunbaria, Eriosema*)

Pest status, Host plants, Biology, Nature of damage and Management options of *Helicoverpa armigera*

Pest status

Helicoverpa armigera is a polyphagous pest occurring throughout Africa, the Middle East, southern Europe, India, central and southeastern Asia, eastern and northern Australia, New Zealand and many Pacific Islands (Fitt, 1989). The cosmopolitan occurrence of this pest has accentuated the problem globally. It is considered as a major

biotic constraint in increasing the pigeonpea production. *Helicoverpa armigera* has attained the key pest status due to its direct attack on fruiting bodies, voracious feeding habits, high mobility and fecundity, multivoltine and overlapping generations with facultative diapause, nocturnal behavior and migration, host selection, and propensity for acquiring resistance against insecticides (Satpute and Sarode, 1995; Sarode, 1999).

Host plants

Helicoverpa armigera has been recorded feeding on 182 plant species, across 47 families in the Indian subcontinent, of which 56 are heavily damaged and 126 are rarely affected (Pawar *et al.*, 1986). Zalucki *et al.*. (1986) recorded 102 potential host plants of *H. armigera*. An extensive survey of host plants, in Australia, found 26 additional host plants (Zalucki *et al.*,(1994).

Nevertheless, it is clear that *H. armigera* has a wide host range. The main host families include Asteraceae, Fabaceae, Leguminaceae, Malvaceae, Poaceae and Solanaceae. However, they were sceptical about accepting all of them as host plants since the completion of full life cycle were not confirmed on all of these species. In addition to the main crops such as cotton, pigeonpea, chickpea, sunflower, maize, sorghum, several weeds and wild plants have been found to be important alternate hosts. *Chenopodium alba* and *Melilotus alba*, the most abundant weeds of chickpea were preferred by *H. armigera* for oviposition compared to chickpea (Bajpai and Shegal, 1993). High adaptability and potential to utilize different host plants enables the *H. armigera* to survive and develop continuously even in the off-season (Bhatnagar *et al.*, 1982). *Helicoverpa armigera* exhibits preference among the host-plant species (Roome, 1975; Hillhouse and Pitre, 1976). Johnson *et al.*. (1975) stated that the "adaptive host-plant shift" occurs with a decrease in primary host-plant number and an increase in suitable secondary host. In Sudan Gezira, groundnut is an important alternate host when sorghum and cotton are not available or are at the non-attractive growth stage (Topper, 1987). Although, cotton is highly susceptible to *H. armigera*, is not a much preferred host since

in many areas, cotton is heavily attacked only after the alternate hosts have senesced (Fitt, 1989; Ramnath *et al.*, 1992). A growth index calculated from the laboratory studies on *H. armigera* to assess the effects of feeding tomato, cabbage, cotton, pigeonpea and chickpea showed that the survival of larvae, emergence of adults and the growth index were greatest for insects reared on pigeon pea (Valand *et al.*, 1992). Different parts of the same host plant may also differ in their suitability for *H. armigera*. Hmimina (1988) found that larval growth was faster on cotton flower buds than on cotton leaves, potato leaves, tomato fruits, maize cobs or synthetic diet. However, no larvae survived on tomato leaves. Young larvae preferred to feed on sorghum flowers, while the older larvae preferred developing grains (Roome, 1975). Under laboratory conditions significant variation in growth, development and survival of larvae were observed by Sison and Shanower (1994), when the suitability of different plant parts (flowers, pods and leaves) of six-short duration pigeonpea genotypes on the growth and survival of *H. armigera* were studied. The larval and pupal weights were significantly higher, developmental time was significantly shorter, and the adult life span was significantly longer for larvae reared on pods compared to flowers and leaves. This significant variation may be due to differences in biochemical constituents.

Biology

The adults of *H. armigera* are nocturnal (Roome, 1975; Topper, 1987; Riley *et al.*, 1992). The moths hide among the leaves and cracks and crevices during the daytime. Females are dark grayish-brown, while males are almost uniform pale cream in color. The emergence of moths starts at dusk and continues until mid-night, after which it virtually ceases (Riley *et al.*, 1992). Female moths are highly fecund and oviposit 24 h, after mating. The pre-oviposition period is 2 to3 days, while the oviposition period lasts for 5 to 9 days (Patel *et al.*, 1968; Singh and Singh, 1975). A single female is capable of laying up to 3000 eggs (Fitt, 1989). Eggs are tiny spherical balls; yellowish white when freshly laid, but become dark brown/black before hatching. Tiny translucent yellowish

white larva emerges form the egg after 2 to 3 days. The larvae pass through five or six instars (Bilapte *et al.*, 1988), but exceptionally seventh instar is also found when larval development is prolonged (Pearson and Darling, 1959). The larval duration varies from 8 to 12 days (Singh and Singh, 1975), and the variation is influenced by temperature and host plant. The larval duration on the short duration pigeonpea genotypes is 21 days (Sison and Shanower, 1994). Larvae prefer to feed on reproductive structures and growing points, and a larva is capable of destroying several bolls or fruits during its development. Pupation takes place in the soil at a depth of 5 to 10 cm below the base of the plants, and the adults emerge in 7 to 10 days (Pearson and Darling, 1959). The length of adult life span is largely determined by the availability of food, in the absence of which depletion of the fat body is rapid and death occurs in a few days (Pearson, 1958). The longevity of females is more compared to the males. Number of generations per year varies according to agro-climatic conditions. In favourable conditions, one generation can be completed in 28 to 30 days. Four generations have been recorded in Punjab (Singh and Singh, 1975), 7 to 8 generations in Andhra Pradesh (Bhatnagar, 1980), and five in Uttar Pradesh (Tripathi, 1985).

Nature of damage

In India, *Helicoverpa* is represented by three species viz; *H armigera* constituting 99.2%, *H peltigera* at 0.6% and *H assulta* at 0.2% (Pawar, 1998). The life history features such as polyphagous nature, multiple generations, high reproductive rate, scattered egg laying, high mobility and facultative diapause has made *H armigera,* as one of the "world's worst pests" (Pimbert *et al*, 1989). Oviposition by *H armigera* females coincides with the flowering stage of the host plants (Roome, 1975). The chances of finding a suitable host by young larvae are low as they cannot move far from their egg shells (Jackson, 1990). The neonate larva wanders about nibbling various parts of the plant, until they find a flower bud or flower and finally feed by scraping the green tissues.

The older larvae eat the developing seeds by boring into the pods and leave characteristic large round holes along the locules of the pod.

Helicoverpa armigera claims a major share in the crop losses every year for crops such as chickpea, pigeonpea, tomato, cotton, tobacco, maize, groundnut, sorghum, etc. (Manjunath *et al.,* 1989). A single larva per 10 plants reduces the pigeonpea yields by 30.9 kg ha^{-1} (Venugopal Rao *et al.,* 1992). Damage from early instars is minor, and foliar damage does not usually result in yield reductions (Sehgal, 1990). The extent of damage caused by this pest in chickpea is up to 84.4% with an average of 7% in different farming systems (Lateef, 1992) and 50 to 60% in pigeonpea (Puri, 1998). During 1997-98, the pigeonpea crop was completely damaged in the telangana region of Andhra Pradesh due to the outbreak of *H armigera.* In the tropics, total annual losses due to this pest on cotton, legumes, vegetables and cereals may exceed $2000 million, and in India, estimates of total losses in both the pulses and cotton exceed $500 million per annum (Sharma, 2001).

Management options

Pest management strategies vary according to the agro-ecosystem, pest incidence and socio-economic conditions of a particular area (Matthews, 1997). Since 1950, the application of pesticides to control *H. armigera* has become a regular practice. Even though various chemical control measures have been devised to minimize the losses caused by the pod borer, their indiscriminate use has resulted in development of resistance to insecticides including pyrethroids. Resistance to pyrethroids in *H. armigera* has been reported through out the world. Integrated Pest Management (IPM) can help to minimize the use of insecticides, and hence, there has been a shift towards the adoption of appropriate IPM strategies rather than use insecticides only for its control (Sharma *et al.,* 1999). Several IPM strategies have been recommended for crops such as cotton, pigeonpea, chickpea, and other crops. Adoption of companion/mixed cropping systems,

application of biopesticides, and biocontrol agents, nuclear polyhedrosis virus (NPV), use of pheromone traps, and development of host plant resistance are some of the IPM tactics that have been evaluated against this pest on several crops.

Biological pest suppression is an important strategy for the management *H. armigera*. The impact of parasitism on *H. armigera* populations has been quantified by Titmarsh (1985). More than 70 species of parasitoids and 60 species of predators are known to attack *H. armigera* in India (Romeis and Shanower, 1996). However, the impact of predators and parasitoids on *H. armigera* is relatively low in pigeonpea as their activity is significantly hindered by trichomes and trichome exudates on pigeonpea buds and pods (Shanower *et al.*, 1999; Romeis *et al.*, 1999).

Host plant resistance (HPR) to insects is one of the easiest and cheapest components of an integrated pest management program. It is an environmentally friendly method of insect management, and is compatible with other control strategies such as biological, cultural and chemical control. Utilization of plant resistance as a control strategy in the developing world has enormous practical relevance and additional emotional appeal (Davies, 1981). Insect resistance has been introduced into several crop varieties during the last 20 years (Smith, 1989) and its importance is increasing as insecticides lose efficacy due to pest adaptation or are removed from use to protect the environment and human health (Eigenbrode and Trumble, 1994). Often, successful crop production is impossible without resistance to insects and pathogens. Much of the screening for host plant resistance (HPR) to *H. armigera* in pigeonpea has been carried out at ICRISAT from the mid-1970s to the early 1990s (Lateef and Pimbert, 1990). Development of pigeonpea varieties resistant to *H. armigera* appears to be a complex problem considering the polyphagous nature of insect.

Mechanisms of resistance

Various aspects of host-plant resistance to insects have been discussed by Painter (1951), Maxwell and Jennings (1980), Smith (1989), and Sharma and Ortiz (2002). The mechanisms of resistance have been classified into three types; a) antixenosis (non-preference to oviposition) b) antibiosis and c) tolerance (Painter, 1951).

Antixenosis (non-preference for oviposition)

Antixenosis is derived from a Greek word, "xenos" which means "guest", and describes the inability of a plant to serve as host to an insect herbivore. This term was proposed by Kogan and Ortman (1978) to replace the term nonpreference, which was proposed earlier by Painter (1951). Antixenosis may be due to morphological or chemical factors that affect the insect behavior adversely, resulting in the selection of an alternative host plant. The morphological characters involved with insect resistance are color, shape, succulence, toughness, spines and trichomes of the host plant, while the biochemical components include sugars, enzymes, fats, amino acids, and secondary metabolites.

Oviposition is an important phenomenon for the dispersal, existence and establishment of an insect population (Saxena, 1969). According to Eherlich and Raven (1964), the selection of the oviposition site by the adult insects is often most crucial for the survival of its offspring, as neonate larvae are usually incapable of moving very far for food. However, *H. armigera* can oviposit freely in captivity even on unsuitable substrates (Roome, 1975).

Several workers (Fitt, 1986; Courtney and Kibota, 1990; Singer *et al,*. 1992) have suggested that the host selection behavior of an insect depends on its physiological state including age, feeding status, mated status and egg load. The preference for a particular host by *H. armigera* is shown by laying more eggs. Presence of certain physiological cues in the host plants is responsible for exhibiting the preference by the insect.

The complete chain of sequences which culminate in oviposition, is guided by multiple sensory cues (Miller and Strickler, 1984), like visual, particularly color (Ilse, 1973; Prokopy and Owens, 1983), shape (Stadler, 1974; Rausher, 1978), plant volatiles (Yamamoto and Fraenkel, 1960; Renwick and Radke, 1983; Salama *et al.*, 1984; Jackson *et al.*, 1984) and surface texture (Callahan, 1957; Robinson *et al.*, 1980; Hagley *et al.*, 1980). There are also reports about the effect of larval food (Hough and Pimentel, 1978; Dhandapani and Balasubramanian, 1980; Arnault and Loevenburck, 1986), and adult feeding (Topper, 1987; Cunningham *et al.*, 1998) on fecundity and distribution of eggs. The influence of flower colour on oviposition preference by *H. armigera* in pigeonpea was studied by Laxmipathi (2000), and it was found that yellow coloured flowers were preferred over red flowers.

Helicoverpa armigera exhibits a hierarchy of host plant preference (Firempong and Zalucki, 1990a; Jallow and Zalucki, 1995, 1996; Jallow 1998). Firempong and Zalucki, (1990b) studied the oviposition preference by *H. armigera* on *Helianthus annus*, *Nicotiana tobaccum* and *Zea mays*. *Helicoverpa armigera* prefers to lay eggs on host plant during the flowering stage (Pearson, 1940; Roome, 1975; Fitt, 1991). In contrast to other hosts, the oviposition on chickpea declines with the onset of flowering (King, 1994). Preference of moths to oviposit on plants during the reproductive growth stage could be due to an increase in chemical attractiveness of the crops (Zalucki *et al.*, 1986). Topper (1987) found a rapid increase in egg laying of *H. armigera* in the dark period succeeding dusk. Studies on the oviposition response of *H. armigera* in different varieties of cotton under caged conditions revealed the preference to lay maximum eggs on *Gossypium hirsutum* varieties than on *Gossypium arboreum* varieties (Butter and Surjit singh, 1996). In chickpea, the resistance is mainly due to oviposition preference rather than larval preference and antibiosis (Srivastava and Srivastava, 1989; Cowgill and Lateef, 1996). In pigeonpea, *H. armigera* prefers to lay eggs on flowers and flower buds, while the leaves are least preferred (Venugopal Rao *et al.*, 1991). On the other hand in chickpea, the leaves are the most favorable substrates for oviposition. In pigeonpea, ICPL

87 was preferred much for oviposition both under no-choice and multi-choice conditions (Sison *et al.*, 1993). Pigeonpea genotypes showing resistance to *H. armigera* under field conditions exhibited oviposition nonprefernce under laboratory conditions (ICRISAT, 1991).

Antibiosis

Antibiosis includes the adverse effects of the physico-chemical characteristics of the plants on the biology of an insect attempting to use that plant as a host. Both chemical and morphological factors mediate antibiosis. The effects of these factors may be acute, often affecting eggs and young larvae, and the chronic effects may lead to the mortality of older larvae, pupae, and adults. Individuals surviving the direct effects of antibiosis may have reduced body size and weight, prolonged period of development, and reduced fecundity.

Laboratory screening of chickpea genotypes for antibiosis to *H. armigera* larvae showed significant variation for pupal weight and larval survival (Srivastava and Srivastava, 1990), and pupae on chickpea pods were heavier and developed more quickly than those reared on chickpea leaves. Sison *et al.*, (1996), reported that larvae reared on leaves or pods of desi chickpea genotypes showed significant variation in pupal weights and larval survival, whereas, there was no variation in these parameters when larvae were reared on kabuli type chickpea genotypes.

In cotton, several genotypes have been screened both in the field and laboratory conditions against *H. armigera* to understand the antibiosis mechanism of resistance. When the second-instar larvae were fed with fresh leaves and bolls or their lyophilized powders of SC 50, SC 70, SC 71, SC 112, SC 163 and st 213 varieties, mixed with artificial diet revealed that the larval and pupal weights of the insects fed on fresh bolls were significantly higher than those fed on fresh leaves and it was vice versa for the larval periods (Yuwadee-Adulyasak, 1989). Similar results were observed on artificial

diet. The presence of physiologically active compounds such as gossypol in cotton and tomatine in tomato lead to antibiotic activity against *H. armigera* (Vilkova and Ivashchenko, 1991; McColl and Noble 1992).

Kashyap *et al.*, (1990) screened nineteen accessions of seven *Lycopersicon* species for resistance against *H. armig*era and maximum resistance was found in the accessions of *L. hirsutum* f. *glabratum*, where the duration for larval development was more, and larval weights and survival rates were low. Screening of 11 pigeonpea genotypes using third-instar larvae of *H. armigera* showed significant gain in larval, pupal and adult weights in genotypes with lower levels of trypsin inhibitors. A significant decline in the larval and pupal weights and longer duration in both the stages were observed for larvae fed on developing pods of resistant varieties, ICPL 270 and ICPL 84060 as compared to those fed on the susceptible variety, BDN2 (Dodia and Patel, 1994).

Flowers and pods of wild species of pigeonpea adversely affect growth and development of *H. armigera*. Dodia *et al.*, (1996) studied the antibiotic effects of flowers of *Cajanus scarbaeoides, C.cajanifolus, C. reticulatus, C. sericeus*, F_1s (*C. scarabaeoides* x *C. cajan*) and cultivated pigeonpea (T15 – 15) on the biology of *H. armigera*. The larval mortality was high during first 7 days, and very few larvae survived to the pupal or adult stages. Adults were small; growth index and fecundity were also adversely affected for the larvae reared on wild species and their F_1s as compared to cultivated pigeonpea. Lateef *et al.*, (1981) studied the life cycle of *H. armigera* on *Atylosia scarabaeoides*, *A. sericeus* and *C. cajan* (ICP 1), and reported that the larvae grew more slowly on *Atylosia spp.*, took longer to pupate, formed smaller pupae, and these adults laid few eggs. The pod walls of *A. scarabaeoides* are relatively tough, and under field conditions, the pod borer damage is often limited to scarification of the pod surface such that seeds are left intact. Developing pods of *C. scarabaeoides* are devoid of glandular hairs and have lignified cells just below the epidermis, suggesting that this species also has a mechanical type of resistance, in addition to antibiosis. The seed coat colour is also one of the factors

influencing growth of *H. armigera* larvae fed on artificial diet containing powdered groundnut seeds. Groundnuts with brown colour seeds showed more antibiosis towards the larvae compared to the groundnuts with white seeds. These results were further confirmed from field observations, where the brown seeded genotypes were less damaged by *H. armigera* than the genotypes with white coloured seeds (ICRISAT, 1985).

Tolerance

The ability of a plant to withstand or recover from the damage caused by insect abundance equivalent to that required to damage a susceptible cultivar is termed 'tolerance mechanism of resistance'. The expression of tolerance is determined by inherent genetic capability to outgrow an insect infestation or to recover and add new plant growth after the recovery from the insect damage

Plants with tolerance mechanism of resistance have a great value in pest management, as such plants prevent the evolution of new insect biotypes, and also help in maintaining the populations of the natural enemies. Effects of tolerance are cumulative as a result of interacting plant growth responses such as plant vigor, inter and intra plant growth compensation, mechanical strength of tissues and organs, and nutrient and growth regulation and partitions (Tingey, 1981). Development of new insect biotypes capable of feeding on resistant cultivars with antixenotic or antibiosis mechanisms of resistance can be delayed or minimized by utilizing tolerance as a polygenic resistance (Tingey, 1981).

Factors associated with resistance to *Helicoverpa armigera*

Trichomes

Trichomes are epidermal appendages of diverse form and structure present on the leaf, stem, flower and pod surfaces of many plant types. The most common morphological resistance mechanism is the presence of trichomes. The role of trichomes as an insect defense mechanism has been studied by Levin (1973), Webster (1975) and

Stipanovic (1983). The variation in forms and functions of trichomes within the same species are frequently the basis of plant resistance to insect attack (Southwood, 1986). Trichomes can be simple unicellular, multicelluar uniseriate, multicellular multiseriate, stellate, pellate, dentritic or arboriform (Jeffree, 1986). Trichomes are either glandular (secrete or contain chemicals) or non-glandular (do not secrete or contain chemicals).

The chemicals in and on the glandular trichomes may either be toxic or may impede the insects ability to move, feed and/or survive (Duffey 1986; David and Easwaramoorthy, 1988; Peter *et al.*, 1995). The volume of the exudate secretion varies with weather, time of day and plant age (Koundal and Sinha, 1981; Rembold *et al.*, 1990), and they play an important role in host selection process of insect herbivores (Bernays and Chapman, 1994). In addition to entrapping, the exudates contain volatile chemicals which act as repellents (Rodriguez *et al.*, 1972; Cantelo *et al.*, 1974; Patterson *et al.*, 1975; Rick *et al.*, 1976).

Non-glandular trichomes usually have hooked tips, which trap the insect, and impede the insect's activity by holding the insect and disallowing a contact with the foliar surface, leading to starvation. Trichomes effect the physiology of insect by interfering with its digestion (Wellso, 1973). Presence of a dense pubescence on the leaves also changes its optical properties (Southwood, 1986), contributes to feeding antixenosis (Khan and Saxena, 1986), serves as an attractive oviposition substrate for some insects (Renwick and Chew, 1994; Bratti, 1994) and affects the walking speed of the predatory insects (Krips *et al.*, 1999). Trichome density exhibits a negative impact on the larval growth and survival (John Peter, 1995; Valverde *et al.*, 2001; Gurr and Mac Grath, 2001). Parnell *et al.*, (1949) reported that the hair length is a more important determinant of resistance than hair density.

Presence of glandular trichomes in annual *Medicago* species confers a high level of resistance to several alfalfa insect pests as exudates increase larval mortality (Shade *et al.*, 1975), inhibits larval mobility (Johnson *et al.*, 1980a,b), and decreases the oviposition

rate (MacLean and Byers, 1983; Brewer *et al.*, 1986). Shade and Kitch (1983) reported a significantly higher population of *Acyrthosiphon pisum* (Harris) on nonglandular alfalfa cultivar compared to glandular species.

In soybean, trichomes have been evaluated as potential resistance mechanism for potato leaf hopper, *Empoasca fabae* (Wolfenbarger and Sleeman, 1963) and lepidopteran insect, *Heliothis zea* (Boddie) (Panda, 1979). The resistance to *E. fabae* is conferred by the orientation of hairs (Broersma *et al.*, 1972), the length of the trichomes (Jonhoson, 1975) and density of trichomes (Turnipseed, 1977). Presence of dense pubescence in soybean resulted in a significant reduction in feeding damage, oviposition and subsequent nymphal populations of potato leafhopper, *E. fabae* (Elden and Lambert, 1992).

A correlation between insect resistance and Type IV and Type VI glandular trichomes in tomato has been reported by several authors (Isaman and Duffey, 1982a,b; Snyder and Carter, 1984; Farrar and Kennedy, 1987; Goffreda *et al.*, 1988; Weston *et al.*, 1989). Catecholic phenols identified in Type IV trichomes (Ave and Tingey, 1986) can act as an additive to inhibit the growth of *H. zea* (Duffey, 1986) and the methyl ketones, 2-tridecanone (Dimock and Kennedy, 1983), 2-undecanone (Farrar and Kennedy, 1987) in Type VI trichomes are acutely toxic to *Aphis gossypii* (Glover), *Epilachma varivestis Leptinotarsa decemlineata* (Say), *Manduca Sexta* (Linneaus) and *H. zea* (Williams *et al.*, 1980; Kenedy and Dimock, 1983). In potato, the polyphenoloxidase (PPO), enzyme present in the exudates of trichomes of Type A play a key role in controlling the damage (Yencho and Tingey, 1994), by hardening the exudates (Gregory *et al.*, 1986), entrapping the insects (Gibson and Turner, 1977), and finally causing mortality.

The cotton cultivars with high trichome density on the lower surface of leaf were more resistant to cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Kamel, 1965). Slow larval development (Stephens and Lee, 1961) and inhibited movement of *Aphis grandis* was observed in cotton varieties with dense hairs (Cook, 1906). Two pairs of genes, H_1 and H_2 appear to play a role in the genetic control of pubescence of leaves in

cotton. The gene H_1 induces the length and density of hair and is incompletely dominant over h_1, while the H_2 allele seems to induce hairiness, but only to a small degree. It acts additively to H_1, giving profuse hairiness to plants.

Trichomes on *Triticum* spp have been reported to confer resistance to cereal leaf beetle, *Oulema melanopus* (Linneaus) (Schillinger, 1969; Webster *et al.*, 1973; Wellso and Hoxie, 1982), hessian fly, *Mayetiola destructor* (Say) (Miller *et al.*, 1960; Roberts *et al.*, 1979), and bird cherry oat aphid, *Rhopalosiphum padi* (Linneaus) (Roberts and Foster, 1983). Genotypes with pubescent leaves suffered a less damage (Webster *et al.*, 1972) as they were not preferred for oviposition by the cereal leaf beetle (Schillinger and Gallun, 1968; Gallun *et al.*, 1973). A negative correlation between larval weights and larval survival of cereal leaf beetle, and pubescence has been reported (Webster and Smith, 1971; Wellso 1973).

Chickpea trichomes have been found to play important role in the resistance against leaf miner, *Liriomyza cicerina* (Rozdani) and *H. armigera* (Rembold *et al.*, 1990). The malic and oxalic acids (Koundal and Sinha, 1981; Rembold and Weinger, 1990) composition in glandular trichome secretions of chickpea varies among genotypes (Santhakumari *et al.*, 1979). A correlation between the levels of resistance and the amounts of malic acid has been reported by several workers (Rembold, 1981; Rembold and Winter, 1982; Lateef, 1985; Rembold *et al.*, 1990). The trichomal exudates of chickpea showed nonpreference to oviposition and antibiosis mechanism of resistance to *H. armigera* (Srivastava and Srivastava, 1990; Weigand and Pimbert, 1993).

Pigeonpea foliar trichomes have been studied by a few workers (Espinoza and Flores, 1977; Sharma *et al.*, 1981; Navasero and Ramaswamy, 1991). Bisen and Sheldrake (1981) reported three types of trichomes in *C. cajan* viz ., simple nonglandular, yellow glandular sacs and tubular glandular trichomes. Shanower *et al.*, (1997) observed five types of trichomes viz., Type A, Type B, Type C, Type D and Type E on pods of *Cajanus* species and reported their importance in mechanism of resistance against *H.*

armigera. The phagostimulant / antifeedant activity of glandular trichomal secretions towards *H. armigera* larvae has been reported (Sharma *et al.*, 2001; Green *et al.*, 2003). Dense nonglandular trichomes on pods of wild pigeonpea act as physical barrier to young *H. armigera* larvae (Romeis *et al.*, 1999), while the glandular trichomes act as attractants to adult moths (Hartleib and Rembold, 1996).

Biochemical basis of resistance

Plants are known to produce certain chemical compounds, in different quantities and proportions, which affect the behavior of phytophagous insects in various ways (Painter 1951, 1958; Beck 1965; Schoonhoven, 1968). These compounds can be attractants (oviposition and feeding stimulants) or repellents (oviposition and feeding deterrents) or antibiotic (reduced survival and growth and development).

The proteiase inhibitors in *Lycopersicon esculentum* and *Solanum tuberosum* leaves (Green and Ryan, 1972) and cucurbitacins in *Cucurbita moschata* and *C. pepo* act as feeding deterrents to *Epilachna* beetles (Carroll and Hoffman, 1980; Tallamy, 1985). The inhibitory effects of caffeoylquinic acids on the larval development of *H. armigera* in wild groundnut species, *Arachis paraguariensis* was reported by Kimmins *et al.*, (1995). Sundararajan and Kumuthakalavalli (2001) observed the antifeedant activity of aqueous leaf extracts of *Gnidia glauca* and *Toddalia asiatica* against the sixth instar larvae of *H. armigera*.

Crude extracts from the pods of wild species of *Vigna* resulted in significantly higher mortality, longer developmental time, and lower growth index of pod bug, *Clavigralla tomentosicollis* than those from their cultivated cowpea (Koona *et al.*, 2003). Feeding bioassay studies against *H. armigera* using filter paper discs impregnated with acetone extracts of pod surface chemicals of pigeonpea cultivar, ICPL 87 and wild *Cajanus scarabaeoides* and *C. platycarpus* indicated the presence of phagostimulants on pod surface of ICPL 87, making it more vulnerable than wild *Cajanus* (Shanower *et al.*,

1997). The chemical compounds or the type and distribution of trichomes on the plant surfaces determine the feeding and food selection behaviour of *H. armigera* larvae (Green *et al.*, 2002 a, b)

Sugars

The pods of pigeonpea belonging to three maturity groups (early, medium and late) were analyzed at green and maturity pod stages for various biochemical parameters (proteins, total sugars, phosphorus and potassium). Early maturing varieties (UPAS 120, ICPL 87 and TAT 10, susceptible to pod borer damage), possessed significantly higher total sugar content (3.56 to 4.70%) than the late maturing cultivars (PT 35, PT 25, C 11, N 290-21) (2.99 to 3.30% sugar content) (Knap *et al.*, 1996). A significant positive correlation between the total sugars and pod borer damage has been reported by several authors (Singh and Jotwani, 1980; Khurana and Verma, 1983) while, the association of lower sugar content with susceptibility to *C. partellus* was observed in sorghum (Swarup and Chaugale, 1962). Higher content of total sugars and lower amount of phenols were observed in groundnut genotypes susceptible to leaf miner (Senguttuvan and Sujatha, 2000).

Pigeonpea is known to contain some antinutritional factors such as proteinase inhibitors, oligosaccharides, phenols, tannins and phytic acid (Singh, 1988). The late maturing cultivars of pigeonpea resistant to pod borer damage have higher content of polyphenols and lower amino acids, sugars and proteins compared to the susceptible medium and early maturity varieties (Mukerji *et al.*, 1993; Sahoo and Patnaik, 2003).

Tannins

Tannins and other secondary plant substances accumulated in plant tissues act as defense mechanism against insects causing damage (Swain, 1979; Ebel 1986; Sharma and Nooris, 1990). Tannins in legume seeds are implicated in decreasing the activities of digestive enzymes and the availability of proteins, amino acids and mineral uptake

(Salunkhe *et al.*, 1982). Sharma *et al.*, (1993) reported the antifeedant activity of tannins in sorghum against insects.

Polyphenols

The presence of polyphenols has been reported in several plant species (Haslam, 1981). Phenolic compounds in sorghum caryopsis are reported to improve resistance to insects, fungi and other pathogens. (Dreyer *et al.*, 1981; Butler, 1988). Annadurai *et al.*, (1990) suggested that the relative concentrations of various phenols play an important role in determining the suitability of pigeonpea plant tissues as insect food. Presence of phloroglucinol in pods stimulates the growth and enhances the survival of larvae. The compound resorcinol may be the cause of poor larval growth and survival on leaves.

Flavonoids

Flavonoids constitute a relatively diverse family of molecules that are derived from Phe and malonyl-coenzyme A (CoA; via the fatty acid pathway). These compounds include six major subgroups viz., the chalcones, flavones, flavonols, flavandiols, anthocyanins and condensed tannins (or pro-anthocyanidins); that are found in higher plants. A seventh group, the aurones, is widespread, but not ubiquitous.

Specialized forms of flavonoids, such as the isoflavonoids, are found in legumes and a small number of non-leguminous plants. Sorghum, maize, and gloxinia are among the few species known to synthesize 3-deoxyanthocyanins (or phlobaphenes in the polymerized form). The stilbenes, which are closely related to flavonoids, are synthesized by plant species such as grape (*Vitis vinifera*), peanut (*Arachis hypogaea*), and pine (*Pinus sylvestris*). Non-polar flavonoid aglycones are usually extracted with chloroform, ether, ethyl acetate, or benzene, while the more polar flavonoids are extracted with acetone, methanol, water or a combination of these (Markham, 1975).

Flavonoids and isoflavonoids are known to confer resistance against insect attack in several plant species (Hedin and Waage, 1986; Grayer *et al.,* 1992). Flavonoids in soybean contribute to genotypic resistance against plant pathogens (Keen *et al.,* 1972; Keen and Paxton, 1975; Ingham *et al.,* 1981; Ebel, 1986) and insects (Chiang *et al.,* 1986; Khan *et al.,* 1986; Sharma and Nooris, 1991). C-glycosyl flavone isolated from the silk of a resistant maize variety was shown to inhibit the growth of the corn ear worm, *H. zea* (Waiss *et a.,l* 1979).The antifeedant activity of flavonoids from the leaf extracts of soybean was reported against cabbage looper, *Trichoplusia ni* Hb. (Sharma and Norris, 1991 &1994), whereas, the flavonol glycosides in horseradish, act as phagostimulants to horseradish flea beetle, *Phyllotreta armoraciae* (Nielsen, 1978). Simmonds and Stevenson (2001) isolated four isoflavonoids from wild relatives of chickpea and reported their antifeedant activity against *Helicoverpa* larvae. The acetone extracts from the pod surface of *C. cajan* stimulated the feeding of third-instar larvae of *H. armigera.* The phagostimulants present in the pod surface extract of ICPL 87 favoured the larval feeding (Romeis *et al.*, 1999; Green *et al.,* 2002 a, b).

Investigations were made on five principal flavonoids viz; quercetin 3-O- β-d-glucoside 7-O-β-d-glucoside, quercetin 3-O-β-d-apiofuranosyl-(1->2)-β-d-galactoside, hyperoside, quercetin and kaempferol, in 40 samples of Semen Cuscutae by using a reversed phase liquid chromatograph system using 0.025 M phosphoric acid-methanol as mobile phase (Ye *et al.*, 2002). Six flavonoid constituents viz; genkwanin 5-O-β-D-primeveroside, genkwanin5-O-β-D-glucoside, genkwanin, potassium apigenin 7-O-β -D-glucuronate, apigenin and tiliroside were determined in Daphnis Genkwae Flos, by a high performance liquid chromatographic method using a Cosmosil 5C18-AR reversed phase column by gradient elution with varied proportion of 1.0 % (v/v) acetic acid and acetonitrile as mobile phase at 254 nm (Jer-Hueilin *et al.,* 2000). The flavonoids (quercetin, myricetin and kaempferol) and stilbenes (cis- and trans-resveratrol) were identified in red wine with a new reversed-phase (RP) high-performance liquid-chromatographic (HPLC) method with UV-absorbance detection at 320 nm for stilbenes

and 377 nm for flavonoids (Stecher *et al.*, 2001). Flavonoids; aspalathin, isoorientin, orientin, rutin, isovitexin, vitexin, isoquercitrin, hyperoside, quercetin, luteolin and chrysoeryol were quantitatively characterized by HPLC/UV method in *Aspalathus linearis*, (Bramati *et al.*, 2003). Chlorogenic acid, quercetin, quercitrin, isoquercitrin, rutin, hyperoside, I3, II8-biapigenin, pseudohypericin, hypericin, hyperforin and adhyperforin were separated by an aqueous phosphoric acid–acetonitrile–methanol gradient within 50 min by using a wide pore RP-18 column and a water–methanol–acetonitrile–phosphoric acid mobile phase system (Brolis *et al.*, 1998).

Materials and Methods

Materials and Methods

Studies on the "Mechanisms of resistance to *Helicoverpa armigera* (Hubner.) in wild relatives of pigeonpea (*Cajanus cajan* (L.) Millspaugh)" were conducted at the International Crop Research Institute for the Semi Arid Tropics (ICRISAT), Patancheru, India. The materials and methods used in conducting these experiments are elucidated below.

A total of 31 accessions (29 wild relatives and two varieties of cultivated pigeonpea listed in Table 2) were used in the present study. Of the 29 accessions of wild realtives of pigeonpea, 12 accessions belongs to *Cajanus scarabaeoides,* two each to *C. acutifolius*, *C. albicans*, *C. cajanifolius*, *C. lineatus*, *C. sericeus*, and one each to *C. platycarpus*, *Rhyncosia bracteata*, *R. aurea*, *Dunbaria ferruginea*, *Flemingia stricta*, *Paracalyx scariosa*, and *F. bracteata*. Two cultivars belonging to cultivated pigeonpea, ICPL 87 (susceptible check) and ICPL 332 (resistant check) were included as controls. The crop was raised during 2000-2003 rainy seasons under rainfed conditions. The seeds were sown with a spacing of 30 cm on ridges, 75 cm apart, on deep black Vertisols in a complete randomized block design. Each entry was sown in a 4-row plot of 2m long. To enhance water absorption and faster germination, the seeds were scarified at base and soaked in water for 24 h and treated with thiram @ 1 g per Kg of seed . Normal agronomic practices were followed for raising the crop (basal fertilizer N: P: K :: 100: 60: 40, and top dressing with urea @50 kg ha^{-1} 40 days after germination). The plants were irrigated occasionally and weeding operations were carried out as and when needed.

Morphological traits

Observations were recorded on the morphological traits as per morphological and taxonomic descriptors (ICRISAT, 1993). Data were recorded on 15 plants selected at random plants from each accession.

Table-2: Accessions of wild relatives of pigeonpea used in the present study

ICP number	ICPW number	Species	Origin
ICP 15602	ICPW 1	*C. acutifolius*	Australia
ICP 15603	ICPW 2	*C. acutifolius*	Australia
ICP 15614	ICPW 13	*C. albicans*	Karnataka, India
ICP 15615	ICPW 14	*C. albicans*	Andhra Pradesh, India
ICP 15629	ICPW 28	*C. cajanifolius*	Madhya Pradesh, India
ICP 15630	ICPW 29	*C. cajanifolius*	Andhra Pradesh, India
ICP 15641	ICPW40	*C. lineatus*	Karnataka, India
ICP 15642	ICPW 41	*C. lineatus*	Tamil Nadu, India
ICP 15760	ICPW 159	*C. sericeus*	Maharastra, India
ICP 15671	ICPW 160	*C. sericeus*	Maharastra, India
ICP 15669	ICPW 68	*C. platycarpus*	Uttar Pradesh, India
ICP 15684	ICPW 83	*C. scarabaeoides*	Maharastra, India
ICP 15691	ICPW 90	*C. scarabaeoides*	Himachal Pradesh,India
ICP 15695	ICPW 94	*C. scarabaeoides*	Sri Lanka
ICP 15717	ICPW116	*C. scarabaeoides*	Sikkim, India
ICP 15726	ICPW 125	*C. scarabaeoides*	Tamil Nadu, India
ICP 15731	ICPW 130	*C. scarabaeoides*	Andhra Pradesh, India
ICP 15738	ICPW 137	*C. scarabaeoides*	Orissa, India
ICP 15742	ICPW 141	*C. scarabaeoides*	Australia
ICP 15753	ICPW 152	*C. scarabaeoides*	Betuta-Rote island, Indonesia
ICP 15879	ICPW 278	*C. scarabaeoides*	Flores Island, Indonesia
ICP 15881	ICPW 280	*C. scarabaeoides*	Flores Island, Indonesia
ICP 15882	ICPW 281	*C. scarabaeoides*	West Tripura, India
ICP 15779	ICPW 178	*D. ferruginea*	Tamil Nadu, India
ICP 15793	ICPW 192	*F. bracteata*	Indonesia
ICP 15803	ICPW 202	*F. stricta*	Andhra Pradesh, India
ICP 15808	ICPW 207	*P. scariosa*	Maharastra, India
ICP 15815	ICPW 214	*R. bracteata*	Andhra Pradesh, India
ICP 15811	ICPW 210	*R. aurea*	Andhra Pradesh, India
ICP 14770	ICPL 87	*C. cajan*	ICRISAT, India
ICP 11543	ICPL 332	*C. cajan*	ICRISAT, India

Fig - 1: Accessions of wild relatives of pigeonpea in the field

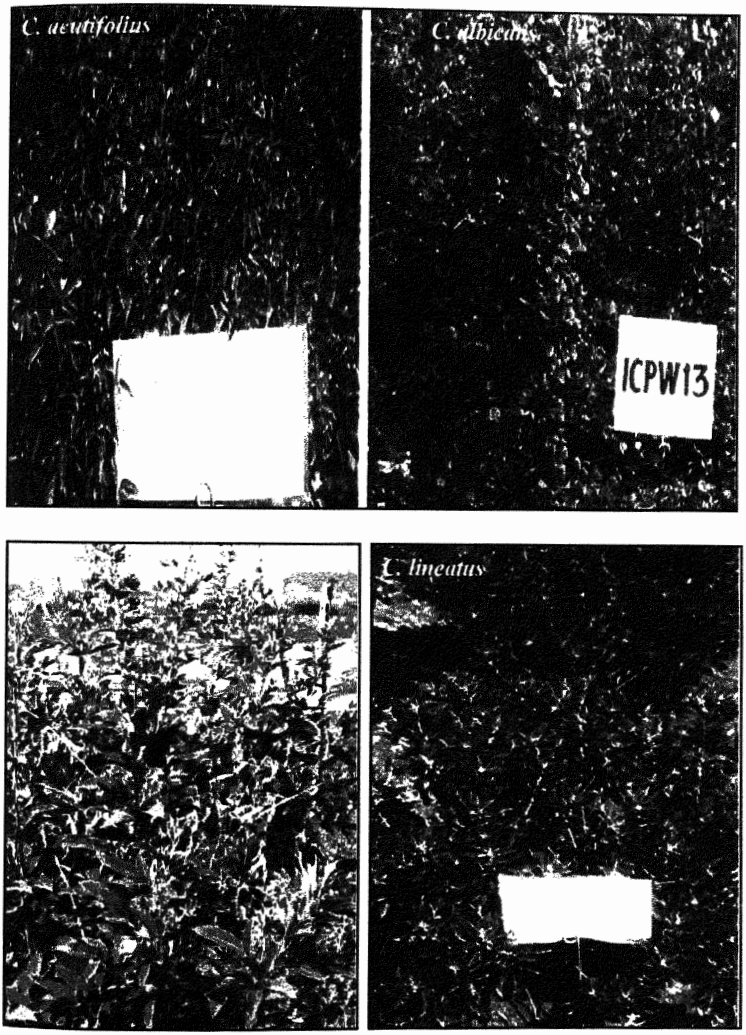

Fig-2: Accessions of wild relatives of pigeonpea

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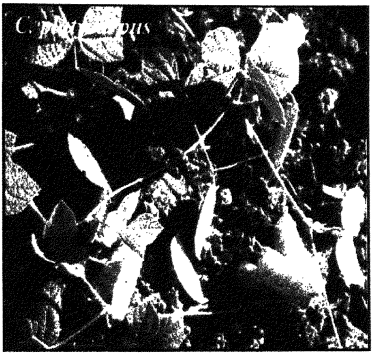
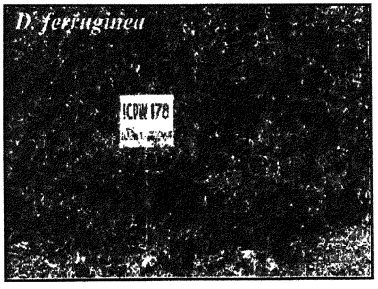
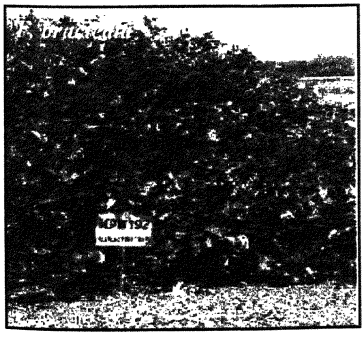

C. pla

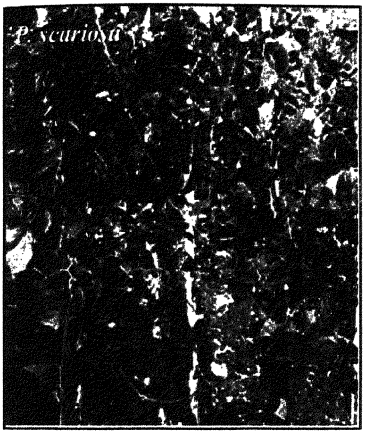

C. scutellaceoides


D. ferruginea
ICRW 178


F. brunescens


F. sinicia

Contd..


P. scuriosa


P. cujan (1C PL 87)

Growth habit

Plant growth habit – climber or erect

Days to 50% flowering

Days to 50% flowering were recorded as the number of days from the day of seedling emergence to 50% of flowering for each accession.

Leaf area

Leaf area was measured in a sample of five fully expanded leaves (from three plants) taken at random from the upper portion of plant at the time of flowering. Leaf area (mm^2) was measured using a Delta–T automatic leaf area meter.

Pod length and width

Pod length and width were measured for fives pods chosen at random from all the five plants. Pod length and width were recorded in centimeters (cm). The average value was taken as the pod length and width for a particular accession.

Pod surface area

Pod surface area was measured using the leaf area meter. Five mature pods were collected from each of the five selected plants and the area was recorded by passing through the Delta–T automatic leaf area meter.

Number of locules per pod

Number of locules per pod were recorded in five mature pods chosen at random from five plants were used in the study, same pods were used for the length and width measurements.

Number of seeds per pod

Number of seeds per pod were collected by spiltting open the pods.

100 seed weight

Seed harvested from plants belonging to the same accession was pooled and the weight of 100 seeds taken at random was recorded using a Mettler balance.

Trichomes

Trichomes are the most common morphological structures, which play an important role in the insect-host plant interactions in pigeonpea, and the variation in their form and function are quite often associated with plant resistance to insect attack (Southwood, 1986). Hence, the study was carried out to identify different types of trichomes and their density in wild relatives of pigeonpea. The presence of trichomes on pods and calyxes was recorded by collecting a minimum of 15 pods and flowers from each accession, and there were three replications. The material was preserved in a fixative (Acetic acid: absolute alcohol:: 1: 3) and examined under a Zeiss Stereomicroscope (Carl Zeiss, Inc., Thornwood, NY) at a magnification of 32X with an ocular measuring grid.

Screening for pod borer resistance under field conditions

In all, thirty one accessions of wild relatives of pigeonpea, including two cultivars (ICPL 332- resistant check, and ICPL 87–susceptible check) were screened in the field under multi-choice conditions to evaluate their relative resistance/susceptibility to *H. armigera*. The material was grouped into three experiments based on maturity (early $\leq$60 days, medium 60 to 120 days, and late $\geq$120 days to flowering). The crop was raised during 2001 to 2003 under rain fed conditions as desribed earlier. Experiment was planted such that the material is exposed to the peak abundance of *H. armigera*. Wooden

pegs (1.5 m) were provided as a support for accessions of *C. scarabaeoides, C. platycarpus* and *R. aurea* which have a creeping habit.

Data on oviposition by *H. armigera* females was recorded for the accessions flowering at same time. In each plot five infloresences of 10 cm long were tagged with a ribbon at the pre-flowering stage. Egg and larval numbers of *H. armigera* were recorded on the tagged portion of the infloresences, on the 5, 7, 9, 20 and 30 days after tagging the inflorescence. The total number of pods and the pods damaged by pod borer were recorded at maturity in pods harvested from tagged inflorescences from each plot.

Statistical analysis

The data recorded for the above traits were subjected to ANOVA

Mechanisms of resistance to *H. armigera*

Maintenance of Insect culture

Larvae of *H. armigera* used in the present experiments were obtained from the laboratory culture maintained at ICRISAT, Patancheru, India. The culture was established by regularly supplementing with field-collected larvae. Larvae were reared on chickpea based diet (Armes *et al.*, 1992) at ambient temperature (27± 2^{O}C) and relative humidity (65± 5%) (Table 3 & Fig 3). Adults were confined in a rearing cage (36 x 36x 30 cm) and provided with nappy liners as substrate for oviposition. The moths were provided with 10% honey as food on cotton wool. Eggs laid on nappy liners were treated with 1% sodium hypochlorite solution. Neonates emerging from these eggs were used for carrying out the experiments (Fig 4).

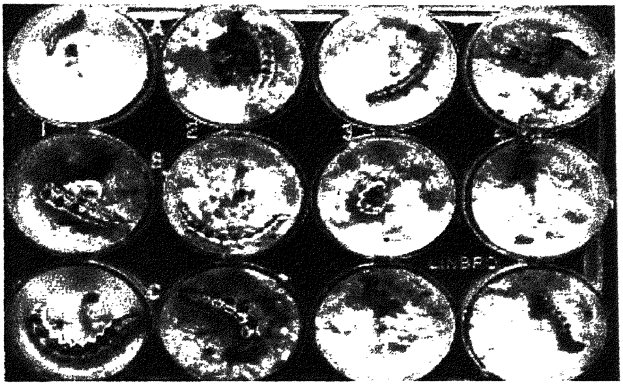
Diet

For preparing the chickpea based diet for insect culture all the ingredients (Table 3) were weighed and placed separately. The ingredients A to F and H were mixed

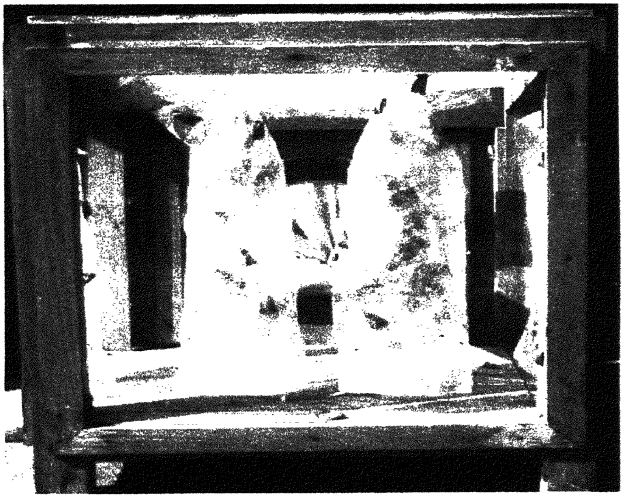
throughly in water (G) in a large bowl of 2 L capacity by using a hand mixer. The agar-agar was mixed with water (J) and heated in saucepan on a hot plate. The boiled agar-agar was mixed with other ingredients in a plastic bowl and stirred until an even consistency was obtained. This hot diet (5 cm layer) was poured into stainless steel trays placed on a level surface. The diet in the trays was allowed to cool, and then the trays were wrapped in a polyethylene sheet to avoid contamination. As and when needed, the diet was cut into 3 cm square pieces and placed in plastic cups (150 ml capacity) for rearing the larvae.

Table-3: Chemical composition of diet for rearing *H. armigera* larvae

Ingredients	**Quantity**
A Chickpea flour	300.0 g
B. Ascorbic acid	4.7 g
C. Methyl-p- hydroxybenzoate	5.0 g
D. Sorbic acid	3.0 g
E. Auromycin powder	11.5 g
F. Vitamin stock solution	10.0 ml
G. Water	450.0 ml
H. Yeast	48.0 g
I. Agar	17.3 g
J. Water (Agar)	800.0 ml
Vitamin stock solution	
Nicotinic acid	1.528 g
Calcium pantothenate	1.528 g
Riboflavine	0.764 g
Aneurine hydrochloride	0.382 g
Pyridoxine hydrochloride	0.382 g
Folic acid	0.382 g
D-Biotin	0.305 g
Cyanocobal amine	0.003 g
Water	500.0 ml



Culture vials



Oviposition cage

Fig- 3: *Helicoverpa armigera* culture vials and oviposition cage

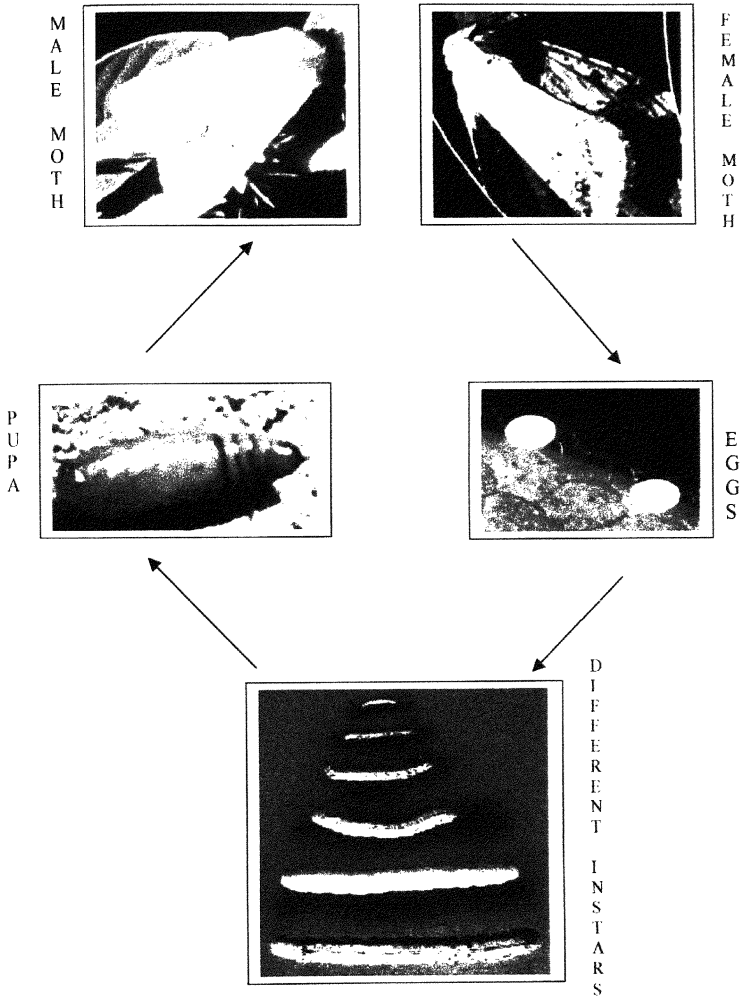


Fig – 4: Life cycle of *H. armigera*

Antixenosis/nonpreference for oviposition

Antixenosis or oviposition non-preference was studied under no-choice, dual-choice and multi-choice conditions (Fig 5).

In the no-choice test, the moths were confined with inflorescences collected from field of the same species/genotype, in a wooden cage (36 x 36 x 30 cm). The sides of the cage were covered with a fine wire-mesh, except in the front, where a wooden door fitted with a cloth bag was provided for releasing the moths. Five inflorescences(10 cm long) were kept in a conical flask filled with water to keep them in a turgid condition. A cotton swab was wrapped around the inflorescences to keep them in an upright position. Five pairs of newly emerged male and female moths were released in each cage. The moths were provided with 10% sucrose solution in a cotton swab as food. Fresh inflorescences were provided for oviposition everyday. Observations on oviposition were recorded for three consecutive days, two days after the releasing moths in the cage (pre-oviposition period).

Oviposition studies were conducted under dual choice conditions by offering a choice to the female moths between the susceptible check, ICPL 87 and the test variety. Experimental details were same as described above. For comparision of each test variety with the susceptible check there were five replications.

Non-preference for oviposition was also studied under multi-choice conditions by keeping the inflorescences of all the 29 test varieties, along with the susceptible and resistant checks, together in a large cage (80 x 70 x 60 cm) in an environmental chamber under controlled conditions (temperature day/night: 26/20^{0}C, relative humidity 70%, and photoperiod 12 h). Fifty pairs of newly emerged moths were released into the cage. The inflorescences were arranged in a randomized block design. Fresh inflorescences, collected from the field were provided to the moths daily for oviposition. Moths were fed with 10% sucrose solution in a cotton swab. Observations on oviposition were recorded 2 days after releasing the moths in a cage on each inflorescence for three consecutive days

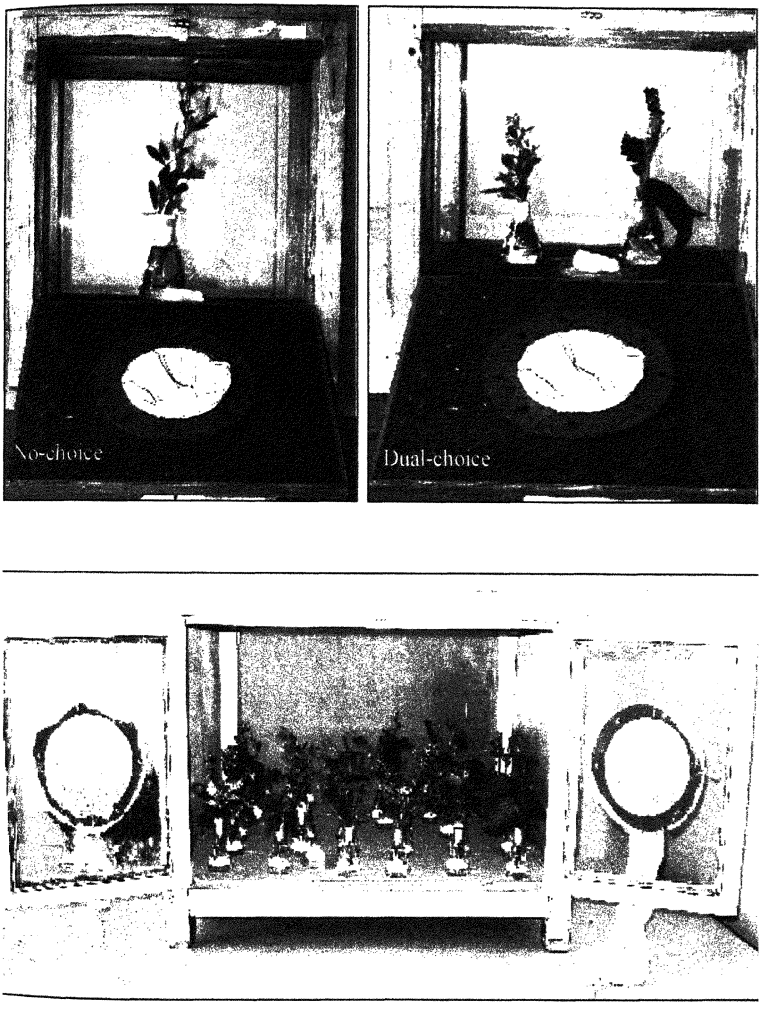


Fig- 5: Antixenosis / non-preference for oviposition under different conditions

Statistical analysis

Data recorded for oviposition under no-choice and multi choice conditions were subjected to ANOVA, while the data for dual-choice test was subjected to paired 't' test.

Antibiosis

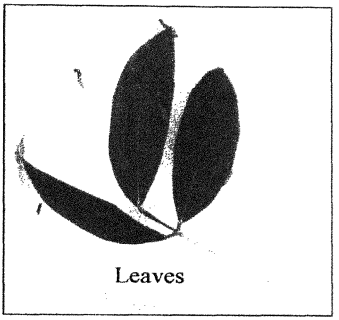
The antibiosis component of resistance was studied under *in vivo* (leaves, and flowers and pods) and *in vitro* (lyophilized leaf and pod powder impregnated in artificial diet) conditions (Fig 6). Data were recorded on larval survival, larval and pupal weights, percentage pupation, and adult emergence, and post-embryonic developmental period.

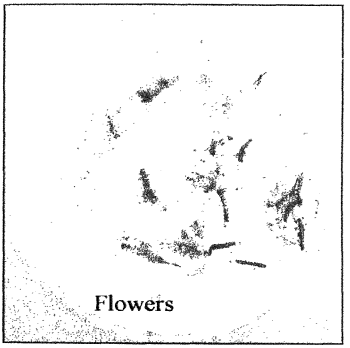
Development and survival of *H. armigera* on leaves

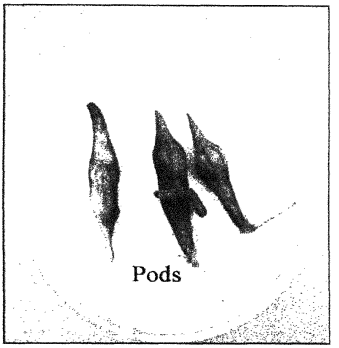
The development and survival of neonate larvae of *H. armigera* were studied on fresh leaves obtained from the upper portion of plants raised in the field. There were five replications for each accession, and there were 10 larvae per replication. The leaves were kept afresh by wrapping the petiole in a wet cotton swab. The first-instar larvae were transferred on to leaves in petri dishes with the help of a fine camel hair brush. First and second instars were kept in groups of five per petridish, while the later instars were reared individually to avoid cannibalism. The leaves were changed on alternate days. Observations were recorded on larval and pupal periods, weights and percentage mortality. Pupal weights were recorded one day after the pupation. The experiment was conducted at 27 ± 3°C. Data were subjected to analysis of variance to test the significance of differences between treatments using the F-test, and the treatment means were compared using least significant difference at $P < 0.05$.

Development and survival of *H. armigera* on flowers and pods

Inflorescences with flowers and pods collected from the test genotypes were placed on a moist filter paper in petri dishes. First-instar larvae were transferred on to flowers in the petri dish with the help of a fine camel hairbrush. The food was changed every alternate







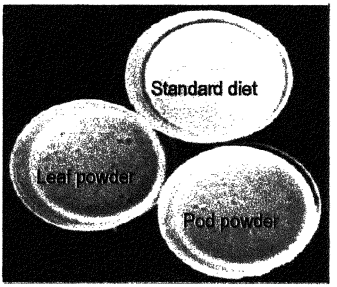


Fig – 6:Antibiosis: Growth and development of *H. armigera* larvae

day. There were five replicates of each variety with 10 larvae per replication.Larvae were first reared on flowers for seven days, and later were fed on pods of the same accession. The first-instar larvae were kept in groups of five per petri dish, whereas the grown up larvae (>7 days old) were reared individually. Observations were recorded on larval and pupal periods, percentage pupation and adult emergence. Observations on larval survival and larval weights were recorded at an interval of five days till 15 days after initiating the experiment. Pupal weights were recorded one day after pupation. Analysis of variance was used to compare differences in development periods and weights of larvae and pupae on the test cultivars as stated above.

Development and survival of *H. armigera* larvae on artificial diet impregnated with lyophilized leaf powder of wild relatives of pigeonpea

The antibiosis component of resistance to *H. armigera* in wild relatives of pigeonpea was evaluated by rearing the neonate larvae on artificial diet impregnated with powdered lyophilized leaves. Observations were recorded on larval survival, larval and pupal weights, percentage pupation and adult emergence, and post-embryonic developmental period.

Leaves of the test genotypes were collected from 50 to 55 day old plants raised in the field. The leaves were freeze-dried in a lyophilizer for 36 h to avoid changes in chemical composition of the leaves. The leaves were then powdered in a Willey mill and stored in a dessicator till used.

To know the optimum amount of leaf powder needed in the artificial diet to measure the antibiotic component of resistance in different accessions of wild relatives of pigeonpea, different proportions of leaf powder (Table 4) of the cultivated pigeonpea genotypes (ICPL 332 - resistant, and ICPL 87- susceptible) and the wild relative, *C. scarabaeoides* (ICPW 83-resistant) were added into 250 ml artificial diet. The lyophilized leaf powder was soaked in 100 ml warm water of fraction B of the artificial diet, and then blended with fraction A (Table 2) for 2 minutes. Agar-agar was boiled in

Table- 4: Composition of artificial diet impregnated with different concentrations of lyophilized leaf / pod powders for assessment of antibiosis to *H. armigera*

Ingredients	Quantity (g)				
Chickpea flour	75	70	65	60	55
Leaf / pod powder	-	5	10	15	20

100 ml of water (fraction B) and then poured into the blender containing fraction A. Finally, all the constituents were blended for 2 minutes, and 10 ml of this diet mixture was poured into small plastic cups (25 ml capacity). Each treatment was replicated thrice (10 larvae in each replication). The larvae were obtained from the insect culture maintained on chickpea flour based diet in the laboratory at ICRISAT (Armes *et al.*, 1992). The first-instar larvae were released into the cups with a fine camel hairbrush. Data were recorded on larval survival, and larval weights on 10^{th} day after releasing the larvae onto the diet.

Maximum differences in larval survival and larval weights were observed when 10 g of lyophilized leaf powder was added into the artificial diet. Hence, it was concluded that 10 g leaf powder could be used to measure the antibiosis mechanism of resistance to *H. armigera.*

For further experiments, leaf powder from all the test genotypes was bioassayed by impregnating 10 g leaf powder into the artificial diet (Table 5). The diet was prepared as described above. There were three replications for each test genotype (10 larvae in each replication). First-instar larvae were released into the cups with the help of a fine camel hairbrush. The rearing cups were kept at 27 ± 2^0C, RH 65 $\pm$ 5%, and 12 h photoperiod. Data were recorded on larval survival and weights on 10^{th} day after releasing the larvae into artificial diet. Pupal weights were recorded one day after pupation. Data were also recorded on larval and pupal periods. Percentage pupation and adult emergence was computed in relation to the total number of larvae released into the

Table-5: Chemical composition of artificial diet impregnated with lyophilized leaf /pod powder for assessment of antibiosis to *H. armigera*

Fraction A	**Quantity**	
Chickpea flour	65.00	g
Ascorbic acid	1.175	g
Methyl-p- hydroxybenzoate	1.25	g
Sorbic acid	0.75	g
Auromycin powder	2.875	g
Vitamin stock solution	2.5	ml
Water	112.5	ml
Yeast	12	g
Agar	17.30	g
Fraction B		
Agar-Agar	4.375	g
Water (for yeast/Agar)	200	ml
Leaf powder / pod powder	10	g

artificial diet. The data were subjected to analysis of variance to test the significance of differences between treatments by F-test, and the treatment means were compared by least significant difference at P = 0.05.

Development and survival of *H. armigera* larvae on artificial diet impregnated with lyophilized pod powder of wild relatives of pigeonpea

To study the antibiosis component of resistance to *H. armigera* in pods, the larvae were reared on artificial diet impregnated with lyophilized pod powder of different accessions of wild relatives of pigeonpea. Ten grams of lyophilized pod powder of different accessions of wild relatives of pigeonpe was impregnated into the artificial diet (Table 5) and data were recorded on larval survival, and larval weights on 10^{th} day after releasing the larvae onto the diet as described above.

Feeding preference of the third-instar larvae of *H. armigera* on the leaves and pods of wild relatives of pigeonpea under no-choice and multi-choice conditions

The relative feeding preference by the third-instar larvae of *H. armigera* towards wild relatives pigeonpea was studied under no choice and multi-choice conditions including both leaves and pods.

No- choice conditions

Under no-choice conditions, the larvae were confined with the leaves or pods of only one genotype. The experiment was carried out by keeping the leaves or pods of a test genotype in a petri dish of 7.5 cm diameter. A single third-instar larva was released into each petri dish. To keep the test material afresh, a moistened filter paper (with 2 ml of water) was placed inside lid of the petri dish. There were twenty replications for each accession. Observations on percentage damage to the leaves and pods were recorded visually on a 1 to 9 scale at 24 and 48 h after initiating the experiment [damage rating (DR); 1 = <10% pod area damaged, 2 = 11 - 20%, 3 = 21 - 30%, 4 = 31 - 40%, 5 = 41 - 50%, 6 = 51 - 60%, 7 = 61 - 70%, 8 = 71 - 80%, and 9 = >80% leaf or pod area damaged]. The data were subjected to analysis of variance as indicated above.

Multi-choice conditions

For multi-choice tests, the test varieties were grouped into 5 sets each with 6 accessions of wild species and one susceptible cultivar, ICPL 87. The experiments were carried out in a glass petri dish (20 cm diameter, and 2.5 cm high). The pods of the test genotypes were kept in a circular arena, and 10 third-instar larvae were released in the center of the petri dish, and allowed a choice to select their food. The larvae were starved for 4 h before releasing into the petri dish arena. Pod feeding was recorded on a 1 to 9 scale at 24 and 48 h after initiating the experiment based on the visual damage to the pods [damage rating (DR); 1 = <10% and 9 = >80% leaf or pod arae damage]. The

experiment was repeated thrice and the data were subjected to analysis of variance as described above.

Role of pod surface chemicals on feeding by the third-instar larvae of *H. armigera*

To study the effect of chemicals on pod surface of pigeonpea and its wild relatives on feeding behavior of *H. armigera* larvae, the field collected pods were washed with polar (water and methanol) and non-polar (hexane) solvents for 2 to 3 minutes to remove the pod surface chemicals by placing the pods in the solvents individually and stirred with a glass rod for 2 minutes. The washed pods were air dried for 3 h in the laboratory to evaporate the solvent from the surface of pods. The pods were then offered to larvae to study their food selection behavior, which was evident from the extent of pod feeding under no-choice and dual-choice conditions.

No-choice assay

No-choice bioassays were carried out by releasing a single third-instar larva in to a 7.5 cm petri dish area with a single washed pod or un washed pod. To keep the pod afresh, a moistened filter paper (with 2 ml of water) was placed inside the lid of the petri dish. There were twenty replications for each accession, and solvent washing treatment. The tests were also carried out with the unwashed pods. Observations on percent pod damage were recorded visually on a 1 to 9 scale at 24 and 48 h after initiating the experiment [damage rating (DR); 1 = <10% pod area damaged, and 9 = >80% pod area damaged]. The data were subjected to analysis of variance as described above.

Dual-choice assay

Dual-choice bioassays were carried out by providing the larvae a choice between the washed and unwashed pods of the same accession. There were twenty replications for each accession (for comparison between washed and unwashed pods of the same accession). Observations were recorded on percentage pod damage at 24 and 48 h after

releasing the larvae. Significance of differences between the treatments was judged by paired 't' test.

Bioassay of pod surface extracts

The pod surface extracts were bioassayed using glass fiber discs of 3.44 mm diameter as feeding substrate for the larvae. The test discs were impregnated with 100 µl of solvent extract by using a micropipette, while the control discs were left un treated. The discs were air dried for 24 h. Later, each disk area was measured by passing through area meter and positioned 5 mm apart in an apposed arrangement on a thin waxy layer in the center of a 9 cm diameter petri dish. The waxy layer was covered with a filter paper. Both the discs were moistened with 100 µl of distilled water, as *H. armigera* larvae were found less likely to feed on the dry discs (Stevenson *et al.*, 2002). The larvae were deprived of food for 4h prior to the bioassay.

The experiment was carried out with three different instars (third, fourth and fifth). To ensure the uniformity of age, the larvae were reared separately on artificial diet. A single larva of known age was released into each petri dish arena, and the experiment was maintained at 27 ± 2°C temperature. Twenty replicates were maintained. After 24 h of initiating the experiment, the larvae were removed from the petri dishes and the discs were dried and the surface area was measured to calculate the area of disc consumed by the larvae. The data was subjected to paired 't'-test.

Biochemical composition in the leaves and pods of wild pigeonpea relatives

Total soluble sugars

For estimating the total soluble sugars in the leaves and pods of pigeonpea and its wild relatives, the material was extracted with hot aqueous-ethyl alcohol. On treatment with phenol sulphuric acid, the sugars produced a stable and sensitive golden yellow color (Dubois *et al.*, 1956). The absorbance of the golden yellow color was measured at

490 nm, which was used to estimate the percentage of total soluble sugars present in the leaves and pods.

The leaves and pods of the test varieties were collected from the crop raised in the field, and were oven dried for 12 h. The oven-dried material was powdered in a Willey mill and defatted by using hexane. 80% ethyl alcohol, 5% phenol, 96% sulphuric acid (specific gravity 1.84), glucose standard (stock solution: 1000 mg/1000 ml) and glucose working standard (12.5 ml of stock standard pipetted into 100 ml volumetric flask, and volume made up to 100 ml, to have the final concentration of 125 µg/ml) were used for estimating the total soluble sugars.

From the defatted material, 100 mg sample was weighed into a boiling test tube, to which 25 ml of 80% hot ethanol was added. The mixture was shaken vigorously on a vortex mixer. The material was allowed to settle for 30 minutes and the supernatant was filtered through Whatman No. 41 filter paper. This step was repeated thrice for complete extraction of sugars. The ethanol was completely evaporated by placing the extract on hot sand bath. After removal of ethanol, 3 ml of water was added to dissolve the contents. One ml of the above solution was pipetted into a test tube, to which 1 ml of 5% phenol and 5 ml of 96% sulphuric acid were added. The mixture was shaken vigorously on a vortex mixer. The tubes were allowed to cool in cold water. A blank was prepared by taking 1 ml of water. Absorbance of the golden yellow color was read at 490 nm using Spectronic 21. Standards with concentrations of 25, 50, 75, 100, and 125 µg of glucose were prepared from the working standard and recorded their absorbance by taking 1 ml aliquotes.

Percent total soluble sugars were calculated by using the formula:

$$\frac{\text{Conc. of std.}}{\text{Absorbance of std.}} \times \text{Absorbance of 1 ml extract} \times \frac{1}{1000000} \times \frac{3\text{ ml}}{0.1\text{g}} \times 100$$

Total polyphenols

The total amounts of polyphenols present in the leaves and pods of pigeonpea and its wild relatives were estimated by Folin Denis method (OAAOAC, 1984).

Folin Denis reagent [100 gm of sodium tungstate (Na_2WO_4 $2H_2O$), 20 g phosphomolybdic acid and 50 ml phosphoric acid were dissolved in 750 ml of water. The mixture was refluxed for 2 h and the final volume was made to 1 L by adding water]; saturated sodium carbonate solution [45 g of anhydrous sodium carbonate was dissolved in 100 ml of water, at 70 – 80°C and allowed to cool overnight. The solution was seed supersaturated with Na_2CO_3 crystals filtered through glass wool after crystallization]; tannic acid standard solution [tannic acid standard was prepared by dissolving 100 mg tannic acid in 1 L water and fresh solution was prepared for each determination]; and methanol-HCl [10 ml concentrated hydrochloric acid was added to methyl alcohol and the final volume was made to 1 litre] were used for estimating the phenols.

To carry out the phenol estimation, 100 ml of methanol-HCl was added to 200 mg of defatted material in a round bottommed flask. This mixture was refluxed for two hours, and allowed to cool. The extract was filtered through Whatman No. 40 filter paper into 100 ml volumetric flask, and the volume was made to 100 ml with methanol-HCl by a few washings.

For estimation of polyphenols, 0.2 ml extract, 0.5 ml of Folin Denis reagent and 1 ml of saturated sodium carbonate solution were added in a test tube and the final volume was made to 10 ml with water and vortexed. After vortexing, the absorbance was read at 760 nm using Spectronic 21. A standard curve was prepared by pipetting 0 - 1 ml aliquots of standard tannic acid solution at intervals of 0.2 ml for expressing the results in terms of milligrams per liter of tannic acid. Using the standard curve, the results were expressed as mg tannic acid equivalent/g sample.

Estimation of tannins

The amount of tannins present in the leaves and pods of wild relatives of pigeonpea were estimated by Vanillin-Hydrochloric acid method (Price *et al.*, 1978) .The following reagents were used in the present study.

1. 8% HCl in methanol (v/v): 8 ml conc HCl in methanol and makde upto 100ml
2. In methanol 1 gm of Vanillin was dissolved and final vol. was made to 100 ml
3. Vanillin-Hydrochloric acid reagent: Equal volumes of solution 1 and 2 are mixed before use.
4. 4% hydrochloric acid in methanol(v/v): 4 ml conc HCl in 96 ml methanol.
5. 1% hydrochloric acid in methanol (v/v): 1 ml conc. HCl in 99 ml methanol.
6. Standard solutions: A stock solution is prepared by dissolving 1 mg of catechin in 1ml of methanol.

From the defatted material, 100 mg is transferred to a centrifuge tube containig 2 ml of 1% acidic-methanol, centrifuged for 10 min. and the aliquot is transferred to a 5 ml volumetric flask. This step was repeated by adding 1 ml of (1%) acidic-methanol. The aliquot was transferred to the first extraction and the final volume of 4 ml.

From the above extract 1 ml was pipetted out into a test tube and to it freshly prepared vanillin-HCl reagent was added slowly. An individual blank was prepared for each extract by adding 5ml of 4% HCl in methanol to 1m ml aliquot. Finally the absorbance was recorded at 500nm against the reagent blank in a spectrophotometer.

Standard curve is prepared by plotting the average absorbance readings of the duplicate determinations of catechin concentrations.The catechin equivalents are caluculated by using the formula

$$CE\ (\%) = \frac{(\text{mg catechin/ml})}{\text{Vol. of extract taken}} \times \frac{\text{volume made up}}{\text{wt. of sample}} \times 100$$

Protein estimation

Protein content in the pods of wild relatives of pigeonpea was estimated by using Lowry's method from 100 mg of defatted material.

The following reagents were used:

Reagent A: 2% Sodium carbonate in 0.1N NaOH.

Reagent B: a) Copper sulphate solution.

b) Sodium potassium tartarate solution

Reagent C: Alkaline copper soluution.

Reagent D: Folin Ciocalten reagent with a dilution of 1:1 (15 ml of distilled water + 15 ml Follins reagent).

Working standards: Bovine serum albumin diluuted to 100 to 1000 fold.

A total of 300 µl of sample was prepared. From the sample, 20µl of the supernatant was pipetted out and to it 2.5 ml of solution D and 250 µl of solution E were added. The ingredients were incubated at room temperature for 30 min and protein was estimated at 600 nm. Bovine Serum Albumin (BSA) was used as standard at a concentration of 2mg/ml. Protein content in each sample was calculated from the standard graph.

High performance liquid chromatography (HPLC) analysis of pod surface extracts of wild relatives of pigeonpea

Preparation of crude extracts

The pigeonpea pods (125 gms) were extracted in 500 ml of methanol and hexane solvents for 2 min at room temperature. The extract was filtered through Whatman No. 1 filter paper and the solvents were evaporated under reduced pressure. This crude was redissolved in 5 ml of solvents. These extracts were used for the analysis of flavonoids by Reverse phase HPLC (Fig 7). The chromatographic system used in this study

consisted of dual Shimadzu (Kyoto, Japan) LC–10 ATVP high-pressure pumps, a Shimadzu SIE–10ADVP automatic injector, a Shimadzu SCL–10AVP integrated system controller, a Symmetry ® C18 reverse-phase analytical column (250 × 4.6 mm, RP-18, 5-μm particle size) and a Shimadzu SPD-M 10 AVP diode array detector with an attached HP analysis computer and data storage system. The gradient elution schedule consisted of an initial 2-min run of 75% of 2% acetic acid and 25% methanol followed by a linear gradient to 100-percent methanol over 55 min at a flow rate of 1 ml/min.

The mobile phase was a mixture of methanol (A) and 2.0 % (v/v) acetic acid with a gradient elution. The condition is shown in the following table (Table 6).

Table - 6: HPLC analysis of compounds in methanol and hexane pod surface extracts of pigeonpea and its wild relatives.

Time (min)	Methanol (%)	Acetic acid (2%) (%)
0	25	75
20	100	0
30	100	0
35	25	75
45	25	75
55	25	75

The flow rate was 1.0 ml/min with a detecting wave length of 254 nm.

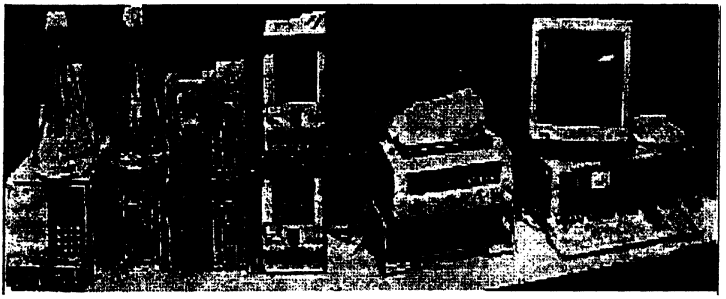


Fig- 7: HPLC instrument

Statistical methods

Analysis of variance

Analysis of variance was done for each parameter separately. The significance of differences between the genotypes was tested by F-test, and the treatment means were compared using LSD (least significant difference) at P>0.05 level (Steele *et al.*, 1997).

Correlations

Changes in one variable may be accompanied by changes in the other, indicating the relationship between the two variables. Correlation coefficient (r) is the measure of direction and degree of closeness of the linear relationship between two variables. Simple correlation coefficients, among different characters were calculated using the formula suggested by Panse and Sukhatme (1967).

$$\text{Correlation coefficient } (r) = \frac{\sigma XY}{\sigma X.\sigma Y}; \quad \sigma XY = \frac{\varepsilon f.\, dx.\, dy}{N}$$

σXY = The co- variance between X and Y

σX = standard deviation of X, σY = standard deviation of Y

dx and dy= deviations.

Significance of correlation coefficient

The significance of correlation coefficients was tested by comparing the observed values of correlation coefficients with that of the table values of correlation coefficients (Gomez and Gomez, 1984) for (n-2) degrees of freedom.

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

r is the estimate obtained from n pairs and compared to the standard 't' value at 5% and 1% levels of significance (Snedecor and Cochran, 1968).

Results

Results

In the present investigation, 31 accessions of wild relatives of pigeonpea, belonging to 14 species (including two cultivated varieties, ICPL 332, the resistant check, and ICPL 87, the susceptible check, of *Cajanus cajan*) were evaluated for mechanisms of resistance to pod borer (Table 2). During the course of this investigation, the morphological evaluation of these accessions, identification of various physico-chemical factors associated with resistance to pod borer, and studies on characterization of different mechanisms of resistance were carried out. The accessions were evaluated for certain morphological and agronomical characteristics viz; growth habit, leaf surface area, days to 50% flowering, pod length, pod width, pod surface area, number of locules and seeds per pod, 100 seed weight and trichomes (Tables 7, 8 & 9).

Morphological characterization

Growth habit

The species; *Cajanus acutifolius, C. lineatus* and *Flemmingia stricta* have upright stems and semi-spreading growth habit, while *C. albicans*, *Dunbaria ferrugeniea*, *Paracalyx scariosa,* and *Rhyncosia bracteata* are climbers. *Cajanus scarabaeoides*, *C. platycarpus,* and *R. aurea* are creepers, and *C. cajanifolius*, *C. serecius, F. bracteata,* and *C. cajan* are upright in habit.

Leaf surface area

Significant differences were observed in the leaf surface areas among the species tested, where the differences were not large within the species. The leaf surface area of *C. sericeus* [ICPW 159 (1.21 mm^2), and ICPW 160 (1.26 mm^2)] was the lowest, followed by *C. acutifolius* [ICPW 1 (2.51 mm^2), and ICPW 2 (2.95 mm^2)]. Leaf surface area was quite large in *R. bracteata* [ICPW 214 (45.87 mm^2)], *P. scariosa* [ICPW 207 (45.96 mm^2)], *D. ferruginea* [ICPW 178 (58.64 mm^2)], and *F. bracteata* [ICPW 192 (47.79 mm^2)]. Maximum leaf surface area (194.24 mm^2) was recorded in *F. stricta* (ICPW 202).

Table - 7: Data on morphological traits of wild relatives of pigeonpea.

Species	Accession number	Habit	Leaf surface area (mm)	Days to 50% flowering	Pod length (cm)	Pod width (cm)	Pod surface area (mm)	No. of locules/ pod	No. of seeds/ pod	100 seed weight (g)
C acutifolius	ICPW 1	Ss	2.51	167	1.84	0.98	2.45	2.8	2.8	2.71
C acutifolius	ICPW 2	Ss	2.95	158	2.16	0.76	2.15	2.6	2.6	2.76
C albicans	ICPW 13	Cl	14.85	160	3.72	1.02	5.78	6.0	6.0	2.30
C albicans	ICPW 14	Cl	14.84	154	3 48	1.04	5.00	5.4	5.4	2.25
C cajanifolius	ICPW 28	Es	17.80	173	3.30	0.86	3.70	3.6	3.6	5.14
C cajanifolius	ICPW 29	Es	17.32	183	3 18	0.76	3.20	3.4	3.4	7.40
C lineatus	ICPW40	Ss	4.44	179	2.06	0 72	2.46	2.6	2.6	2 08
C lineatus	ICPW 41	Ss	3.98	187	1.94	0.70	2.05	2.4	2.4	1.14
C sericeus	ICPW 159	Es	1.21	174	1 38	0.56	1.29	2 0	2 0	1.82
C sericeus	ICPW 160	Es	1.26	173	1.34	0.56	1.23	2.0	2.0	1.76
C platycarpus	ICPW 68	Cr	17.02	37	4.00	1.46	8.75	4.0	4.0	6.08
C scarabaeoides	ICPW 83	Cr	6.90	158	2.50	0.74	3.04	4.4	4.4	2.44
C scarabaeoides	ICPW 90	Cr	6.40	150	2.26	0.66	3.01	5.0	5.0	1.84
C scarabaeoides	ICPW 94	Cr	9.89	58	2.38	0.64	2.72	5.0	5.0	2.78
C scarabaeoides	ICPW116	Cr	8.74	140	2.56	0.80	3.17	4.8	4.8	2.61
C scarabaeoides	ICPW 125	Cr	10.99	139	2 40	0.74	3.06	5.2	5.2	2.18
C scarabaeoides	ICPW 130	Cr	7.64	58	2.38	0.80	2.94	4.4	4.4	2.66
C scarabaeoides	ICPW 137	Cr	6.89	59	2 34	0.76	3.17	5.0	5.0	2.01
C scarabaeoides	ICPW 141	Cr	8.28	139	2.38	0.70	2.85	4.6	4.6	2.30
C scarabaeoides	ICPW 152	Cr	8.79	58	2.40	0.70	2.86	4.6	4.6	2.92
C scarabaeoides	ICPW278	Cr	11.18	139	2.42	0.74	2.83	5.0	5.0	2.10
C scarabaeoides	ICPW 280	Cr	9.00	140	2.40	0.78	3.10	5.0	5.0	2.13
C scarabaeoides	ICPW 281	Cr	11.94	148	2.50	0.70	2.84	5.2	5.2	2.24
D ferruginea	ICPW 178	Cl	58.64	230	3.24	1.00	3.71	2.6	2.6	1.75
F bracteata	ICPW 192	Es	47.79	197	0.90	0.50	0.68	1.0	2.0	0.97
F stricta	ICPW 202	Ss	194.24	172	1.44	0.56	1.23	1.0	2.0	0.91
P scariosa	ICPW 207	Cl	45.96	167	1.10	0.66	0.73	1.0	1.0	3.98
R aurea	ICPW 210	Cr	12.72	53	1.04	1.00	1.46	2.0	2.0	2.41
R bracteata	ICPW 214	Cl	45.87	154	3.34	0.94	2.34	2.0	2.0	6.20
C cajan (S)	ICPL 87	Es	24.14	142	5.40	1.08	9.21	4.6	4.6	9.60
C cajan (R)	ICPL 332	Es	18.49	174	4.52	0.78	4.24	3.4	3.4	8.50
Mean			21.05	142.60	2.51	0.79	3.13	3.63	3.69	3.16
SE ±			1.37	0.19	0.07	0.02	0.25	0.17	0.17	0.07
LSD at 5%			3.83	0.54	0.22	0.06	0.7	0.48	0.48	0.19
Prob			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Cl – Climber. Cr – Creeper. Es - Erect stem. Ss - Upright stem and semi-spreading habit.
S - Susceptible check. R - Resistant check.

Leaf surface area of *C. platycarpus* [ICPW 68 (17.02 mm^2)], *C. cajanofolius* [ICPW 29 (17.32 mm^2) and ICPW 28 (17.80 mm^2)] was similar to that of the cultivated pigeonpeas, [18.49 mm^2 in ICPL 332 and 24.14 mm^2 in ICPL 87].

Days to 50% flowering

Days to 50% flowering varied significantly among the species, and sometimes within a species. Among the short-duration pigeonpeas, the number of days to 50% flowering were least in *C. platycarpus* [ICPW 68 (37 days)] followed by *R aurea* [ICPW 210 (53 days)], and *C. scarabaeoides* [ICPW 94, ICPW 130 and ICPW 152 (58 days)] compared to 65days of the cultivated variety, ICPL 87. Among the medium-duration accessions, the number of days to 50% flowering was recorded in ICPW 125, ICPW 141and ICPW 278 (139 days), followed by ICPW 116 and ICPW 280 (140 days) of *C. scarabaeoides*. Highest number of days to 50% flowering was recorded in *D. ferruginea* [ICPW 178 (230 days)] among the long-duration wild accessions.

Pod length

Data on pod length, width, surface area and number of locules were collected in all the accessions (Fig 8)

The pod length varied significantly among the species tested. The pod length was significantly low incase of *F. bracteata* [ICPW 192 (0.90cm)] followed by *R. aurea* [ICPW 210 (1.04 cm)], *P. scariosa* [ICPW 207 (1.10 cm)], and *C. sericeus* [ICPW 160 (1.34) and ICPW 159 (1.38)] as compared to that of ICPL 87 (5.40 cm).

Pod width

The pod width varied significantly among the species. The pod width was maximum in *C. platycarpus* [ICPW 68 (1.46 cm)] as compared to ICPL 87 (1.08 cm). The pods were narrower in *F. bracteata* [ICPW 192 (0.50 cm)]. The pod width of 0.76 cm was similar in *C. acutifolius* (ICPW 2), *C. cajanifolius* (ICPW 29), and *C. scarabaeoides* (ICPW 137).

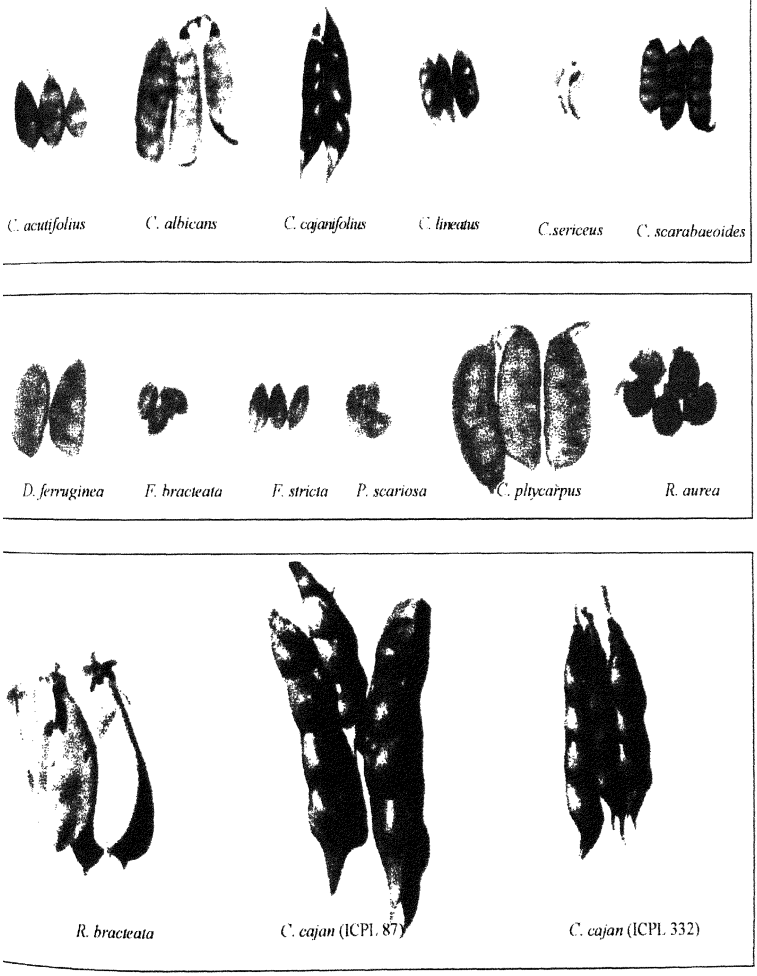


Fig – 8 : Pods of different accessions of wild and cultivated pigeonpeas

Pod surface area

There were significant differences in pod surface area among the species. However, the variation within the species was quite small.The pod surface area was smaller in *F. bracteata* [ICPW 192 (0.68 mm^2)], followed by *P. scariosa* [ICPW 207 (0.73 mm^2)] compared to the cultivated pigeonpea ICPL 87 (9.21 mm^2).

Number of locules/seeds per pod

The numbers of locules per pod varied significantly among the species. *F. bracteata* (ICPW 192), *F. stricta* (ICPW 202), and *P. scariosa* (ICPW 207) had only one locule, whereas *C. sericeus* (ICPW 159 and ICPW 160) of, *R. aurea* (ICPW 210) and *R. bracteata* (ICPW 214) had two locules per pod. The number of locules per pod was more in *C. albicans* [ICPW 13 (6.0), and ICPW 14 (5.4)] compared to the cultivated pigeonpea, ICPL 87 (4.6).

100 seed weight

Among the species tested, the lowest 100 seed weight was observed in *F. stricta* [ICPW 202 (0.91 g)] followed by *F. bracteata* [ICPW 192 (0.97 g)] compared to the cultivated pigeonpea, ICPL 87 (9.60 g).

Trichomes

Studies were conducted on physical components associated with resistance to *H. armigera*. Data were recorded on trichomes, the hairy structures, on flowers (calyx) and pods.

Trichomes, the hairy out growths, were observed on the calyxes and pod wall surfaces. The calyx and pod wall surfaces were scanned under Zeiss Sterio microscope (carl Zeiss, inc; Thornwood, NY) and under an Electron microscope. Four types of trichomes: type A, type B, type C, and type D (Fig 9) were observed. Type A and type B were glandular trichomes whereas, type C and type D were non-glandular trichomes. The type A trichome had a long tubular neck with 4 to 8 cells, and an enlarged base

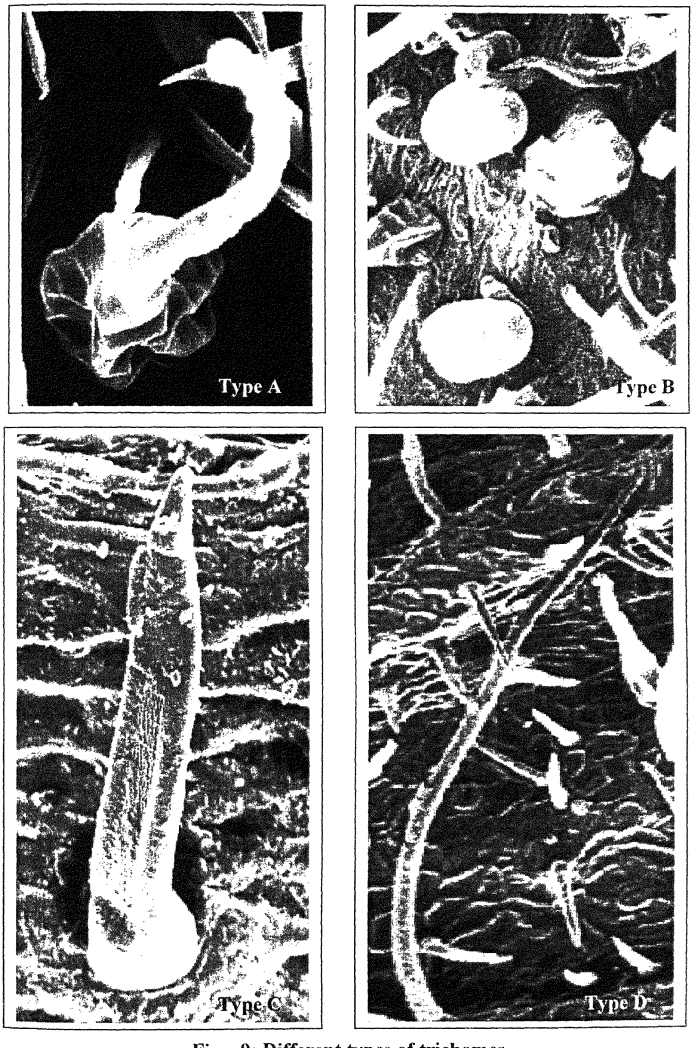


Fig – 9: Different types of trichomes

with 6 to 10 cells. It secretes clear exudates visible as droplets at the top and along the shaft of the trichome. Type B trichome is a sac like structure containing yellow, oily substance. The secretions in the type B trichomes are liberated only when the cell wall is ruptured. Type C and D trichomes were unsegmented and nonglandular. The type C trichome was short and type D trichome was 4 to 11 times longer than type C trichome. Density and distribution of different types of trichomes varied significantly in different accessions of wild relatives of pigeonpea.

Trichomes on calyx

The density and distribution of trichomes; type A, type B, type C, and type D varied significantly on the calyxes among the species, but there was little variation within the species (Table 8). There was no significant variation in the density of type A trichomes in *C. acutifolius* [ICPW 1 (27.70) and ICPW 2 (27.30)], *C. cajanifolius* [ICPW 29 (27.00)], *C. lineatus* [ICPW 41 (29.70)], and on cultivated pigeonpea, ICPL 87 (27.3).Very high trichome density was observed in cultivated pigeonpea variety, ICPL 332 (47.00). The density of type A trichomes was very low on *C. albicans* (ICPW 14), *C. scarabaeoides* (ICPW 116, ICPW 141, ICPW 152, ICPW 280, and ICPW 281), *R.aurea* (ICPW 210), *C. albicans* (ICPW 13), and *C. sericeus* (ICPW 159).

The numbers of type B trichomes were lower compared to other types of trichomes in all the species, except in *C. albicans* and *R. bracteata*. The highest numbers of type B trichomes (15) were recorded on ICPL 332, but they were completely absent in *D.* ferruginea and *C. scarabaeoides* (except ICPW 152).

Density of type C trichomes varied both among and within the species. Density of type C trichomes was significantly high in *C. scarbaeoides* [ICPW 281 (70.33)], followed by *C. albicans* [ICPW 14 (67.67), and ICPW 13 (61.67)]. There was little variation in the density of type C trichomes in *F. bracteata* [ICPW 192 (40.33)], *P. scariosa* [ICPW 207 (41.33)], *R. aurea* [ICPW 210 (40.67)], and *R. bracteata* [ICPW 214 (40.67)]. The density of type C trichomes was the lowest in the cultivated pigeonpea varieties, ICPL 87(10.00) and ICPL 332 (12.33).

Table - 8: Density of different types of trichomes on calyxes of wild relatives of pigeonpea.

Species	Accession number	Trichome type			
		A	B	C	D
C.acutifolius	ICPW 1	27.70	7.33	47.33	0.00
C.acutifolius	ICPW 2	27.30	5.00	42.00	0.00
C. albicans	ICPW 13	0.70	2.67	61.67	25.33
C. albicans	ICPW 14	0.30	1.67	67.67	32.00
C. cajanifolius	ICPW 28	29.30	1.33	32.67	16.33
C. cajanifolius	ICPW 29	27.00	1.00	27.67	25.33
C.lineatus	ICPW40	34.00	1.33	59.33	16.00
C.lineatus	ICPW 41	29.70	4.33	50.67	22.33
C. sericeus	ICPW 159	0.70	0.67	26.67	86.00
C. sericeus	ICPW 160	1.00	0.33	32.67	66.00
C. platycarpus	ICPW 68	5.00	0.67	33.67	0.67
C. scarabaeoides	ICPW 83	0.00	0.00	46.00	71.00
C. scarabaeoides	ICPW 90	0.00	0.00	35.33	76.33
C. scarabaeoides	ICPW 94	0.00	0.00	52.67	99.33
C. scarabaeoides	ICPW116	0.70	0.00	46.67	84.00
C. scarabaeoides	ICPW 125	1.00	0.00	42.00	49.00
C. scarabaeoides	ICPW 130	0.00	0.00	56.67	63.67
C. scarabaeoides	ICPW 137	0.00	0.00	33.00	82.00
C. scarabaeoides	ICPW 141	0.70	0.00	31.33	66.00
C. scarabaeoides	ICPW 152	0.30	0.67	55.33	36.00
C. scarabaeoides	ICPW278	2.30	0.00	32.67	53.33
C. scarabaeoides	ICPW 280	0.30	0.00	52.00	72.33
C. scarabaeoides	ICPW 281	0.70	0.00	70.33	48.67
D. ferruginea	ICPW 178	5.00	0.00	31.33	3.33
F.bracteata	ICPW 192	12.00	2.67	40.33	1.33
F. stricta	ICPW 202	2.30	4.33	34.67	2.33
P. scariosa	ICPW 207	2.00	1.67	41.33	0.67
R. aurea	ICPW 210	0.30	5.00	40.67	0.33
R. bracteata	ICPW 214	5.00	4.67	40.67	0.00
C. cajan (S)	ICPL 87	27.33	1.00	10.00	30.67
C. cajan (R)	ICPL 332	47.00	15.00	12.33	56.67
Mean		2.81	1.95	4.89	9.70
SE ±		0.92	0.69	1.73	3.43
LSD at 5%		10.13	1.98	41.53	38.29
F prob		<0.001	<0.001	<0.001	<0.001

S - Susceptible check. R - Resistant check.

Density of type D trichomes was significantly lowest in *R. aurea* [ICPW 210 (0.33)], followed by *C. platycarpus* [ICPW 68 (0.67)], and *P. scariosa* [ICPW 207 (0.67)]. The type D trichome density was significantly high in *C. sericeus* [ICPW 159 (86.00)], and *C. scarbaeoides* [ICPW 94 (99.33), ICPW116 (84.00) and ICPW 137 (82.00)] as compared to the cultivated ICPL 332(56.67) and ICPL 87 (30.67). Type D trichomes were completely absent in the accessions of *C. acutifolius* and *R. bracteata.*

Trichomes on pods

Four types of trichomes; type A, type B, type C, and type D were recorded on the pods of all the wild species of pigeonpea except type A trichome in *C. sericeus* and *C. scarabaeooides.* Density of diffrent trichomes on pods was studied in all the accessions (Table 9 & Fig 10). Density of type A trichome was significantly higher on the pods of *R. bracteata* [ICPW 214 (53.33)], followed by *C. platycarpus* [ICPW 68 (26.33)] as compared to that on the pods of *P. scariaosa* [ICPW 207 (0.67)], *F. stricta* [ICPW 202 (1.00)], and the cultivated pigeonpea varieties, ICPL 332 (18.67) and ICPL 87 (21.67). Type A trichomes were not recorded on the pods of *C. scarabaeoides*

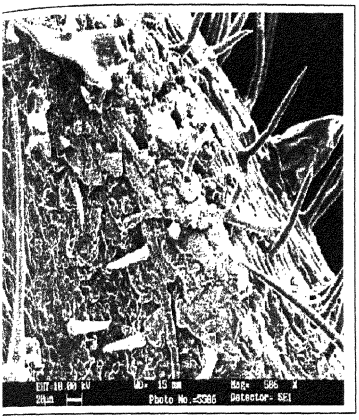
There were significant differences in the density of type B trichome between the species. Significantly lower numbers of type B trichome were observed on the pods of *C. platycarpus* [ICPW 68 (0.33)], and *F. bracteata* [ICPW 192 (0.33)] as compared to that on the pods of *C. lineatus* [ICPW 40 (61.33), and ICPW 41 (48.33)], *C. albicans* [ICPW 13 (36.67), and ICPW14 (25.67)], *C. cajanifolius* [ICPW 28 (23.67)and ICPW 29 (23.33)], and the cultivated pigeonpea variety, ICPL 87 (5.33).

The numbers of type C trichome on the pods varied significantly among the species. The density of type C trichome was low on pods of *C. albicans* [ICPW 13 (18.67)], and *C. acutifolius* [ICPW 1 (21.00)] as compared to the pigeonpea variety, ICPL 87 (40.33). The density of type C trichome on the pods of *C. scarabaeoides* was very high (>100) in all the accessions.

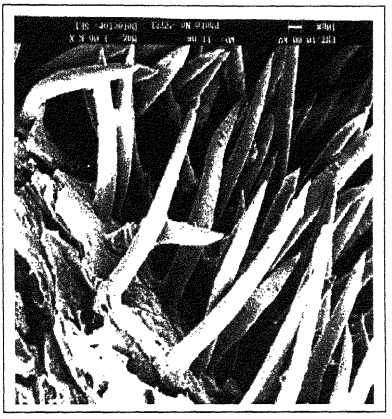
Table - 9: Density of different types of trichomes on pods of wild relatives of pigeonpea.

Species	Accession number	Trichome type			
		A	B	C	D
C.acutifolius	ICPW 1	21.33	5.00	21.00	1.33
C.acutifolius	ICPW 2	14.33	5.33	31.33	1.00
C. albicans	ICPW 13	4.33	36.67	18.67	2.33
C. albicans	ICPW 14	2.67	25.67	27.67	1.00
C. cajanifolius	ICPW 28	23.00	23.67	28.33	0.22
C. cajanifolius	ICPW 29	20.33	23.33	36.00	0.33
C.lineatus	ICPW40	23.33	61.33	52.67	26.67
C.lineatus	ICPW 41	20.67	48.33	42.33	29.00
C. sericeus	ICPW 159	0.00	17.67	26.67	141.67
C. sericeus	ICPW 160	0.00	13.33	28.00	122.33
C. platycarpus	ICPW 68	26.33	0.33	31.67	7.67
C. scarabaeoides	ICPW 83	0.00	5.67	141.67	22.30
C. scarabaeoides	ICPW 90	0.00	4.67	138.33	25.00
C. scarabaeoides	ICPW 94	0.00	2.67	117.00	20.26
C. scarabaeoides	ICPW116	0.00	7.33	148.67	22.42
C. scarabaeoides	ICPW 125	0.00	5.00	134.67	15.63
C. scarabaeoides	ICPW 130	0.00	2.00	150.00	28.26
C. scarabaeoides	ICPW 137	0.00	1.33	102.00	15.52
C. scarabaeoides	ICPW 141	0.00	4.00	156.33	16.85
C. scarabaeoides	ICPW 152	0.00	4.67	152.00	18.53
C. scarabaeoides	ICPW278	0.00	3.00	118.33	17.53
C. scarabaeoides	ICPW 280	0.00	7.00	140.33	15.63
C. scarabaeoides	ICPW 281	0.00	5.33	133.33	22.15
D. ferruginea	ICPW 178	11.67	41.00	52.00	22.33
F .bracteata	ICPW 192	8.67	0.33	53.67	0.33
F. stricta	ICPW 202	1.00	22.33	123.67	0.00
P. scariosa	ICPW 207	0.67	3.33	108.00	1.00
R. aurea	ICPW 210	4.33	9.33	37.00	49.67
R. bracteata	ICPW 214	53.33	11.33	51.00	1.33
C. cajan (S)	ICPL 87	21.67	5.33	40.33	8.00
C. cajan (R)	ICPL 332	18.67	26.67	135.67	10.50
SE ±		1.701	1.45	3.61	2.29
LSD at 5%		4.81	4.114	10.21	6.49
F prob		<0.001	<0.001	<0.001	<0.001

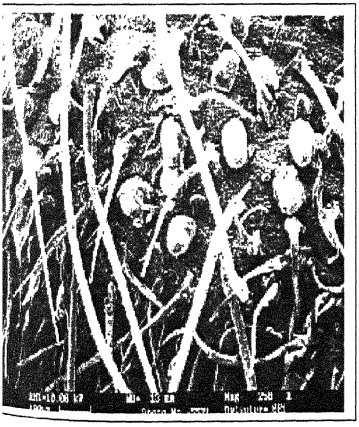
S - Susceptible check. R - Resistant check.



C. acutifolius



C. albicans



C. lineatus

C. cajanifolius

Fig-10: Density of different types of trichomes on the pods of wild relatives of pigeonpea

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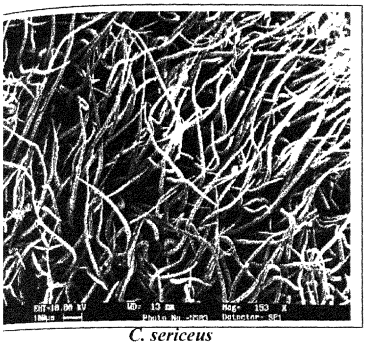
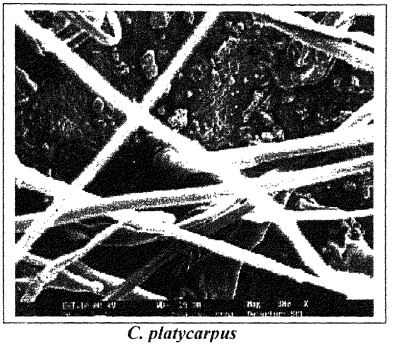
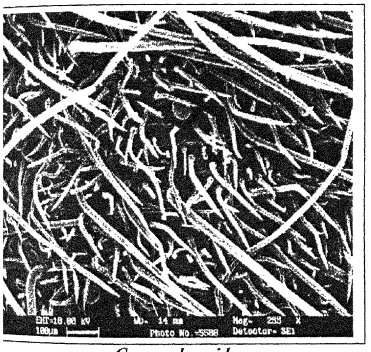


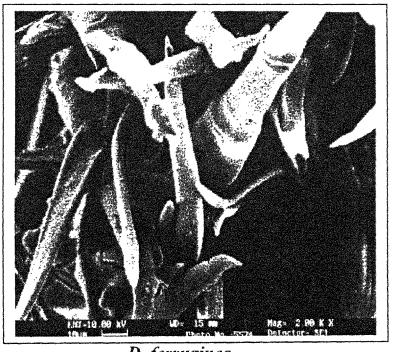
C. sericeus



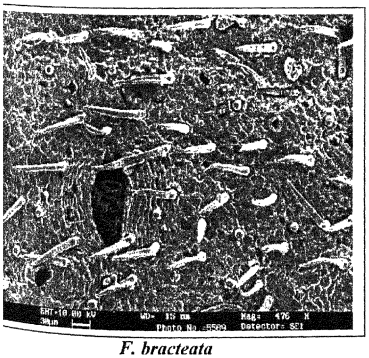
C. platycarpus



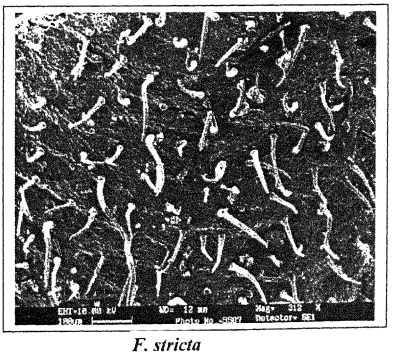
C. scarabaeoides



D. ferruginea

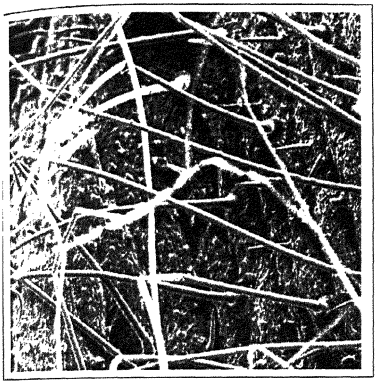


F. bracteata

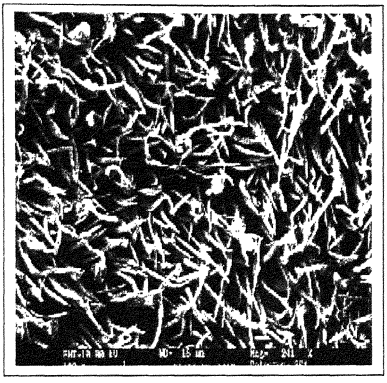


F. stricta

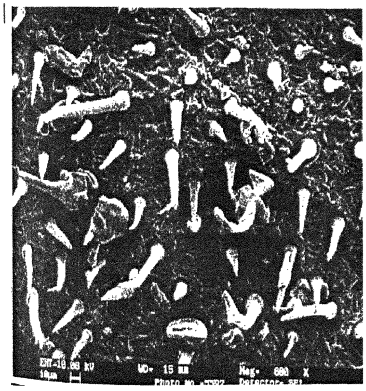
Contd.,



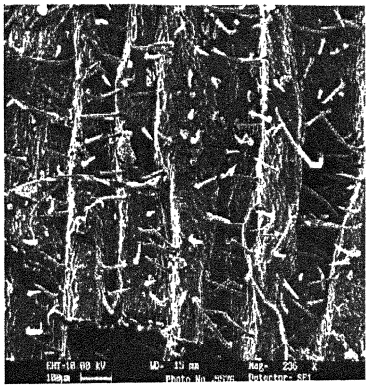
R. aurea



R. bracteata



C. cajan (ICPL 87)



C. cajan (ICPL 332)

Density of type D trichome was significantly higher on the pods of *C. sericeus* (122.33 to 141.67) followed by *R. aurea* (49.67), *C. lineatus* (26.67 to 29) and in the accessions of *C. sacarabaeoides* (15.52 to 28.26) compared to the cultivated pigeonpeas, ICPL 332 (10.50) and ICPL 87 (8.00). Number of type D trichomes was very low on pods of *C. acutifolius* (1 - 1.33) , *C. albicans* (1 - 2.33), *C. cajanifolius* (0.22 - 0.33), *F. bracteata* (0.33), *F. stricta* (0.00), *P. scariaosa* (1.00) and *R. bracteata* (1.33) (Table 9).

A significant and positive correlation was observed between the number of eggs laid, larval abundance, pod damage and the density of type A trichomes on calyxes and pods, while for the number of eggs laid, larval abundance, pod damage, and the density of type C and type D trichomes was significant and negative. Type B, trichomes showed no association with egg laying, larval abundance, and pod damage (Table 10).

Evaluation of wild relatives of pigeonpea for resistance to *H. armigera*

To identify diverse sources of resistance to *H. armigera*, 29 accessions of wild relatives of pigeonpea (6 short-duration, 13 medium-duration, and 10 long-duration), and 2 varieties of cultivated (ICPL 332, medium-duration and ICPL 87, short-duration) were evaluated for resistance to this pest under field conditions. (Tables 11 to 16).

In the accessions of short-duration group, the number of flowers in tagged portion ranged from 15.13 in *C. scarabaeoides* (ICPW 94) to 31.93 in ICPL 87. There was no egg laying on ICPW 137, and ICPW 152, while a few eggs (0.07) were recorded on ICPW 94, and ICPW 130 (*C. scarabaeoides*). There were 6.38 eggs per 5 inflorescences of the pigeonpea variety ICPL 87. There were no larvae on *C. scarabaeoides* (ICPW 94, ICPW 137, and ICPW 152), while low larval numbers were recorded on *R. aurea* [ICPW 210 (0.30)], and *C. platycarpus* [ICPW 68 (0.87)] compared to ICPL 87 (8.40). Number of pods in the tagged portion were low in *C. platycarpus* [ICPW 68 (19.40)] and high in ICPW 210 (32.87) of *R. aurea* compared to ICPL 87 (29.93). *Helicoverpa armigera* damage in the pods of early-duration wild relatives of pigeonpea ranged from 0.0% in *C. scarabaeoides* (ICPW 137) to 4.12% in *C. platycarpus* (ICPW 68) compared to 83.83% damage in the pods of ICPL 87 of *C. cajan.*

Table - 10: Correlation coefficient between different types of trichomes and *H. armigera* abundance and pod damage.

		Calyx				Pods				No. of eggs	No. of larvae	Pod damage %
		Type A	Type B	Type C	Type D	Type A	Type B	Type C	Type D			
Calyx	Type A	1.00										
	Type B	0.63**	1.00									
	Type C	-0.33*	-0.26*	1.00								
	Type D	-0.36*	-0.35*	0.00	1.00							
Pods	Type A	0.60**	0.41**	-0.23*	-0.57**	1.00						
	Type B	0.46**	0.22*	0.13	-0.27*	0.31*	1.00					
	Type C	-0.36*	-0.18	0.10	0.56**	-0.52**	-0.39*	1.00				
	Type D	-0.24*	-0.19	-0.14	0.42*	-0.29*	0.05	-0.15	1.00			
	No. of eggs	0.45**	-0.06	-0.39*	-0.18	0.36*	0.13	-0.36*	-0.16	1.00		
	No.of larvae	0.42**	0.00	-0.51**	-0.15	0.40*	0.02	-0.32*	-0.11	0.81**	1.00	
	Pod damage %	0.61**	0.17	-0.56**	-0.18	0.43*	0.20	-0.27*	-0.22*	0.91**	0.83**	1.00

*,** Correlation coefficients significant at P = 0.05 and 0.01, respectively.

In the medium-duration group the number of flowers in the tagged portion were lower in *C. scarabaeoides* [ICPW 90 (12.73)], *D. ferrugenia* [ICPW 178 (22.67)], *C. scarabaeoides* [ICPW 141 (27.07)], and *C. cajanifolius* [ICPW 28 (27.13)] as compared to the cultivated pigeonpea varieties, ICPL 332 (66.67) and ICPL 87 (54.87). There was no egg laying on ICPW 83, ICPW 90, ICPW 116, ICPW 125, ICPW 141, ICPW 278, ICPW 280, and ICPW 281 of *C. scarabaeoides*. Egg laying was quite high on *C. cajanifolius* [ICPW 28 (10.60)] as compared to ICPL 87 (4.53). There were no larvae on ICPW 90, ICPW 125, ICPW 278, ICPW 280, and ICPW 281; while 0.07 larvae per 5 inflorescences were recorded on ICPW 83, and ICPW 141 (*C. scarabaeoides*) compared to 4.73 larvae on ICPL 87. Number of pods in the tagged inflorescences were significantly lower in *C. cajanifolius* [ICPW 28 (9.00), and ICPW 29 (25.70)] compared to *C. scarabaeoides* [ICPW 141(29.6)] and ICPL 87 (45.00). The pod damage was high in the cultivated ICPL 87 (83.02%) and ICPW 28 (93.33%) of *C. cajanifolius,* while no damage was observed in ICPW 83, and ICPW 90 (*C. scarabaeoides*).

In the long-duration group, the number of flowers in the tagged portion was low in *C. acutifolius* [ICPW 1 (20.87), and ICPW 2 (24.53)], and high incase of *F. stricta* [ICPW 202 (157.27)] and ICPL 87 (44.00) of *C. cajan*. There were no significant differences in pod damage between the accessions belonging to *C. albicans,* and *C. lineatus*. Egg laying was not observed on *C. acutifolius* (ICPW 1) and was lower on *R. bracteata* [ICPW 214 (0.94)] as compared to ICPL 87 (1.81) of *C. cajan*. There were no larvae on ICPW 2, while a few larvae were recorded on ICPW 1 (0.07) of *C. acutifolius,* followed by ICPW 14 (0.14) of *C. albicans* and ICPW 41 (0.14) of *C. lineatus* compared to ICPL 87 (1.87). Number of pods were significantly high in *C. albicans* [ICPW 13 (158.00), and ICPW 14 (153.33)], followed by *R. bracteata* [ICPW 214(131.67)], *P. scariosa* [ICPW 207 (103.33)], and ICPL 87 (11.67) of *C. cajan*. There was no pod damage in ICPW 14, while low pod damage was observed in ICPW 13 (0.30%) of *C. albicans,* followed by ICPW 192 (0.38%) of *F. bracteata,* and ICPL 87 (80.00%) of *C. cajan*.

Table - 11: **Oviposition and abundance of *H. armigera* larvae in short-duration wild relatives of pigeonpea.**

Species	Accession number	No. of flowers	Eggs inflorescence^{-1}					Larvae inflorescence^{-1}				
			5th day	7th day	9th day	20th day	30th day	5th day	7th day	9th day	20th day	30th day
C. platycarpus	ICPW 68	16.67	0.60	0.40	0.00	0.00	0.00	0.00	0.27	0.20	0.40	0.00
C. scarabaeoides	ICPW 94	15.13	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C. scarabaeoides	ICPW 130	21.60	0.07	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.07	0.00
C. scarabaeoides	ICPW 137	13.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C. scarabaeoides	ICPW 152	21.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R. aurea	ICPW 210	19.67	0.13	0.33	0.00	0.00	0.00	0.00	0.07	0.27	0.00	0.00
C. cajan (S)	ICPL 87	31.93	2.40	1.87	0.87	0.13	1.13	1.10	1.73	3.87	1.47	0.20
SE ±		1.38	0.13	0.12	0.14	0.07	0.03	0.07	0.05	0.26	0.19	0.04
LSD at 5%		6.39	0.402	0.358	0.430	0.204	0.077	0.205	0.158	0.786	0.599	0.134
F-test		<0.001	<0.001	<0.001	<0.001	<0.001	0.020	<0.001	<0.001	<0.001	0.001	0.05

- Susceptible check.

Table - 12: **Pod damage by *H. armigera* in short-duration wild relatives of pigeonpea.**

Species	Accession number	No. of eggs	No. of larvae	No. of pods	No. of damaged pods	Pod damage (%)
C. platycarpus	ICPW 68	1.00	0.87	19.40	0.80	4.12
C. scarabaeoides	ICPW 94	0.07	0.00	28.67	0.13	0.43
C. scarabaeoides	ICPW 130	0.07	0.20	29.40	0.27	0.91
C. scarabaeoides	ICPW 137	0.00	0.00	32.40	0.00	0.00
C. scarabaeoides	ICPW 152	0.00	0.00	30.47	0.20	0.60
R. aurea	ICPW 210	0.00	0.34	32.87	0.33	1.07
C. cajan (S)	ICPL 87	6.38	8.40	29.93	25.07	83.83
SE ±		0.154	0.19	1.37	0.41	10.18
LSD at 5%		0.476	0.591	4.247	1.276	31.36
F-test		<0.001	<0.001	<0.001	<0.001	<0.001

S-Susceptible check.

Table - 13: Oviposition and abundance of *H. armigera* larvae in medium-duration wild relatives of pigeonpea.

Species	Accession number	No. of flowers	Eggs inflorescence^{-1}					Larvae inflorescence^{-1}				
			5th day	7th day	9th day	20th day	30th day	5th day	7th day	9th day	20th day	30th day
C. cajanifolius	ICPW 28	27.13	5.33	3.13	2.13	0.00	0.00	0.27	1.87	1.80	0.27	0.13
C. cajanifolius	ICPW 29	38.27	4.47	1.00	0.00	0.00	0.00	0.20	0.47	0.67	0.27	0.07
C. sericeus	ICPW 159	40.87	0.20	0.40	0.33	0.00	0.00	0.00	0.27	0.27	0.27	0.00
C. sericeus	ICPW 160	44.73	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.20	0.07	0.00
C. scarabaeoides	ICPW 83	32.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07
C. scarabaeoides	ICPW 90	12.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C. scarabaeoides	ICPW116	31.80	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00
C. scarabaeoides	ICPW 125	34.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C. scarabaeoides	ICPW 141	27.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00
C. scarabaeoides	ICPW278	28.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C. scarabaeoides	ICPW 280	35.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C. scarabaeoides	ICPW 281	41.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D. ferruginea	ICPW 178	22.67	0.00	0.07	0.00	0.00	0.00	0.00	0.07	0.00	0.13	0.00
C. cajan (S)	ICPL 87	54.87	1.13	0.67	2.73	0.00	0.00	0.33	1.40	2.53	0.33	0.13
C. cajan (R)	ICPL 332	66.67	0.00	0.20	0.20	0.00	0.00	0.13	0.40	0.27	0.13	0.00
SE ±		2.71	0.37	0.36	0.13	0.03		0.0659	0.086	0.155	0.091	0.031
LSD at 5%		7.784	1.067	1.053	0.379	NS		0.189	0.248	0.446	0.262	0.09
F-test		<0.001	<0.001	<0.001	<0.001	0.064		<0.001	<0.001	<0.001	<0.001	0.033

S- Susceptible check; R- Resistant check. ; NS – Non-significant at p =0.05.

Table - 14: Pod damage by *H. armigera* in medium-duration wild relatives of pigeonpea.

Species	Accession number	No. of eggs	No. of larvae	No. of pods	No. of damaged pods	Pod damage (%)
C. cajanifolius	ICPW 28	10.60	4.33	9.00	8.00	93.33
C. cajanifolius	ICPW 29	5.47	1.67	25.70	16.33	65.83
C. sericeus	ICPW 159	0.93	0.80	216.70	0.67	0.27
C. sericeus	ICPW 160	0.20	0.27	256.00	0.33	0.16
C. scarabaeoides	ICPW 83	0.00	0.07	181.70	0.00	0.00
C. scarabaeoides	ICPW 90	0.00	0.00	195.30	0.67	0.34
C. scarabaeoides	ICPW116	0.00	0.13	290.30	0.67	0.24
C. scarabaeoides	ICPW 125	0.00	0.00	296.70	1.67	0.57
C. scarabaeoides	ICPW 141	0.00	0.07	257.30	0.33	0.13
C. scarabaeoides	ICPW278	0.00	0.00	134.30	0.67	0.58
C. scarabaeoides	ICPW 280	0.00	0.00	191.70	0.33	0.17
C. scarabaeoides	ICPW 281	0.00	0.00	223.30	0.67	0.26
D. ferruginea	ICPW 178	0.07	0.20	205.00	56.33	27.47
C. cajan (S)	ICPL 87	4.53	4.73	45.00	37.36	83.02
C. cajan (R)	ICPL 332	0.40	0.93	125.00	61.00	49.00
SE ±		0.89	1.041	21.04	2.31	5.094
LSD at 5%		1.818	2.126	42.97	4.710	10.403
F-Test		<0.001	<0.001	<0.001	<0.001	<0.001

S - Susceptible check. R - Resistant check.

Table - 15: **Oviposition and abundance of *H. armigera* larvae in long-duration wild relatives of pigeonpea.**

Species	Accession number	No.of flowers	Eggs inflorescence^{-1}					Larvae inflorescence^{-1}				
			5th day	7th day	9th day	20th day	30th day	5th day	7th day	9th day	20th day	30th day
C.acutifolius	ICPW 1	20.87	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07
C.acutifolius	ICPW 2	24.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C. albicans	ICPW 13	33.07	0.20	0.33	0.00	0.13	0.00	0.00	0.13	0.07	0.13	0.00
C. albicans	ICPW 14	33.73	0.07	0.00	0.00	0.07	0.00	0.00	0.00	0.07	0.07	0.00
C.lineatus	ICPW40	35.67	0.00	0.47	0.00	0.00	0.00	0.00	0.20	0.00	0.13	0.07
C.lineatus	ICPW 41	37.40	0.00	0.27	0.00	0.00	0.00	0.00	0.07	0.00	0.07	0.00
F. bracteata	ICPW 192	63.53	0.00	0.13	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00
F. stricta	ICPW 202	157.27	0.40	0.07	0.00	0.07	0.00	0.00	0.13	0.00	0.07	0.00
P. scariosa	ICPW 207	68.80	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00
R. bracteata	ICPW 214	58.33	0.60	0.20	0.00	0.07	0.07	0.13	0.33	0.20	0.13	0.27
C. cajan (S)	ICPL 87	44.00	0.67	0.73	0.27	0.07	0.07	0.20	0.73	0.60	0.20	0.07
SE ±		4.70	0.08	0.11	0.02	0.04	0.023	0.04	0.07	0.09	0.07	0.04
LSD at 5%		13.87	0.25	0.33	0.06	NS	NS	0.1141	0.1958	0.2769	NS	0.1230
F-test			< 0.001	< 0.001	0.004	< 0.001	0.389	0.5840	0.013	< 0.001	0.006	0.420	0.006

S - Susceptible check. NS – Non-significant at P=0.05

Table - 16: **Pod damage by *H. armigera* in long-duration wild relatives of pigeonpea.**

Species	Accession number	No. of eggs	No. of larvae	No. of pods	No. of damaged pods	Pod damage (%)
C.acutifolius	ICPW 1	0.13	0.07	77.33	1.00	1.32
C.acutifolius	ICPW 2	0.00	0.00	82.00	0.33	0.45
C. albicans	ICPW 13	0.68	0.33	158.00	0.67	0.30
C. albicans	ICPW 14	0.14	0.14	153.33	0.00	0.00
C.lineatus	ICPW40	0.47	0.40	66.33	2.67	3.90
C.lineatus	ICPW 41	0.27	0.14	60.00	2.00	3.27
F. bracteata	ICPW 192	0.13	0.20	82.67	0.33	0.38
F. stricta	ICPW 202	0.54	0.20	77.33	0.67	0.64
P. scariosa	ICPW 207	0.07	0.13	103.33	3.67	3.61
R. bracteata	ICPW 214	0.97	1.06	131.67	9.33	7.14
C. cajan (S)	ICPL 87	1.81	1.87	11.67	9.33	80.00
SE ±		0.03	0.03	11.32	1.47	3.74
LSD at 5%		0.78	0.09	33.38	4.34	11.04
F-test		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

S - Susceptible check.

Mechanisms of resistance to *H. armigera* in wild relatives of pigeonpea

In the present investigation, 31 accessions were evaluated for mechanisms of resistance to pod borer, *H. armigera*. Two types of resistance mechanisms; antixenosis and antibiosis were recorded and studied.

Antixenosis/ non-preference for oviposition

The antixenosis mechanism of resistance was studied under no-choice, dual-choice and multi-choice conditions.

No-choice conditions

Under no-choice conditions, five inflorescences from each of the 31 test genotypes were kept separately in conical flasks and placed inside the oviposition cages. Five pairs of moths were released into each cage and observations were recorded on number of eggs laid on each accession on 3rd, 4th and 5th day after release of moths. Each female moth laid 56 to 425 eggs on different test genotypes. A female moth laid 190 eggs per 5 inflorescences in ICPL 332 (resistant check), compared to 334 eggs on the susceptible check, ICPL 87. There was considerable variation in oviposition preference even within a species, e.g., ICPW 1 (151) and ICPW 2 (236) of *C. acutifolius*, ICPW 13 (65) and ICPW 14 (150) of *C. albicans*, ICPW 28 (151) and ICPW 29 (236) of *C. cajanifolius*, ICPW 40 (132) and ICPW 41 (425) of *C. lineatus*, and ICPW 159 (161) and ICPW 160 (250) of *C sericeus*.

All the accessions of *C. scarabaeoides* (except ICPW 280) were least preferred by moths for oviposition. Also, the accessions of *C. acutifolius* (ICPW 1), *C. albicans* [(ICPW 13), and (ICPW 14)], *C. lineatus* (ICPW 41), *C. serecius* (ICPW 159), *F. stricta* (ICPW 202), *P. scariosa* (ICPW 207), *C. platycarpus* (ICPW 68), and *R. aurea* (ICPW 210) were less preferred for ovipositon compared to the resistant check, ICPL 332. However, all the wild relatives of pigeonpea were found to be less preferred (except *C. lineatus* (ICPW 40), *D. ferrugenia* (ICPW 178), *C. cajanifolius* (ICPW 29)) compared to the susceptible check, ICPL 87 (Table 17 & Fig 11).

Table - 17: Oviposition preference by *H. armigera* towards wild relatives of pigeonpea under no-choice conditions.

Species	Accession number	No. of eggs female^{-1}	ROP
C. acutifolius	ICPW 1	151 (12.27 ± 0.40)	-32.6
C. acutifolius	ICPW 2	236 (15.35 ± 0.25)	-15.7
C. albicans	ICPW 13	65 (8.04 ± 0.28)	-55.8
C. albicans	ICPW 14	150 (12.20 ± 0.53)	-33.0
C. cajanifolius	ICPW 28	258 (15.86 ± 1.28)	-13.2
C. cajanifolius	ICPW 29	347 (18.60 ± 0.57)	2.2
C. lineatus	ICPW 40	132 (11.43 ± 0.51)	-37.2
C. lineatus	ICPW41	425 (20.42 ± 1.46)	12.1
C. sericeus	ICPW 159	161 (12.66 ± 0.27)	-30.4
C. sericeus	ICPW 160	250 (15.60 ± 1.16)	-14.3
C. platycarpus	ICPW 68	141 (11.59 ± 1.09)	-40.7
C. scarabaeoides	ICPW 83	114 (10.57 ± 0.73)	-42.3
C. scarabaeoides	ICPW 90	56 (7.47 ± 0.31)	-59.0
C. scarabaeoides	ICPW 94	76 (8.70 ± 0.21)	-52.2
C. scarabaeoides	ICPW116	89 (9.30 ± 0.68)	-48.9
C. scarabaeoides	ICPW 125	120 (10.86 ± 0.66)	-40.7
C. scarabaeoides	ICPW 130	125 (11.14 ± 0.44)	-38.8
C. scarabaeoides	ICPW 137	84 (9.15 ± 0.27)	-49.7
C. scarabaeoides	ICPW 141	167 (12.85 ± 0.61)	-29.4
C. scarabaeoides	ICPW 152	155 (12.38 ± 0.57)	-32.0
C. scarabaeoides	ICPW278	179 (13.31± 0.65)	-26.9
C. scarabaeoides	ICPW 280	159 (12.56 ± 0.49)	-31.0
C. scarabaeoides	ICPW 281	245 (15.59 ± 0.64)	-14.3
D. ferruginea	ICPW 178	357 (18.80 ± 0.57)	3.3
F. bracteata	ICPW 192	307 (17.38 ± 0.14)	-4.9
F. stricta	ICPW 202	149 (12.10 ± 0.45)	-33.5
P. scariosa	ICPW 207	182 (13.45 ± 0.44)	-26.4
R. aurea	ICPW 210	89 (9.30 ± 0.68)	-48.9
R. bracteata	ICPW 214	190 (13.75 ± 0.34)	-24.7
C. cajan (S)	ICPL 87	334 (18.2 ± 0.34)	0.0
C. cajan (R)	ICPL 332	190 (13.69 ± 0.72)	-24.8

Figures in parentheses are square root transformed values.
R - Resistant check. S - Susceptible check.
ROP - Relative oviposition preference with respect to ICPL 87.

Table - 17: Oviposition preference by *H. armigera* towards wild relatives of pigeonpea under no-choice conditions.

Species	Accession number	No. of eggs female^{-1}	ROP
C. acutifolius	ICPW 1	151 (12.27 ± 0.40)	-32.6
C. acutifolius	ICPW 2	236 (15.35 ± 0.25)	-15.7
C. albicans	ICPW 13	65 (8.04 ± 0.28)	-55.8
C. albicans	ICPW 14	150 (12.20 ± 0.53)	-33.0
C. cajanifolius	ICPW 28	258 (15.86 ± 1.28)	-13.2
C. cajanifolius	ICPW 29	347 (18.60 ± 0.57)	2.2
C. lineatus	ICPW40	425 (20.42 ± 1.46)	12.1
C. lineatus	ICPW 41	132 (11.43 ± 0.51)	-37.2
C. sericeus	ICPW 159	161 (12.66 ± 0.27)	-30.4
C. sericeus	ICPW 160	250 (15.60 ± 1.16)	-14.3
C. platycarpus	ICPW 68	141 (11.59 ± 1.09)	-40.7
C. scarabaeoides	ICPW 83	114 (10.57 ± 0.73)	-42.3
C. scarabaeoides	ICPW 90	56 (7.47 ± 0.31)	-59.0
C. scarabaeoides	ICPW 94	76 (8.70 ± 0.21)	-52.2
C. scarabaeoides	ICPW116	89 (9.30 ± 0.68)	-48.9
C. scarabaeoides	ICPW 125	120 (10.86 ± 0.66)	-40.7
C. scarabaeoides	ICPW 130	125 (11.14 ± 0.44)	-38.8
C. scarabaeoides	ICPW 137	84 (9.15 ± 0.27)	-49.7
C. scarabaeoides	ICPW 141	167 (12.85 ± 0.61)	-29.4
C. scarabaeoides	ICPW 152	155 (12.38 ± 0.57)	-32.0
C. scarabaeoides	ICPW278	179 (13.31± 0.65)	-26.9
C. scarabaeoides	ICPW 280	159 (12.56 ± 0.49)	-31.0
C. scarabaeoides	ICPW 281	245 (15.59 ± 0.64)	-14.3
D. ferruginea	ICPW 178	357 (18.80 ± 0.57)	3.3
F. bracteata	ICPW 192	307 (17.38 ± 0.14)	-4.9
F. stricta	ICPW 202	149 (12.10 ± 0.45)	-33.5
P. scariosa	ICPW 207	182 (13.45 ± 0.44)	-26.4
R. aurea	ICPW 210	89 (9.30 ± 0.68)	-48.9
R. bracteata	ICPW 214	190 (13.75 ± 0.34)	-24.7
C. cajan (S)	ICPL 87	334 (18.2 ± 0.34)	0.0
C. cajan (R)	ICPL 332	190 (13.69 ± 0.72)	-24.8

Figures in parentheses are square root transformed values.
R - Resistant check. S - Susceptible check.
ROP - Relative oviposition preference with respect to ICPL 87.

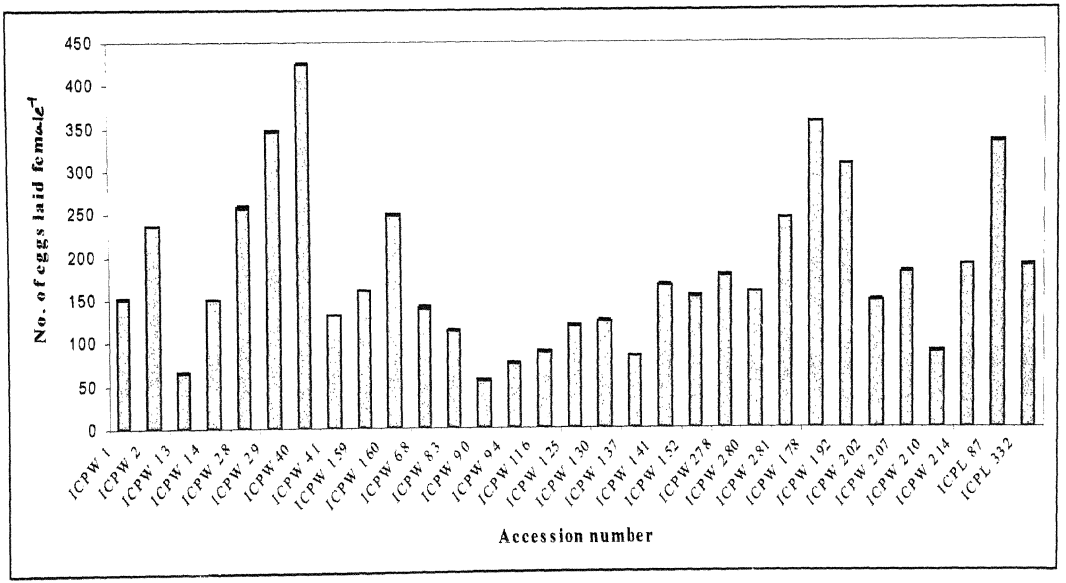


Fig 11 : Oviposition preference by *H. armigera* towards wild relatives of pigeonpea under no-choice conditions

Dual-choice conditions

Under dual choice conditions, the female moths showed high preference for oviposition towards the cultivated pigeonpea than the wild relatives of pigeonpea. Significant differences in oviposition preference were observed in all species, except *F. stricta* (ICPW 202) when the moths were provided with a choice between the cultivated and the wild relatives of pigeonpea (Table 18 & Fig 12).

Multi-choice conditions

Under multi-choice conditions, significant differences were observed in oviposition preference between and within a species. All the wild relatives were less preferred for oviposition (except *C. Cajanifolius*, C. *lineatus* and *F. stricta*) as compared to resistant pigeonpea cultivar ICPL 332. The species; *C. cajnifolius, C. lineatus* and *F. stricta* were also less preferred for oviposition compared to the subceptible check, ICPL 87 (Table 19 & Fig 13).

Antibiosis

To study the antibiosis mechanism of resistance, observations were recorded on development of *H. armigera* on the leaves, flowers and pods; and on artificial diet impregnated with lyophilized leaf and pod powder.

Development and survival of *H. armigera* on leaves

There were no differences in larval and pupal weights of the insects reared on the leaves of pigeonpea genotypes ICPL 87 (susceptible check) and ICPL 332 (resistant check) (Table 20). However, significant differences were observed in the larval and pupal weights in insects reared on the leaves of the wild relatives of pigeonpea. The larval weights on the wild species were significantly lower than those on the cultivated pigeonpea (except on *C. lineatus* (ICPW 40), and *F. stricta* (ICPW 202) at 10 days after emergence). Pupal weights on the wild species were significantly lower (except on *C. albicans* and *C. lineatus*) than on the cultivated pigeonpea, ICPL 87.

Table - 18: Oviposition preference by *H. armigera* towards wild relatives of pigeonpea under dual-choice conditions.

Species	Accession number	No. of eggs female^{-1}		t-value	Probability
		Control (ICPL 87)	Test genotype		
C. acutifolius	ICPW 1	98.20 ± 9.68	26.13 ± 3.25	8.60**	< 0.001
C. acutifolius	ICPW 2	121.80 ± 11.19	54.13 ± 5.74	7.88**	< 0.001
C. albicans	ICPW 13	115.20 ± 12.92	23.80 ± 3.51	7.12**	< 0.001
C. albicans	ICPW 14	128.50 ± 15.31	41.33 ± 4.85	6.66**	< 0.001
C. cajanifolius	ICPW 28	76.60 ± 11.71	36.60 ± 8.39	4.21**	<0.001
C. cajanifolius	ICPW 29	96.93 ± 12.66	54.73 ± 7.22	4.96**	<0.001
C. lineatus	ICPW40	96.40 ± 10.31	79.40 ± 8.97	3.00**	0.010
C. lineatus	ICPW 41	95.93 ± 11.47	63.53 ± 8.26	4.55**	<0.001
C. sericeus	ICPW 159	87.73 ± 10.15	59.47 ± 8.31	7.60**	<0.001
C. sericeus	ICPW 160	95.80 ± 11.54	61.00 ± 7.61	3.80**	0.002
C. platycarpus	ICPW 68	95.93 ± 10.02	63.50 ± 6.62	4.02**	<0.001
C. scarabaeoides	ICPW 83	98.80 ± 10.46	27.27 ± 4.13	7.14**	< 0.001
C. scarabaeoides	ICPW 90	86.53 ± 10.22	23.73 ± 2.13	6.28**	< 0.001
C. scarabaeoides	ICPW 94	85.27 ± 10.38	31.87 ± 4.55	6.34**	< 0.001
C. scarabaeoides	ICPW116	104.10 ± 10.59	54.87 ± 7.52	8.03**	< 0.001
C. scarabaeoides	ICPW 125	101.70 ± 9.21	40.07 ± 4.21	8.67**	< 0.001
C. scarabaeoides	ICPW 130	85.27 ± 10.72	31.87 ± 4.05	4.73**	< 0.001
C. scarabaeoides	ICPW 137	100.30 ± 9.88	33.07 ± 4.05	7.53**	< 0.001
C. scarabaeoides	ICPW 141	81.33 ± 9.93	32.87 ± 4.61	5.42**	< 0.001
C. scarabaeoides	ICPW 152	91.07 ± 12.17	26.00 ± 2.74	5.57**	< 0.001
C. scarabaeoides	ICPW278	75.20 ± 6.68	32.53 ± 3.62	5.42**	< 0.001
C. scarabaeoides	ICPW 280	79.00 ± 10.35	25.67 ± 2.79	5.54**	< 0.001
C. scarabaeoides	ICPW 281	76.60 ± 10.07	36.60 ± 4.95	6.72**	<0.001
D. ferruginea	ICPW 178	87.60 ± 10.06	67.20 ± 9.32	3.50**	0.004
F. bracteata	ICPW 192	73.00 ± 8.57	57.53 ± 7.91	2.43*	0.029
F. stricta	ICPW 202	60.33 ± 9.73	49.93 ± 7.25	1.59	0.135
P. scariosa	ICPW 207	68.20 ± 8.35	38.07 ± 7.77	2.97**	0.010
R. aurea	ICPW 210	76.60 ± 10.70	57.00 ± 6.58	3.42**	<0.001
R. bracteata	ICPW 214	78.80 ± 9.84	49.33 ± 6.79	3.28**	0.005
C. cajan (R)	ICPL 332	79.87 ± 11.51	40.73 ± 5.36	4.48**	<0.001

R - Resistant check.

*,**= t -value significant at P=0.05 and 0.01, respectively.

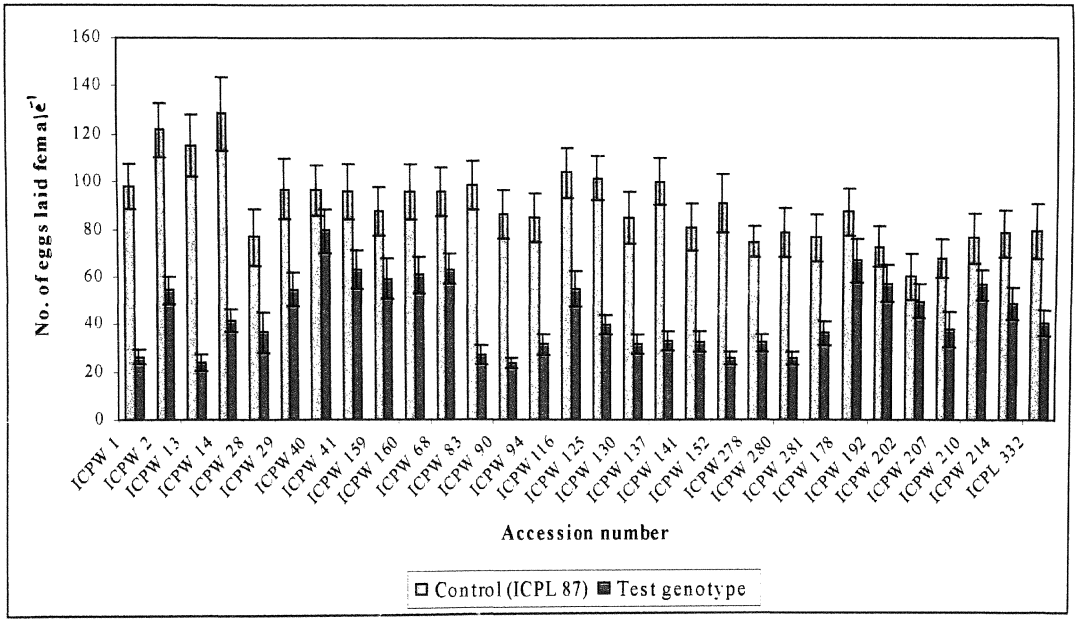


Fig -12 : **Oviposition preference by *H. armigera* towards wild relatives of pigeonpea under dual-choice conditions**

Table – 19: Oviposition preference by *H. armigera* towards wild relatives of pigeonpea under multi-choice conditions.

Species	Accession number	No. of eggs laid female^{-1}	ROP
C. acutifolius	ICPW 1	139 (11.65±1.09)	-41.9
C. acutifolius	ICPW 2	179 (13.30±1.09)	-33.4
C. albicans	ICPW 13	84 (8.57±1.09)	-57.1
C. albicans	ICPW 14	87 (9.00±1.09)	-54.9
C. cajanifolius	ICPW 28	260 (16.09±1.09)	-19.4
C. cajanifolius	ICPW 29	313 (17.65±1.09)	-11.6
C. lineatus	ICPW40	202 (14.15±1.09)	-29.1
C. lineatus	ICPW 41	257 (16.01±1.09)	-19.8
C. sericeus	ICPW 159	74 (8.52±1.09)	-57.3
C. sericeus	ICPW 160	89 (9.41±1.09)	-52.9
C. platycarpus	ICPW 68	141 (11.59±1.09)	-41.9
C. scarabaeoides	ICPW 83	123 (11.00±1.09)	-44.9
C. scarabaeoides	ICPW 90	93 (9.56±1.09)	-52.1
C. scarabaeoides	ICPW 94	141 (11.59±1.09)	-41.9
C. scarabaeoides	ICPW116	168 (12.70±1.09)	-36.4
C. scarabaeoides	ICPW 125	93 (9.52±1.09)	-52.3
C. scarabaeoides	ICPW 130	155 (12.37±1.09)	-38.0
C. scarabaeoides	ICPW 137	82 (8.80±1.09)	-55.9
C. scarabaeoides	ICPW 141	121 (11.00±1.09)	-44.9
C. scarabaeoides	ICPW 152	154 (12.24±1.09)	-38.7
C. scarabaeoides	ICPW 280	175 (9.38±1.09)	-53.0
C. scarabaeoides	ICPW 281	166 (13.20±1.09)	-33.9
C. scarabaeoides	ICPW278	88 (12.88±1.09)	-35.5
D. ferruginea	ICPW 178	139 (11.79±1.09)	-40.9
F. bracteata	ICPW 192	77 (8.65±1.09)	-56.7
F. stricta	ICPW 202	202 (14.20±1.09)	-28.9
P. scariosa	ICPW 207	95 (9.71±1.09)	-51.4
R. aurea	ICPW 210	74 (8.52±1.09)	-57.3
R. bracteata	ICPW 214	105 (9.86±1.09)	-50.6
C. cajan (S)	ICPL 87	399 (13.92±1.09)	0.0
C. cajan (R)	ICPL 332	196 (19.96±1.09)	-30.3

Figures in parentheses are square root transformed values.
S - Susceptible check. R - Resistant check.
ROP - Relative oviposition preference with respect to ICPL 87.

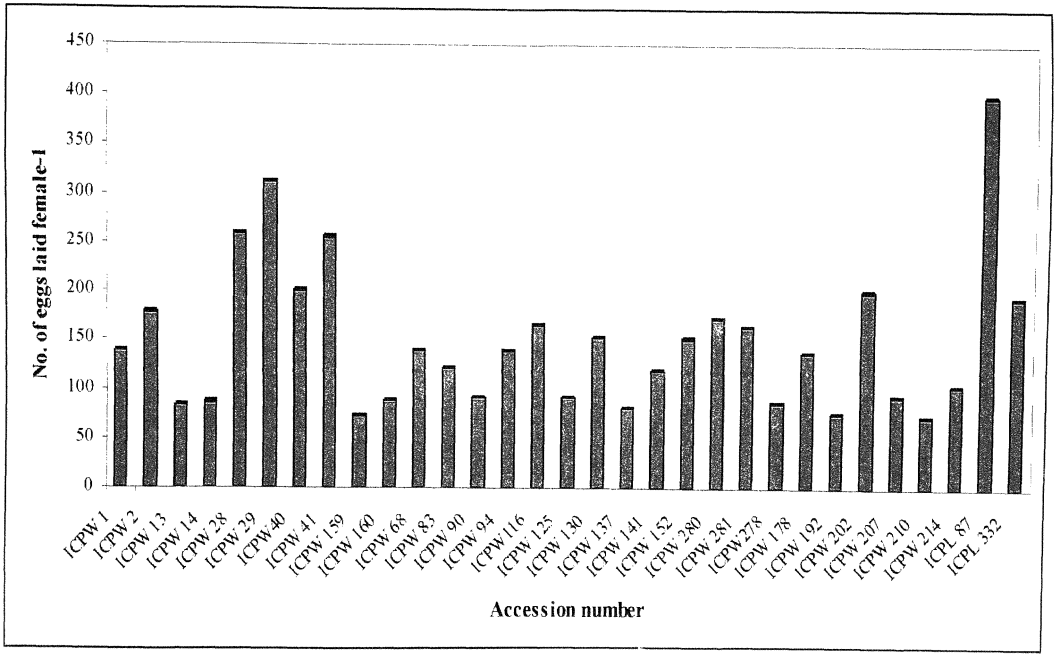


Fig 13 : **Oviposition preference by *H. armigera* towards wild relatives of pigeonpea under multi-choice conditions**

Table - 20: Larval and pupal weights of *H. armigera* reared on the leaves of wild relatives of pigeonpea.

Species	Accession nmber	Larval weight (mg)			Pupal wt. (mg)
		5th day	10th day	15th day	
C. acutifolius	ICPW 1	1.3	11.0	61.5	153.3
C. acutifolius	ICPW 2	1.1	7.2	45.1	142.1
C. albicans	ICPW 13	0.6	8.0	17.4	261.2
C. albicans	ICPW 14	0.7	15.0	39.9	279.0
C. cajanifolius	ICPW 28	1.2	64.3	96.6	164.3
C. cajanifolius	ICPW 29	0.8	11.7	63.3	125.2
C. lineatus	ICPW40	1.4	82.3	118.0	270.9
C. lineatus	ICPW 41	0.5	31.4	64.1	266.3
C. sericeus	ICPW 159	2.7	13.8	210.8	183.6
C. sericeus	ICPW 160	1.6	8.0	68.9	137.1
C. platycarpus	ICPW 68	2.0	17.0	98.0	129.0
C. scarabaeoides	ICPW 83	1.4	17.8	59.1	126.8
C. scarabaeoides	ICPW 90	2.3	51.1	79.5	114.2
C. scarabaeoides	ICPW 94	1.8	35.4	79.7	123.1
C. scarabaeoides	ICPW116	1.5	20.8	73.6	137.8
C. scarabaeoides	ICPW 125	0.8	24.2	75.3	150.0
C. scarabaeoides	ICPW 130	1.6	33.2	81.2	145.0
C. scarabaeoides	ICPW 137	1.4	28.8	98.7	112.0
C. scarabaeoides	ICPW 141	1.4	36.9	69.8	136.0
C. scarabaeoides	ICPW 152	1.7	32.1	63.4	150.1
C. scarabaeoides	ICPW 280	1.5	40.6	88.0	137.4
C. scarabaeoides	ICPW 281	1.6	33.1	102.0	142.0
C. scarabaeoides	ICPW278	1.9	41.1	113.2	160.6
C. scarabaeoides	ICPW 178	1.3	38.2	80.2	194.3
D. ferruginea	ICPW 192	2.0	26.2	148.6	144.7
F. bracteata	ICPW 202	2.6	100.2	182.7	212.9
F. stricta	ICPW 207	0.4	11.4	28.7	124.7
R. aurea	ICPW 210	1.0	15.0	75.0	129.0
P. scariosa	ICPW 214	0.9	15.0	132.1	167.3
C. cajan (S)	ICPL 87	4.8	78.3	249.1	252.8
C. cajan (R)	ICPL 332	4.7	72.1	254.1	227.4
SE ±		0.33	4.22	3.84	2.43
LSD at 5%		12.8	14.7	20.9	8.5
F-test		<0.001	<0.001	<0.001	<0.001

S - Susceptible check. R - Resistant check.

The larval weights at 5 (<2 mg per larva), 10 (<25 mg per larva), and 15 (<100 mg per larva) days after emergence, and the pupal weights (<150 mg per pupa) were significantly lower when the larvae were reared on the leaves of *C. acutifolius* (ICPW 2), *C. cajanifolius* (ICPW 29), *C. sericeus* (ICPW 160), *C. scarabaeoides* (ICPW 83, ICPW 116, and ICPW 125), and *P. scariosa* (ICPW 207) compared to the insects reared on the cultivated pigeonpea varieties, ICPL 87 (larval weights 4.7, 72.1, and 254.1 mg per larva at 5, 10, and 15 days after emergence, respectively; and pupal weight 227.4 mg per pupa).

Significantly higher larval mortality was observed on the wild relatives of pigeonpea (except ICPW 40 of *C. lineatus*) compared to the susceptible check. Larval mortality at 5 days after initiating the experiment was >60% on the leaves of *C. scarabaeoides* (ICPW 83, ICPW 116, ICPW 130, ICPW 137, ICPW 141, ICPW152, ICPW 280, and ICPW 281) compared to 40% mortality on the leaves of ICPL 332 and ICPL 87 (Table 21). At 20 days after initiating the experiment, >70% larval mortality was recorded on the leaves of *C. scarabaeoides* (ICPW 83, ICPW 116, ICPW 130, ICPW 137, ICPW 141, ICPW 152, and ICPW 281) compared to 50% mortality on the leaves of ICPL 87. The larval mortality on the leaves of ICPL 332 was as high (70%) as that on the leaves of certain accessions of *C. scarabaeoides*.

The larval period lasted for 24.1 days on the leaves of ICPL 87 to 39.6 days on the leaves of *C. scarabaeoides* (ICPW 83) (Table 22). Larvae took >35 days to complete development when reared on the leaves of *C. albicans* (ICPW 13 and ICPW 14) and *C. scarabaeoides* (ICPW 83, ICPW 94, ICPW 116, ICPW 130, ICPW 137, ICPW 141, ICPW 152, ICPW 280, and ICPW 281) as compared to 24.1 days on ICPL 87 and 29.1 days on ICPL 332. The pupal period lasted for >18 days when the larvae were reared on the leaves of *C. albicans* (ICPW 13), *C. scarabaeoides* (ICPW 83 and ICPW 130), *D. ferruginea* (ICPW 178), *F. stricta* (ICPW 202) and *P. scariosa* (ICPW 207) as compared to 14.7 days on ICPL 87 and 17.2 days on ICPL 332. Lower pupation and adult emergence (<30%) were recorded in larvae reared on the leaves of *C. scarabaeoides* (ICPW 83, ICPW 90, ICPW 116, ICPW 130, ICPW 137, ICPW 141, ICPW 152, ICPW 280, and ICPW 281), and *P. scariosa* (ICPW 207) compared to 42% adult emergence on

Table - 21: Mortality of *H. armigera* larvae reared on the leaves of wild relatives of pigeonpea.

Species	Accession number	Larval mortality (%)			
		5th day	10th day	15th day	20th day
C. acutifolius	ICPW 1	44 (41)	46 (42)	54 (47)	68 (56)
C. acutifolius	ICPW 2	56 (49)	66 (55)	66 (55)	68 (56)
C. albicans	ICPW 13	52 (46)	58 (52)	60 (51)	62(52)
C. albicans	ICPW 14	56 (49)	56 (49)	56 (49)	62(52)
C. cajanifolius	ICPW 28	56 (49)	60 (51)	60 (51)	64 (54)
C. cajanifolius	ICPW 29	56 (48)	60 (51)	60 (51)	68 (56)
C .lineatus	ICPW40	32 (34)	38 (37)	56 (49)	56 (49)
C .lineatus	ICPW 41	54 (47)	58 (50)	60 (51)	60 (51)
C. sericeus	ICPW 159	34 (35)	48 (44)	48 (44)	52 (46)
C. sericeus	ICPW 160	48 (44)	52 (46)	52 (46)	54 (47)
C. platycarpus	ICPW 68	40(39)	44(41)	52 (46)	64 (53)
C. scarabaeoides	ICPW 83	66 (55)	70 (57)	74 (59)	76(60)
C. scarabaeoides	ICPW 90	48 (43)	58 (50)	68 (56)	68 (56)
C. scarabaeoides	ICPW 94	44 (41)	54 (47)	58 (50)	60 (51)
C. scarabaeoides	ICPW116	72 (58)	72 (58)	72 (58)	74(59)
C. scarabaeoides	ICPW 125	48 (44)	50 (45)	50 (45)	56 (49)
C. scarabaeoides	ICPW 130	66 (55)	72 (58)	72 (58)	74(59)
C. scarabaeoides	ICPW 137	62 (52)	64 (53)	66 (55)	70(57)
C. scarabaeoides	ICPW 141	68 (56)	72 (59)	72 (58)	74(59)
C. scarabaeoides	ICPW 152	66 (55)	68 (56)	68 (56)	76(60)
C. scarabaeoides	ICPW278	56 (49)	58 (50)	58 (50)	68 (56)
C. scarabaeoides	ICPW 280	62 (53)	64 (54)	64 (54)	64 (54)
C. scarabaeoides	ICPW 281	60 (51)	62 (52)	62 (52)	70(57)
D. ferruginea	ICPW 178	40 (39)	56 (48)	58 (50)	58 (50)
F. bracteata	ICPW 192	54 (47)	54 (47)	58 (50)	58 (50)
F. stricta	ICPW 202	44 (41)	56 (49)	56 (49)	56 (49)
P. scariosa	ICPW 207	58 (50)	64 (53)	64 (54)	64 (54)
R. aurea	ICPW 210	34 (36)	46 (43)	54 (47)	68 (56)
R. bracteata	ICPW 214	46 (42)	50 (45)	66 (55)	66 (55)
C. cajan (S)	ICPL 87	40 (38)	44 (41)	50 (45)	50(45)
C. cajan (R)	ICPL 332	40 (36)	50 (45)	70 (57)	70(67)
SE ±		7.03(3.38)	6.09(2.49)	4.91(1.43)	4.54(1.30)
LSD at 5%		19.70(12.74)	17.06(10.5)	13.77(8.42)	12.73(7.94)
F-test		0.002(0.004)	0.004(0.05)	0.003(0.002)	<0.001

Figures in parentheses are Angular transformed values.
S - Susceptible check. R - Resistant check.

Table - 22: Development of *H. armigera* on the leaves of wild relatives of pigeonpea.

Species	Accession number	Larval period (days)	Pupal period (days)	Pupation (%)	Adult emergence (%)
C. acutifolius	ICPW 1	29.9	17.3	32 (34)	24 (29)
C. acutifolius	ICPW 2	32.8	16.9	32 (34)	32 (34)
C. albicans	ICPW 13	38.4	18.5	38 (38)	34 (35)
C. albicans	ICPW 14	35.2	17.6	32 (34)	30 (33)
C. cajanifolius	ICPW 28	30.8	17.1	36 (37)	34 (36)
C. cajanifolius	ICPW 29	34.6	17.6	32 (34)	26 (30)
C. lineatus	ICPW40	31.4	17.5	42 (40)	38 (38)
C. lineatus	ICPW 41	33.5	16.7	36 (37)	34 (36)
C. sericeus	ICPW 159	26.3	15.8	48 (44)	42 (40)
C. sericeus	ICPW 160	30.7	16.2	40 (39)	38 (38)
C. platycarpus	ICPW 68	30.4	16.4	28 (32)	18(25)
C. scarabaeoides	ICPW 83	39.6	17.9	18 (25)	18 (25)
C. scarabaeoides	ICPW 90	33.2	16.9	30 (33)	22 (27)
C. scarabaeoides	ICPW 94	34.9	17.1	32 (34)	24 (26)
C. scarabaeoides	ICPW116	35.4	17.7	18 (25)	18 (25)
C. scarabaeoides	ICPW 125	33.9	17.4	36 (37)	32 34)
C. scarabaeoides	ICPW 130	37.4	18.1	26 (30)	24 (29)
C. scarabaeoides	ICPW 137	36.1	17.2	30 (33)	24 (29)
C. scarabaeoides	ICPW 141	37.3	14.9	26 (30)	24 (29)
C. scarabaeoides	ICPW 152	34.9	14.5	22 (28)	18 (25)
C. scarabaeoides	ICPW278	32.1	16.0	32 (34)	22 (27)
C. scarabaeoides	ICPW 280	36.9	13.5	30 (33)	22 (28)
C. scarabaeoides	ICPW 281	35.8	16.6	30 (33)	26 (28)
D. ferruginea	ICPW 178	33.4	18.0	42 (40)	34 (35)
F. bracteata	ICPW 192	32.9	17.0	36 (37)	28 (31)
F. stricta	ICPW 202	25.7	18.3	34 (35)	28 (32)
P. scariosa	ICPW 207	34.3	18.2	30 (33)	16 (23)
R. aurea	ICPW 210	33.6	16.8	20(27)	12(21)
R. bracteata	ICPW 214	32.2	17.2	34 (35)	26 (30)
C. cajan (S)	ICPL 87	24.1	14.7	48 (44)	36 (37)
C. cajan (R)	ICPL 332	29.1	17.2	30 (33)	22 (27)
SE ±		0.49	0.97	4.60(3.39)	2.29(1.69)
LSD at 5%		1.91	2.02	12.8(8.11)	12.87(9.42)
F-test		<0.001	<0.001	<0.001	0.002(0.010)

Figures in parentheses are Angular transformed values.
S - Susceptible check. R - Resistant check.

leaves of *C. sericeus* (ICPW 159) (Fig 14). The larval and pupal weights, and pupation were significantly and positively correlated (r = 0.20 to 0.22) (Table 26). Larval weight showed a significant and negative association with larval mortality, larval period, and pupal period.

Development and survival of *H. armigera* on flowers and pods of wild relatives of pigeonpea

The larval and pupal weights were significantly lower in the larvae reared on the wild relatives of pigeonpea compared to the cultivated pigeonpea varieties, ICPL 332 (resistant check) and ICPL 87 (susceptible check) (except the larval weights at 5 days on *C. lineatus*, *P. scariosa* and *R. bracteata*).

The larval weights were lower at 5 (<5 mg per larva compared to 11.4 mg on ICPL 87)), 10 (<50 mg per larva compared to 237.7 mg on ICPL 87), and 15 days (<102.4 mg per larva compared to 325.2 mg on ICPL 87) in the larvae reared on the flowers/pods of *C. sericeus* (ICPW 160) and *C. scarabaeoides* (ICPW 83, ICPW 90, ICPW 94, ICPW 116, ICPW 125, ICPW 130, ICPW 137, ICPW 141, ICPW 280, ICPW 281, and ICPW 278) (Table 23).

Five days after initiating the experiment, larval mortality was >50% in the larvae reared on the flowers/pods of *C. acutifolius* (ICPW 2), *C. lineatus* (ICPW 40 and ICPW 41), *C. scarabaeoides* (ICPW 125), and *P. scariosa* (ICPW 207) compared to 26% larval mortality in larvae reared on ICPL 87 and 32% in larvae reared on ICPL 332 (Table 24). After 20 days, the larval mortality was >70% in larvae reared on flowers/pods of *C. scarabaeoides* (ICPW 83, ICPW 94, ICPW 280, and ICPW 281) compared to 36% larval mortality on flowers/pods of ICPL 87, and 46% on ICPL 332. Larvae reared on different accessions of *C. scarabaeoides* took 32.7 to 42.5 days to complete development compared to 24.3 days on ICPL 332 and 21.7 days on ICPL 87.

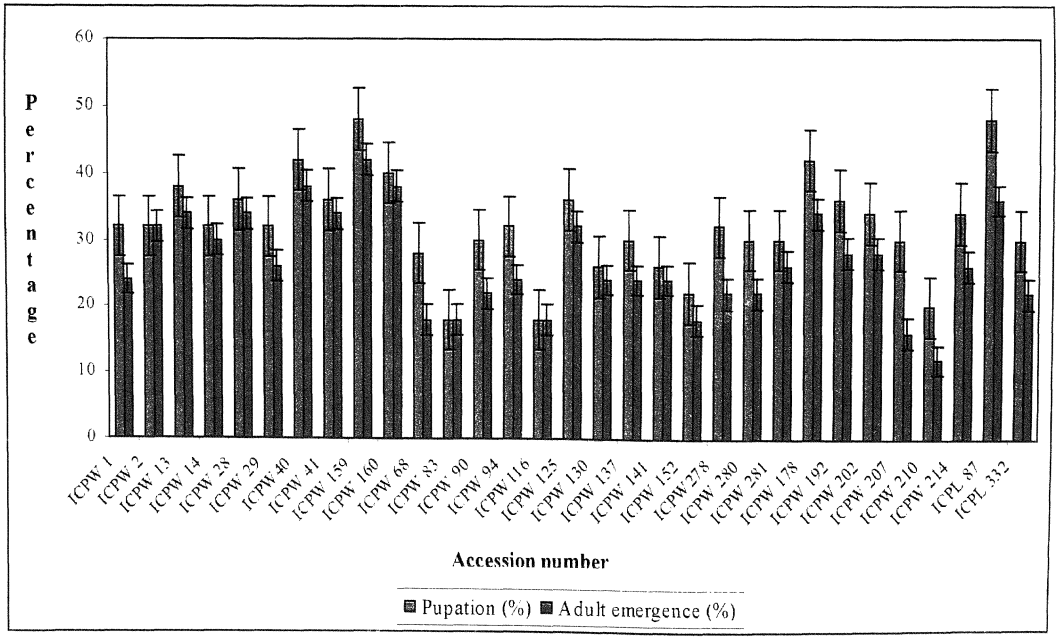


Fig 14 : Pupation and adult emergence of *H. armigera* reared on the leaves of wild relatives of pigeonpea

Table - 23: Larval and pupal weights of *H. armigera* reared on the flowers and pods of wild relatives of pigeonpea.

Species	Accession number	Larval weight (mg)			Pupal wt. (mg)
		5th day	10th day	15th day	
C. acutifolius	ICPW 1	5.5	26.8	154.5	140.5
C. acutifolius	ICPW 2	4.5	51.8	175.6	162.2
C. albicans	ICPW 13	9.8	57.2	197.1	189.3
C. albicans	ICPW 14	10.3	87.8	257.8	241.4
C. cajanifolius	ICPW 28	6.1	109.4	136.3	205.1
C. cajanifolius	ICPW 29	4.2	100.3	126.9	169.1
C. lineatus	ICPW40	17.1	85.4	170.5	232.4
C. lineatus	ICPW 41	13.3	59.2	134.8	219.3
C. sericeus	ICPW 159	4.3	39.6	112.1	165.8
C. sericeus	ICPW 160	4.3	25.9	102.4	148.5
C. platycarpus	ICPW 68	4.9	53.2	113.2	160.7
C. scarabaeoides	ICPW 83	3.1	13.2	33.3	127.6
C. scarabaeoides	ICPW 90	2.1	11.0	34.3	134.0
C. scarabaeoides	ICPW 94	2.2	9.2	29.3	92.5
C. scarabaeoides	ICPW116	2.5	10.0	32.3	124.1
C. scarabaeoides	ICPW 125	2.6	17.6	46.4	140.6
C. scarabaeoides	ICPW 130	3.0	16.6	33.1	124.6
C. scarabaeoides	ICPW 137	2.0	17.0	48.6	123.7
C. scarabaeoides	ICPW 141	3.2	22.1	60.7	126.1
C. scarabaeoides	ICPW 152	3.7	28.4	112.1	123.2
C. scarabaeoides	ICPW 278	2.5	19.6	71.6	134.2
C. scarabaeoides	ICPW 280	2.6	14.0	54.1	128.5
C. scarabaeoides	ICPW281	2.6	23.2	60.8	131.6
D. ferruginea	ICPW 178	3.8	51.4	109.0	147.6
F. bracteata	ICPW 192	4.6	72.2	278.8	227.9
F. stricta	ICPW 202	10.2	105.4	263.5	248.0
P. scariosa	ICPW 207	36.3	134.9	226.5	175.8
R. aurea	ICPW 210	2.4	11.5	25.7	125.2
R. bracteata	ICPW 214	13.3	140.8	300.6	233.7
C. cajan (S)	ICPL 87	11.4	237.7	325.2	271.2
C. cajan (R)	ICPL 332	7.1	181.5	294.5	245.4
SE ±		4.00	3.00	6.00	7.00
LSD at 5%		11.05	8.34	16.68	19.46
F-test		<0.001	<0.001	<0.001	<0.001

S - Susceptible check. R - Resistant check.

Table - 24: Mortality of *H. armigera* larvae reared on the flowers and pods of wild relatives of pigeonpea.

Species	Accession number	Larval mortality (%)			
		5th day	10 th day	15 th day	20 th day
C. acutifolius	ICPW 1	26 (31)	52 (46)	62 (52)	62 (52)
C. acutifolius	ICPW 2	50 (45)	53 (44)	53 (44)	53 (44)
C. albicans	ICPW 13	40 (39)	48 (44)	48 (44)	52 (46)
C. albicans	ICPW 14	38 (38)	38 (38)	40 (39)	40 (39)
C. cajanifolius	ICPW 28	40 (39)	44 (42)	48 (44)	48 (44)
C. cajanifolius	ICPW 29	38 (38)	44 (42)	44 (42)	48 (44)
C. lineatus	ICPW40	52 (46)	58 (52)	58 (52)	60 (51)
C. lineatus	ICPW 41	52 (46)	53 (44)	53 (44)	56 (48)
C. sericeus	ICPW 159	16 (24)	50 (62)	58 (52)	58 (52)
C. sericeus	ICPW 160	32 (34)	48 (44)	48 (44)	48 (44)
C. platycarpus	ICPW 68	30 (33)	44 (41)	50 (45)	56 (48)
C. scarabaeoides	ICPW 83	48 (44)	60 (51)	72 (58)	74 (59)
C. scarabaeoides	ICPW 90	28(32)	36 (37)	62 (52)	62 (52)
C. scarabaeoides	ICPW 94	44 (42)	44 (42)	66 (55)	70 (57)
C. scarabaeoides	ICPW116	34 (36)	42 (44)	60 (51)	60 (51)
C. scarabaeoides	ICPW 125	52 (46)	62(52)	62 (52)	62 (52)
C. scarabaeoides	ICPW 130	44 (42)	46 (43)	53 (44)	60 (51)
C. scarabaeoides	ICPW 137	32 (34)	44 (42)	53 (44)	53 (44)
C. scarabaeoides	ICPW 141	34 (36)	58 (52)	58 (52)	60 (51)
C. scarabaeoides	ICPW 152	34 (36)	34 (36)	40 (39)	48 (44)
C. scarabaeoides	ICPW278	36 (37)	46 (43)	64 (53)	64 (53)
C. scarabaeoides	ICPW 280	32 (34)	52 (46)	72 (58)	72 (58)
C. scarabaeoides	ICPW 281	26 (31)	46 (43)	70 (57)	76 (61)
D. ferruginea	ICPW 178	22 (28)	38 (38)	40 (39)	50 (45)
F. bracteata	ICPW 192	32 (34)	40 (39)	42 (44)	42 (44)
F. stricta	ICPW 202	44 (42)	48 (44)	48 (44)	66 (55)
P. scariosa	ICPW 207	50 (45)	52 (46)	56 (48)	58 (52)
R. aurea	ICPW 210	38 (38)	52 (46)	56 (48)	68 (56)
R. bracteata	ICPW 214	24 (29)	34 (36)	34 (36)	34 (36)
C. cajan (S)	ICPL 87	26 (31)	30 (33)	32 (34)	36 (37)
C. cajan (R)	ICPL 332	32 (34)	44 (42)	46 (43)	46 (43)
SE ±		1.302	1.503	1.682	1.005
LSD at 5%		10.74	9.15	8.978	7.849
F-test		<0.001	<0.001	<0.001	<0.001

Figures in parenthesis are Angular transformed values.
S - Susceptible check. R - Resistant check.

There were no significant differences in developmental period in larvae reared on flowers/pods of *C. acutifolius*, *C. albicans*, *C. cajanifolius*, *C. sericeus*, *F. bracteata*, *F. stricta*, *P. scariosa*, and *R. bracteata*. Pupation was <20% in insects reared on *C. scarabaeoides* (ICPW 83, ICPW 90, ICPW 94, and ICPW 130) compared to 50% on ICPL 332, and 60% on ICPL 87. Among wild accessions, high pupation (42 to 64%) was recorded in larvae reared on the flowers/pods of *C. albicans* (ICPW 14), *C. cajanifolius* (ICPW 28 and ICPW 29), *C. lineatus* (ICPW 41), *C. scarabaeoides* (ICPW 152), *F. stricta* (ICPW 202), *P. scariosa* (ICPW 207), and *R. bracteata* (ICPW 214) (Table 25 & Fig 15). Larval and pupal weights, pupation, and adult emergence were significant and positively correlated (r = 0.04 to 0.55). Larval mortality, and larval and pupal periods showed a significant and negative correlation (Table 26).

Development and survival of *H. armigera* on the artificial diet impregnated with lyophilized leaf powder of different wild relatives of pigeonpea

For standardization of protocol to assess the antibiosis component of resistance, a pilot experiment was conducted involving ICPW 83 (*C. scarabaeoides*), ICPL 87 (susceptible check) and ICPL 332 (resistant check). The larvae of *H. armigera* were reared on artificial diet impregnated with different quantities of lyophilized leaf powder.

Pilot experiment

There were significant differences in larval and pupal weights in the larvae reared on the diet impregnated with different amounts of lyophilized leaf powder. The larvae weighed <100 mg when reared on diet with leaf powder of ICPW 83 compared to > 100 mg on the diet impregnated with 5 g and 10 g of lyophilized leaf powder of the cultivated pigeonpeas, ICPL 332 and ICPL 87. The pupal weight (255.4 mg) was significantly lower on the diet impregnated with 10 g of ICPW 83 compared to pupal weight on standard diet (295.9 mg). Pupal weight (315.4 mg) of insects reared on ICPL 87 was significantly high compared to pupal weight of larvae reared on standard diet.

Table - 25: Development of *H. armigera* larvae on the flowers and pods of wild relatives of pigeonpea.

Species	Accession number	Larval period (days)	Pupal period (days)	Pupation (%)	Adult emergence (%)
C. acutifolius	ICPW 1	25.4	12.2	34 (36)	30 (33)
C. acutifolius	ICPW 2	24.7	12.4	38 (38)	28 (30)
C. albicans	ICPW 13	27.3	12.8	36 (37)	30 (33)
C. albicans	ICPW 14	25.5	13.2	52 (46)	24 (29)
C. cajanifolius	ICPW 28	21.8	12.2	46 (43)	28 (30)
C. cajanifolius	ICPW 29	24.1	12.8	48 (44)	26 (30)
C. lineatus	ICPW40	29.9	13.6	38 (38)	38 (38)
C. lineatus	ICPW 41	32.5	13.8	42 (40)	24 (29)
C. sericeus	ICPW 159	22.7	12.2	38 (38)	34 (36)
C. sericeus	ICPW 160	26.4	12.2	40 (39)	24 (29)
C. platycarpus	ICPW 68	24.8	12.8	30 (33)	24 (29)
C. scarabaeoides	ICPW 83	42.5	13.6	18 (24)	16 (23)
C. scarabaeoides	ICPW 90	37.3	13.8	20 (26)	18 (24)
C. scarabaeoides	ICPW 94	32.7	13.8	16 (23)	12 (20)
C. scarabaeoides	ICPW116	39.8	12.6	26 (30)	20 (26)
C. scarabaeoides	ICPW 125	33.7	12.2	38 (38)	22 (28)
C. scarabaeoides	ICPW 130	39.8	13.0	20 (26)	22 (28)
C. scarabaeoides	ICPW 137	40.0	13.0	32 (26)	28 (30)
C. scarabaeoides	ICPW 141	37.9	12.2	32 (26)	22 (28)
C. scarabaeoides	ICPW 152	36.1	12.8	46 (43)	30 (33)
C. scarabaeoides	ICPW278	42.1	12.8	22 (28)	22 (28)
C. scarabaeoides	ICPW 280	36.2	13.0	26 (30)	26 (30)
C. scarabaeoides	ICPW 281	38.1	13.2	22 (28)	18 (24)
D. ferruginea	ICPW 178	36.0	12.8	44 (42)	36 (37)
F. bracteata	ICPW 192	24.1	12.4	38 (38)	38 (38)
F. stricta	ICPW 202	26.4	12.6	46 (43)	36 (37)
P. scariosa	ICPW 207	27.1	13.6	44 (42)	18 (24)
R. aurea	ICPW 210	35.0	14.0	24 (29)	16 (23)
R. bracteata	ICPW 214	25.9	12.0	64 (53)	44 (42)
C. cajan (S)	ICPL 87	21.7	10.8	60 (51)	44 (42)
C. cajan (R)	ICPL 332	24.3	12.8	50 (45)	30 (33)
SE ±		1.63	1.28	4.40	259(1.67)
LSD at 5%		4.50	9.92	9.39(5.77)	9.12(6.09)
F-test		<0.001	<0.001	<0.001	<0.001

Figures in parenthesis are angular transformed values.
S - Susceptible check. R - Resistant check

Table - 25: Development of *H. armigera* larvae on the flowers and pods of wild relatives of pigeonpea.

Species	Accession number	Larval period (days)	Pupal period (days)	Pupation (%)	Adult emergence (%)
C. acutifolius	ICPW 1	25.4	12.2	34 (36)	30 (33)
C. acutifolius	ICPW 2	24.7	12.4	38 (38)	28 (30)
C. albicans	ICPW 13	27.3	12.8	36 (37)	30 (33)
C. albicans	ICPW 14	25.5	13.2	52 (46)	24 (29)
C. cajanifolius	ICPW 28	21.8	12.2	46 (43)	28 (30)
C. cajanifolius	ICPW 29	24.1	12.8	48 (44)	26 (30)
C. lineatus	ICPW40	29.9	13.6	38 (38)	38 (38)
C. lineatus	ICPW 41	32.5	13.8	42 (40)	24 (29)
C. sericeus	ICPW 159	22.7	12.2	38 (38)	34 (36)
C. sericeus	ICPW 160	26.4	12.2	40 (39)	24 (29)
C. platycarpus	ICPW 68	24.8	12.8	30 (33)	24 (29)
C. scarabaeoides	ICPW 83	42.5	13.6	18 (24)	16 (23)
C. scarabaeoides	ICPW 90	37.3	13.8	20 (26)	18 (24)
C. scarabaeoides	ICPW 94	32.7	13.8	16 (23)	12 (20)
C. scarabaeoides	ICPW116	39.8	12.6	26 (30)	20 (26)
C. scarabaeoides	ICPW 125	33.7	12.2	38 (38)	22 (28)
C. scarabaeoides	ICPW 130	39.8	13.0	20 (26)	22 (28)
C. scarabaeoides	ICPW 137	40.0	13.0	32 (26)	28 (30)
C. scarabaeoides	ICPW 141	37.9	12.2	32 (26)	22 (28)
C. scarabaeoides	ICPW 152	36.1	12.8	46 (43)	30 (33)
C. scarabaeoides	ICPW278	42.1	12.8	22 (28)	22 (28)
C. scarabaeoides	ICPW 280	36.2	13.0	26 (30)	26 (30)
C. scarabaeoides	ICPW 281	38.1	13.2	22 (28)	18 (24)
D. ferruginea	ICPW 178	36.0	12.8	44 (42)	36 (37)
F. bracteata	ICPW 192	24.1	12.4	38 (38)	38 (38)
F. stricta	ICPW 202	26.4	12.6	46 (43)	36 (37)
P. scariosa	ICPW 207	27.1	13.6	44 (42)	18 (24)
R. aurea	ICPW 210	35.0	14.0	24 (29)	16 (23)
R. bracteata	ICPW 214	25.9	12.0	64 (53)	44 (42)
C. cajan (S)	ICPL 87	21.7	10.8	60 (51)	44 (42)
C. cajan (R)	ICPL 332	24.3	12.8	50 (45)	30 (33)
SE ±		1.63	1.28	4.40	259(1.67)
LSD at 5%		4.50	9.92	9.39(5.77)	9.12(6.09)
F-test		<0.001	<0.001	<0.001	<0.001

Figures in parenthesis are angular transformed values.
S - Susceptible check. R - Resistance check

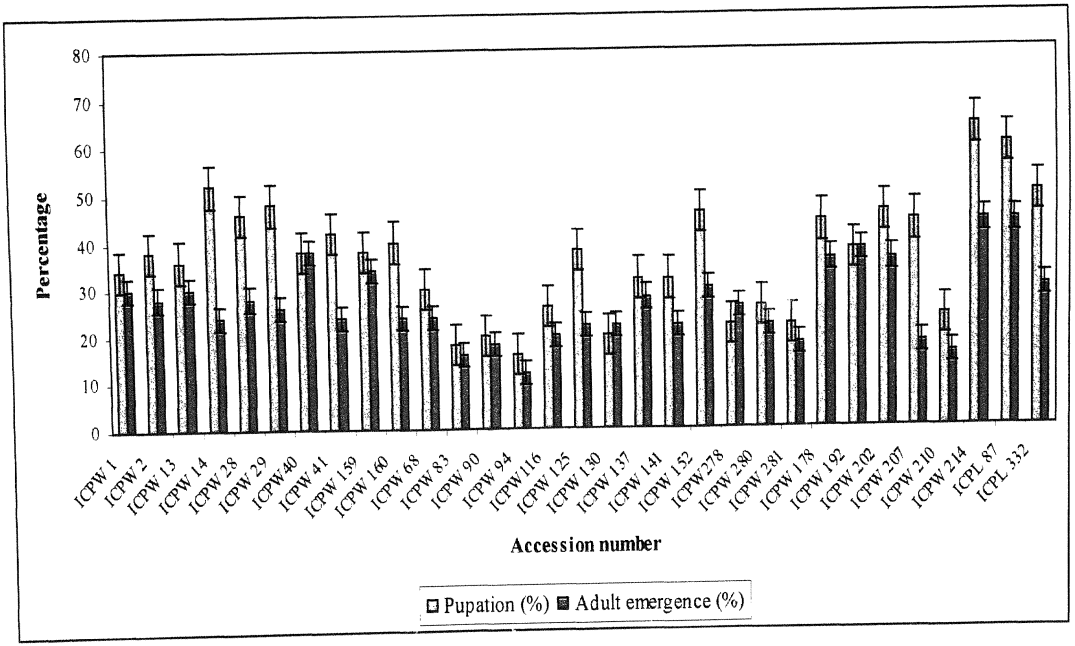


Fig 15 : Pupation and adult emergence of *H. armigera* reared on the flowers and pods of wild relatives of pigeonpea

Table - 26: Association between different developmental parameters of *H. armigera* reared on leaves and flowers and pods of wild relatives of pigeonpea

		Leaves							Flowers and pods						
		Larval weight	Pupal weight	Larval Mortality	Larval period	Pupation %	Pupal period	Adult emergence	Larval weight	Pupal weight	Larval Mortality	Larval period	Pupation %	Pupal period	Adult emergence
Leaves	Larval weight	1.000													
	Pupal weight	0.22*	1.00												
	Larval mortality	-0.24*	-0.69**	1.00											
	Larval period	-0.43**	-0.11	-0.43*	1.00										
	Pupation %	0.22	0.51	-0.69**	-0.11	1.00									
	Pupal period	-0.09	0.01	0.15	0.25*	-0.11	1.00								
	Adult emergence	0.20	0.91**	-0.64**	-0.50**	0.91**	-0.10	1.00							
Flowers and pods	Larval weight	0.59**	0.55**	0.59**	-0.51**	0.42*	-0.01	0.41*	1.00						
	Pupal weight	0.52**	0.73	0.52**	-0.47**	0.62**	0.02	0.59**	0.87	1.00					
	Larval mortality	-0.26*	-0.23*	0.26*	0.21	-0.22*	0.13	-0.22*	-0.15	-0.09	1.00				
	Larval period	-0.16	-0.42*	-0.16	0.59**	-0.62**	0.08	-0.57**	-0.72**	-0.76**	0.06	1.00			
	Pupation %	0.37*	0.53**	0.37*	-0.57**	0.62**	-0.04	0.65**	0.72**	0.70**	-0.44**	-0.66**	1.00		
	Pupal period	-0.19	-0.15	-0.19	0.43*	-0.50**	0.28*	-0.52**	-0.48**	-0.34*	0.50**	0.56**	-0.60**	1.00	
	Adult emergence	0.30*	0.38*	-0.59**	-0.58**	0.57**	-0.34*	0.53**	0.04	-0.32*	-0.48**	0.55**	0.55**	-0.30*	1.00

*,** Correlation coefficients significant at P = 0.05 and 0.01 respectively

The highest larval mortality of 16.7% was recorded on artificial diet impregnated with leaf powder of ICPW 83, followed by 10% on ICPL 332, 6.7% on ICPL 87, and 10% on standard diet. There was a gradual decrease in larval and pupal weights with an increase in the amount of lyophilized leaf powder impregnated in the diet. However, there was an increase in the larval mortality with increase of concentrations. A significant delay was noticed in the larval developmental period with the increase in the concentration of leaf powder in all the genotypes. However, such trend was not observed in the case of pupal periods. A significant reduction in percent pupation and adult emergence was observed with the increase in the concentration of lyophilized leaf powder in the artificial diet in all the genotypes (Fig 16 & 17). The highest reduction, both in the percent pupation and adult emergence was recorded in ICPW 83 of *C. scarabaeoides* compared to both the cultivated checks (Table 27).

Main experiment

The larval and pupal weights of larvae reared on the diet impregnated with lyophilized leaf powder of wild relatives were significantly lower compared to the larvae reared on the diet impregnated with leaf powder of cultivated pigeonpea, and the standard diet (Fig 18). Larval weights were <50 mg per larvae when reared on the diet with lyophilized leaf powder of pigeonpea and its wild relatives (except on ICPL 87 - 53.3 mg) compared to 469.6 mg in the larvae reared on the standard diet. Larval weights were <20 mg in the larvae reared on diets having leaf powder of *C. acutifolius*, *C. sericeus* (ICPW 160), *C. scarabaeoides* (except ICPW 137, ICPW 141, and ICPW 152), *P. scariosa*, *C. platycarpus*, and *R. aurea* compared to 53.3 mg on ICPL 87 and 44.0 mg on ICPL 332. The weights of pupae from the larvae reared on the diet with lyophilized leaf powder of *C. albicans* (ICPW 13), *C. cajanifolius* (ICPW 28 and ICPW 29), *C. lineatus* (ICPW 41), *C. scarabaeoides* (ICPW 125, ICPW 130, ICPW 141, and ICPW 152), *D. ferruginea* (ICPW 178), *F. stricta* (ICPW 202), *R. bracteata* (ICPW 214)), *C. platycarpus* (ICPW 68) and *C. cajan* (ICPL 332 and ICPL 87) were >300 mg compared to <250 mg of the pupae on diets containing leaf powder of *C. sericeus* (ICPW 159 and ICPW 160), and *C. scarabaeoides* (ICPW 137) (Table 28).

Table - 27: Assessment of antibiosis component of resistance in the wild relatives of pigeonpea against *H. armigera* through the artificial diet impregnated with lyophilized leaf powder.

Accession number	Larval wt.(mg)	Pupal Wt.(mg)	Larval mortality (%)	Larval period (days)	Pupal period (days)	Pupation (%)	Adult emergence (%)
Test genotype							
ICPL 83(5g)	30.73	255.4	16.67(23.86)	24.00	12.67	53.33(46.92)	39.23(40.00)
ICPL 83(10g)	9.4	260.7	36.67(37.22)	27.00	15.00	33.33(35.22)	28.29(23.33)
ICPL 83(15g)	5.63	124.8	73.33(59.00)	34.33	14.33	3.33(6.14)	*
ICPL 83(20g)	2.4	66.7	100.00(90.00)	39.72	*	*	*
Resistant check							
ICPL 332(5g)	51.73	290.3	10.00(18.43)	21.67	12.33	63.33(52.78)	45.00(50.00)
ICPL 332(10g)	47.13	293.2	23.33(28.29)	25.67	13.67	50.00(45.00)	39.23(40.00)
ICPL 332(15g)	6.63	141.2	53.33(46.92)	26.33	12.33	33.3(35.22)	15.00(10.00)
ICPL 332(20g)	4.03	109.4	56.67(48.85)	25.33	12.67	33.6(36.08)	31.00(26.67)
Susceptible check							
ICPL 87(5g)	71.67	313.3	6.67(12.29)	16.33	10.67	66.67(54.78)	48.85(56.67)
ICPL 87(10g)	57.03	315.4	16.67(23.86)	17.67	12.33	60.00(50.85)	45.00(50.00)
ICPL 87(15g)	15.40	231.3	56.67(48.85)	21.67	11.67	53.33(46.92)	33.21(30.00)
ICPL 87(20g)	8.60	169.6	56.67(48.85)	20.00	12.33	43.33(41.07)	33.21(30.00)
Standard diet	237.23	295.9	10.00(18.43)	11.67	10.33	73.33(59.00)	52.78(63.33)
SE ±	7.22	378.9	3.332.773	0.492	0.385	3.88(2.82)	2.93(3.14)
LSD at 5%	21.07	< 0.001	9.7298.092	1.442	1.129	11.38(8.29)	8.593(9.204)
F prob	< 0.001		< 0.001 < 0.001	< 0.001	< 0.001	< 0.001 (< 0.001)	< 0.001(0.001)

Figures in parenthesis are Angular transformed values. ; * Not observed.

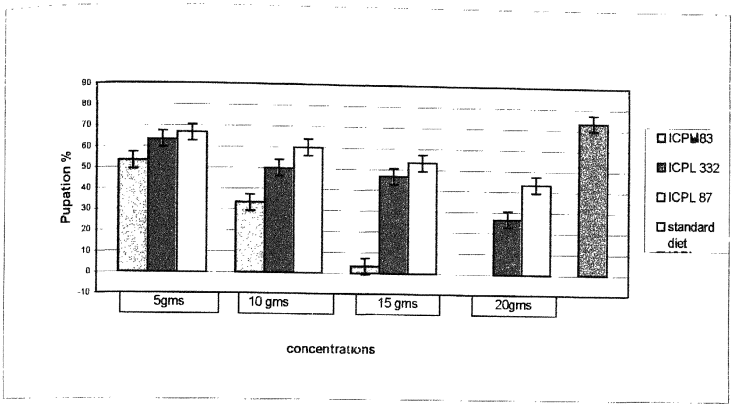


Fig-16 : Pupation of *H. armigera* on the diet impregnated with lyophilized leaf powder of pigeonpeas (ICPL 87 and ICPL 332) and its wild relative *C. scarabaeoides* (ICPW 83)

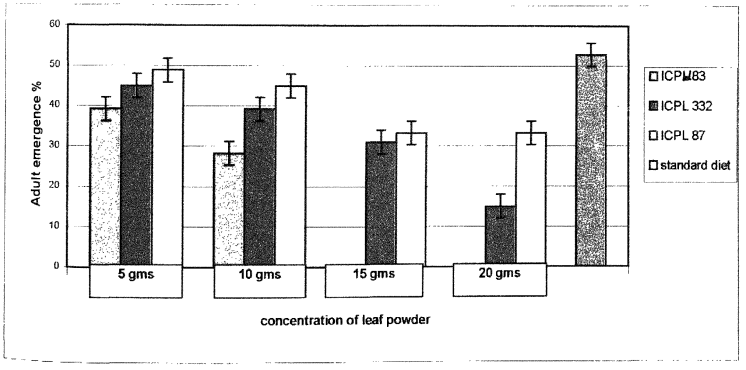


Fig-17 : Adult emergence of *H. armigera* on the diet impregnated with lyophilized leaf powder of pigeonpeas (ICPL 87 and ICPL 332) and its wild relative *C. scarabaeoides* (ICPW 83)

Table - 28: Larval and pupal weights and mortality of *H. armigera* reared on the artificial diet impregnated with lyophilized leaf powder of wild relatives of pigeonpea.

Species	Accession number	Larval wt. (mg)	Pupal wt. (mg)	Larval mortality (%)
C. acutifolius	ICPW 1	12.5	288.3	26.7 (31.0)
C. acutifolius	ICPW 2	12.6	254.6	30.0 (33.2)
C. albicans	ICPW 13	34.3	300.1	26.7 (30.8)
C. albicans	ICPW 14	37.6	266.0	26.7 (31.0)
C. cajanifolius	ICPW 28	41.4	317.4	20.0 (26.6)
C. cajanifolius	ICPW 29	26.8	313.9	33.3 (35.2)
C. lineatus	ICPW40	27.4	297.1	33.3 (35.2)
C. lineatus	ICPW 41	22.3	310.6	33.3 (35.2)
C. sericeus	ICPW 159	24.0	230.4	20.0 (26.6)
C. sericeus	ICPW 160	12.5	243.9	26.7 (31.0)
C. platycarpus	ICPW 68	15.0	307.7	26.7 (31.0)
C. scarabaeoides	ICPW 83	9.5	289.9	33.3 (35.2)
C. scarabaeoides	ICPW 90	10.4	278.1	36.7 (37.2)
C. scarabaeoides	ICPW 94	14.1	292.0	30.0 (33.0)
C. scarabaeoides	ICPW116	13.2	275.3	30.0 (33.2)
C. scarabaeoides	ICPW 125	14.5	301.7	26.7 (31.0)
C. scarabaeoides	ICPW 130	12.1	305.3	33.3 (35.2)
C. scarabaeoides	ICPW 137	22.6	213.5	26.7 (31.0)
C. scarabaeoides	ICPW 141	28.1	307.9	36.7 (37.2)
C. scarabaeoides	ICPW 152	38.1	311.4	33.3 (35.2)
C. scarabaeoides	ICPW278	17.2	299.4	26.7 (31.0)
C. scarabaeoides	ICPW 280	18.2	267.7	40.0 (39.1)
C. scarabaeoides	ICPW 281	15.5	267.2	33.3 (35.2)
D. ferruginea	ICPW 178	26.8	312.1	30.0 (33.2)
F. bracteata	ICPW 192	27.2	296.7	20.0 (26.6)
F. stricta	ICPW 202	48.1	325.6	26.7 (31.0)
P. scariosa	ICPW 207	12.0	270.7	33.3 (35.2)
R. aurea	ICPW 210	39.8	296.0	30.0 (33.2)
R. bracteata	ICPW 214	13.1	322.7	16.7 (23.9)
C. cajan (S)	ICPL 87	53.3	352.5	13.3 (21.1)
C. cajan (R)	ICPL 332	44.0	341.8	26.7 (31.0)
Artificial diet		469.6	334.4	23.3 (28.8)
SE±		6.85	19.93	3.38 (2.17)
LSD at 5%		19.0	56.0	9.455 (6.152)
F-test		<0.001	<0.001	<0.001(<0.001)

Figures in parenthesis are Angular transformed values.
S - Susceptible check. R - Resistant check.

C. cajan (ICPL 87)

C. cajan (ICPL 332)

C. scarabaeoides

R. bracteata

Fig-18 : Growth of *H. armigera* on artificial diet impregnated with lyophilized leaf powder of wild relatives of pigeonpea

The larvae took significantly longer time to complete development on diet impregnated with leaf powder of wild relatives of pigeonpea compared to the larvae reared on cultivated pigeonpea and the standard diet. However, there were no differences in pupal period. Larvae took >25 days for pupation when reared on diet impregnated with lyophilized leaf powder of *C. cajanifolius, C. lineatus, C. sericeus, C. scarabaeoides, D. ferruginea, F. bracteata, F. stricta, C. platycarpus, R. aurea,* and *P. scariosa* compared to, 18.7 days in ICPL 87, 25.3 days in ICPL 332, and 12.3 days on the standard artificial diet (Table 29). There were no significant differences in pupal period (10.7 days on the standard diet, and 11 to 14 days on diets with leaf powder of pigeonpea and its wild relatives). Pupation was >50% when the larvae were reared on the diets with leaf powder of ICPL 87, and ICPL 332, *C. sericeus* (ICPW 159) and *F. stricta* (ICPW 202), and 30 to 36.7% pupation on *C. sericeus* (ICPW 160), *C. scarabaeoides* (ICPW 83 and ICPW 141), *P. scariosa* (ICPW 207), and *R. aurea* (ICPW 210) compared to 63.3% pupation on standard artificial diet. Adult emergence was <30% when the larvae were reared on artificial diet impregnated with lyophilized leaf powder of *P. scariosa* (ICPW 207), and *R. aurea* (ICPW 210) (Fig 19). The larval weight was significant and positively correlated with pupal weight and adult emergence. Larval mortality, larval period and pupal period were negatively correlated (Table 33).

Development and survival of *H. armigera* on the artificial diet impregnated with lyophilized pod powder of wild relatives of pigeonpea

Pilot experiment

Maximum differences in the larval weights and survival were observed in the diet containing 10 g of lyophilized pod powder (Table 30). Therefore, based on this data, the concentration of 10 g of lyophilized pod powder was used to assess the antibiosis mechanism of resistance to *H. armigera* in wild relatives of pigeonpea.

The larval weight was <100 mg when the diet was impregnated with 5 g of lyophilized pod powder of ICPW 83, 244.4 mg in diet with ICPL 332 pod powder and 329.5 mg in diet with ICPL 87 pod powder. Differences in the larval weights were not significant between the larvae reared on the standard diet and the diet with 5 g of ICPL 87 pod

Table - 29: Development of *H. armigera* larvae reared on the artificial diet impregnated with lyophilized leaf powder of wild relatives of pigeonpea.

Species	Accession number	Larval period (days)	Pupal period (days)	Pupation (%)	Adult emergence (%)
C. acutifolius	ICPW 1	19.3	12.5	50.0 (45.1)	46.7 (43.1)
C. acutifolius	ICPW 2	21.0	13.3	46.7 (43.1)	36.7 (37.2)
C. albicans	ICPW 13	22.0	12.7	46.7 (43.1)	43.3 (41.2)
C. albicans	ICPW 14	23.3	14.0	40.0 (39.1)	33.3 (35.2)
C. cajanifolius	ICPW 28	24.7	11.3	46.7 (43.1)	40.0 (39.1)
C. cajanifolius	ICPW 29	25.7	12.3	40.0 (39.1)	30.0 (33.2)
C. lineatus	ICPW40	26.0	12.3	46.7 (43.1)	40.0 (39.2)
C. lineatus	ICPW 41	25.7	13.3	40.0 (39.1)	33.3 (35.2)
C. sericeus	ICPW 159	26.0	13.3	56.7 (48.8)	46.7 (43.1)
C. sericeus	ICPW 160	27.3	13.3	36.7 (37.2)	33.3 (35.2)
C. platycarpus	ICPW 68	26.0	13.3	46.7 (43.1)	30.0 (33.0)
C. scarabaeoides	ICPW 83	27.7	14.3	33.3 (35.2)	26.7 (30.8)
C. scarabaeoides	ICPW 90	27.3	12.3	40.0 (39.2)	33.3 (35.2)
C. scarabaeoides	ICPW 94	26.7	12.7	46.7 (43.1)	30.0 (33.0)
C. scarabaeoides	ICPW116	25.0	12.7	43.3 (41.2)	33.3 (35.0)
C. scarabaeoides	ICPW 125	24.7	11.3	50.0 (45.0)	43.3 (41.1)
C. scarabaeoides	ICPW 130	27.0	13.3	46.7 (43.1)	43.3 (41.2)
C. scarabaeoides	ICPW 137	25.3	12.3	50.0 (45.0)	40.0 (39.1)
C. scarabaeoides	ICPW 141	27.3	13.0	36.7 (37.2)	26.7 (30.8)
C. scarabaeoides	ICPW 152	25.7	12.7	46.7 (43.1)	33.3 (35.2)
C. scarabaeoides	ICPW278	26.7	12.3	50.0 (45.0)	43.3 (41.2)
C. scarabaeoides	ICPW 280	28.7	13.0	46.7 (43.1)	30.0 (33.0)
C. scarabaeoides	ICPW 281	27.0	13.0	46.7 (43.1)	40.0 (39.1)
D. ferruginea	ICPW 178	27.0	13.0	50.0 (45.0)	50.0 (45.0)
F. bracteata	ICPW 192	28.3	11.3	46.7 (43.1)	36.7 (37.2)
F. stricta	ICPW 202	28.0	12.3	53.3 (46.9)	46.7 (43.1)
P. scariosa	ICPW 207	33.3	13.7	30.0 (33.2)	23.3 (28.8)
R. aurea	ICPW 210	27.7	13.7	33.3 (35.2)	16.7 (23.9)
R. bracteata	ICPW 214	23.3	11.7	50.0 (45.0)	46.7 (43.0)
C. cajan (S)	ICPL 87	18.7	12.3	63.3 (52.9)	50.0 (45.0)
C. cajan (R)	ICPL 332	25.3	13.7	53.3 (46.9)	33.3 (35.2)
Artificial diet		12.3	10.7	63.3 (52.8)	60.0 (50.8)
SE ±		0.66	0.46	4.14 (2.42)	4.75 (2.89)
LSD at 5%		1.907	1.273	11.72(6.83)	13.63 (8.29)
F-test		<0.001	<0.001	<0.001 (<0.001)	<0.001(<0.001)

Figures in parentheses are angular transformed values

S- Susceptible check, R- Resistant check

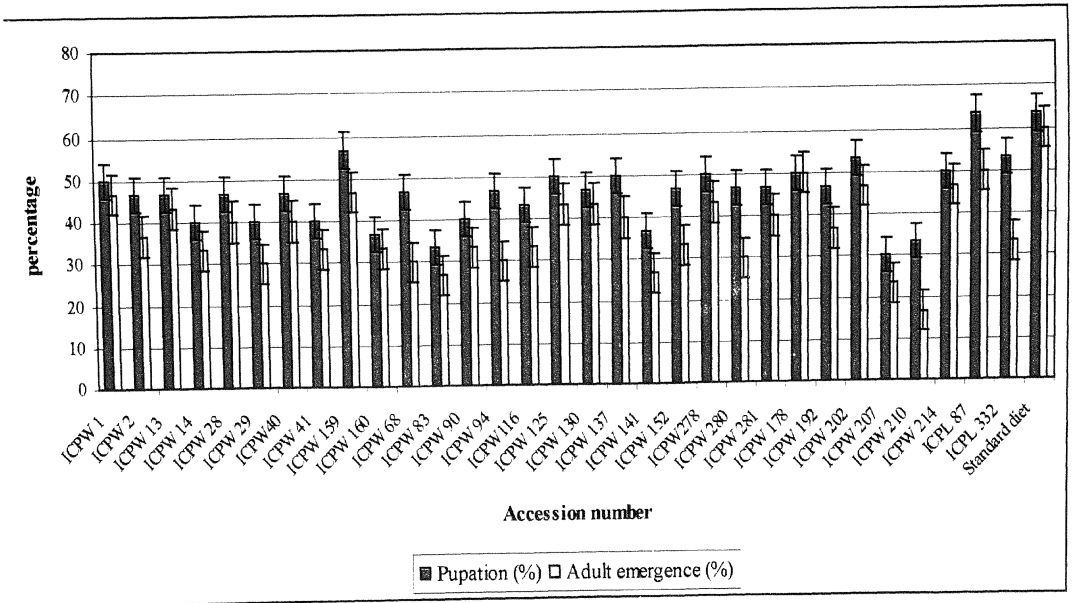


Fig 19 : Pupation and adult emergence of *H. armigera* reared on artificial diet impregnated with lyophilized leaf powder of wild relatives of pigeonpea

Table - 30: Assessment of antibiosis component of resistance in the wild relatives of pigeonpea against *H. armigera* through the artificial diet impregnated with lyophilized pod powder.

Accession .number	Larval wt.(mg)	Pupal wt.(mg)	Larval mortality (%)	Larval period (days)	Pupal period (days)	Pupation (%)	Adult emergence (%)
Test Genotype							
ICPL 83(5g)	97.9	308.2	16.67(23.86)	20.00	11.67	56.67(40.84)	43.33(41.15)
ICPL 83(10g)	34.5	296.3	26.67(31.00)	26.33	14.00	36.67(21.96)	26.67(30.99)
ICPL 83(15g)	8.3	160.9	53.33(21.96)	35.33	15.00	26.67(30.99)	16.67(30.99)
ICPL 83(20)	4.0	*	53.33(46.92)	38.00	*	3.33(6.14)	*
Resistant Check							
ICPL 332(5g)	244.4	307.9	13.33(21.14)	18.33	11.67	60(50.85)	50.00(45.00)
ICPL 332(10)	140.0	327.4	20.00(26.57)	21.67	12.33	50(20.54)	46.67(43.07)
ICPL 332(15g)	78.8	276.8	28.00(20.55)	25.00	13.67	53.33(26.56)	46.67(43.07)
ICPL 332(20g)	12.1	223.0	40.00(18.10)	27.00	16.00	33.33(21.14)	33.33(56.78)
Susceptible check							
ICPL 87(5g)	329.5	344.5	13.33(21.14)	14.33	9.33	66.67(54.99)	66.67(54.79)
ICPL 87(10g)	314.6	326.5	10.00(15.00)	16.00	12.00	56.67(20.25)	50.00(45.00)
ICPL 87(15g)	109.3	291.5	30.00(20.25)	18.67	11.67	50(15.00)	46.67(45.00)
ICPL 87(20g)	57.6	229.2	33.33(35.22)	21.67	11.67	36.67(37.22)	30.00(33.00)
Standard diet	347.7	278.5	13.33(21.14)	12.33	9.67	76.67(61.21)	70.00(56.79)
SE ±	84.7	14.45	4.31(2.03)	0.784	1.517	4.133.102	4.27(3.66)
LSD at 5%	247.1	42.37	12.57(5.932)	2.287	NS	12.079.05	12.46(10.68)
F prob	0.018	< 0.001	< 0.001(< 0.001)	< 0.001	0.117	< 0.001(< 0.001)	< 0.001(<0.001)

Figures in parenthesis are Angular transformed values. ; * Not observed.; NS – Non-significant at P = 0.05.

powder. The pupal weights were >300 mg in the larvae reared on the diets with pod powder compared to the 278.5 mg in larvae reared on the standard artificial diet.

Larval mortality increased with an increase in the amount of pod powder impregnated in artificial diet. Larval mortality was 53.33% in larvae reared on the diet impregnated with 15 and 20 g of lyophilized pod powder of ICPW 83 and was 26.66% in the diet with 10 g of pod powder. Larval mortality was 20% and 10% in the larvae reared on the artificial diet with 10 g of ICPL 332 and ICPL 87 pod powder, respectively.

Larval developmental period varied between and within the species tested. Longest larval period (38 days) was observed in the larvae reared on diet with 20 g of ICPW 83 pod powder compared to 12.33 days in larvae reared on the standard diet. The larval period was 26.33, 21.76, 16.00 days in the larvae reared on diet with 10 g of pod powder of ICPW 83, ICPL 332, ICPL 87, respectively. Pupal period was 16 days in insects reared on diet with 20 g of ICPL 332 pod powder, 9.67 days in insects reared on standard diet. Pupal period was 14.00, 12.33 and 12.00 days in insects reared on the artificial diet with 10 g pod powder of ICPW 83, ICPL 332, and ICPL 87, respectively.

Significant reduction in pupation and adult emergence was observed in the insects reared on diet with 20 g of pod powder. Lower pupation (3.33%) was observed in the insects reared on artificial diet with 20 g of pod powder of ICPW 83 compared to 76.67 % pupation in the insects reared on standard diet (Fig 20 & 21).

Main experiment

Larval weights were <50 mg when reared on the artificial diet impregnated with lyophilized pod powder of *C. acutifolius* (ICPW 1), *C. lineatus* (ICPW 40 and ICPW 41), *C. scarabaeoides* (ICPW 83), *C. platycarpus* (ICPW 68), and *R. aurea* (ICPW 210) as compared to 339.6 g on diets with pod powders of ICPL 87, 137.1 g on ICPL 332, and 407.7 g on standard artificial diet (Table 31). Weights of the larvae reared on *F. stricta* (ICPW 202) and *R. bracteata* (ICPW 214) were similar to those reared on the cultivated pigeonpea (Fig 22). Pupal weights ranged from 258.7 mg on ICPW 281 to 385.7 mg on ICPL 87 as compared to 324.0 mg on the standard artificial diet.

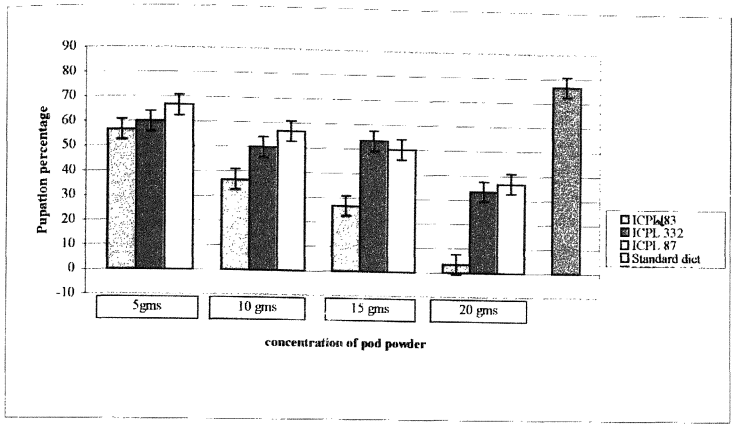


Fig-20 : Pupation of *H. armigera* on the diet impregnated with lyophilized pod powder of pigeonpeas (ICPL 87 and ICPL 332) and its wild relative *C. scarabaeoides* (ICPW 83)

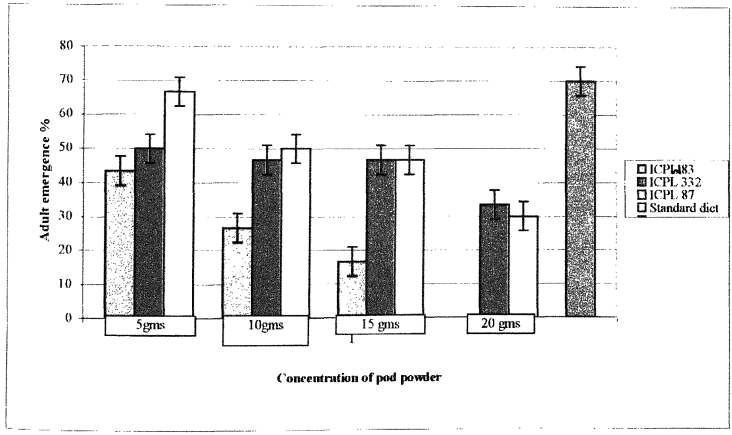


Fig-21 : Adult emergence of *H. armigera* on the diet impregnated with lyophilized pod powder of pigeonpeas (ICPL 87 and ICPL 332) and its wild relative *C. scarabaeoides* (ICPW 83)

Table - 31 : Larval and pupal weights and mortality of *H. armigera* reared on the artificial diet impregnated with lyophilized pod powder of wild relatives of pigeonpea.

Species	Accession number	Larval wt. (mg)	Pupal wt. (mg)	Larval mortality (%)
C. acutifolius	ICPW 1	32.6	284.8	30.0 (33.2)
C. acutifolius	ICPW 2	52.5	299.5	36.7 (37.2)
C. albicans	ICPW 13	137.5	324.8	30.0 (33.2)
C. albicans	ICPW 14	127.6	323.4	33.3 (35.2)
C. cajanifolius	ICPW 28	120.6	318.5	30.0 (33.2)
C. cajanifolius	ICPW 29	131.1	300.7	36.7 (37.2)
C. lineatus	ICPW40	45.0	272.7	40.0 (39.2)
C. lineatus	ICPW 41	40.4	291.5	40.0 (39.2)
C. sericeus	ICPW 159	58.5	320.5	30.0 (33.2)
C. sericeus	ICPW 160	54.4	311.3	30.0 (33.0)
C. platycarpus	ICPW 68	27.9	302.6	26.7 (31.0)
C. scarabaeoides	ICPW 83	31.9	314.6	36.7 (37.2)
C. scarabaeoides	ICPW 90	58 .0	283.1	33.3 (35.2)
C. scarabaeoides	ICPW 94	71.9	299.7	26.7 (31.0)
C. scarabaeoides	ICPW116	60.2	334.2	30.0 (33.0)
C. scarabaeoides	ICPW 125	87.0	312.6	30.0 (33.0)
C. scarabaeoides	ICPW 130	108.0	277.5	40.0 (39.1)
C. scarabaeoides	ICPW 137	78.0	311.3	30.0 (33.0)
C. scarabaeoides	ICPW 141	64.2	288.6	36.7 (37.0)
C. scarabaeoides	ICPW 152	105.4	304.1	36.7 (37.2)
C. scarabaeoides	ICPW278	54.6	300.0	30.0 (33.0)
C. scarabaeoides	ICPW 280	74.6	308.2	30.0 (33.0)
C. scarabaeoides	ICPW 281	77.4	258.7	30.0 (33.0)
D. ferruginea	ICPW 178	104.6	312.1	30.0 (33.3)
E. bracteata	ICPW 192	97.9	303.3	26.7 (31.0)
E. stricta	ICPW 202	216.0	317.8	30.0 (33.0)
P. scariosa	ICPW 207	95.9	281.5	40.0 (39.2)
R. aurea	ICPW 210	26.5	281.4	43.3 (41.2)
R. bracteata	ICPW 214	215.7	355.5	10.0 (18.4)
C. cajan (S)	ICPL87	339.6	385.7	16.7 (23.9)
C. cajan (R)	ICPL 332	137.1	328.1	33.3 (35.2)
Artificial diet	-	407.7	324.0	26.7 (31.0)
SE ±		40.0	9.71	3.83 (2.42)
LSD at 5%		114.0	028.0	10.853 (6.847)
F-test		<0.001	<0.001	<0.001 (<0.001)

Values in parenthesis are Angular transform

S - Susceptible check. R – Resistant check.

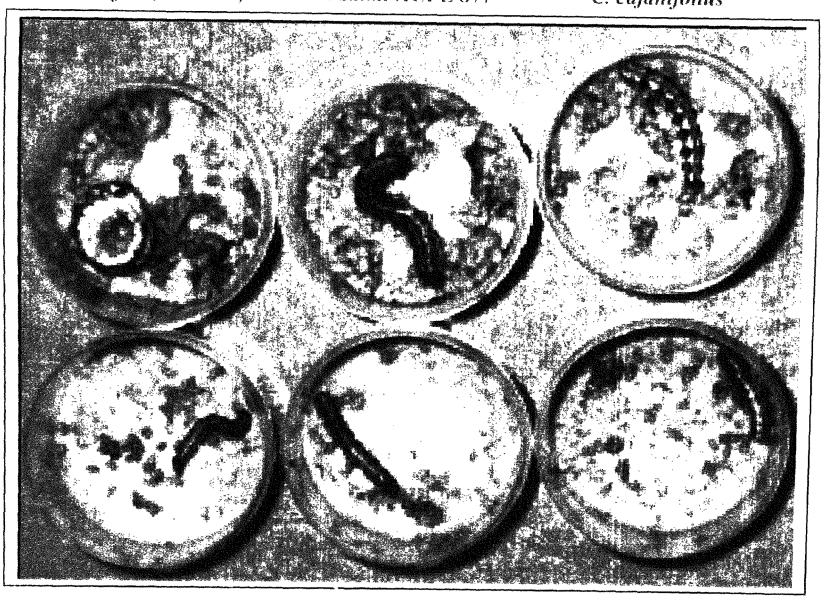


Fig-22 : Growth of *H. armigera* on the diet impregnated with lyophilized pod powder of wild relatives of pigeonpea

The larvae took significantly longer time to complete their development than on the cultivated pigeonpea, and the standard artificial diet. However, there were no differences in pupal period. Larvae took >25 days to complete the development when reared on artificial diet impregnated with lyophilized pod powder of *C. acutifolius* (ICPW 2), *C. lineatus* (ICPW 41), *C. sericeus*, *C. scarabaeoides* (except on ICPW 125), *P. scariosa* (ICPW 207), *R. aurea* (ICPW 210), *D.* ferruginea (ICPW 178) and *C. platycarpus* (ICPW 68) as compared to 15.7 days on ICPL 87, 23.3 days on ICPL 332 and 12.7 days on the standard artificial diet (Table 32). Pupal period was 8.7 days for insects reared on the diet impregnated with lyophilized pod powder of *C. cajanifolius* (ICPW 29), 14.7 days on ICPW 83, 14.0 days on ICPW 280 (*C. scarabaeoides*) as compared to 12 days in ICPL 87, 11.7 days in ICPL 332, and 10.7 days on the standard artificial diet. Pupation was considerably lower on the artificial diet impregnated with pod powder of wild relatives of pigeonpea compared to that on the cultivated pigeonpea and the standard artificial diet. Pupation was <40% in the larvae reared on the artificial diet impregnated with lyophilized pod powder of *C. cajanifolius* (ICPW 29), *C. sericeus* (ICPW 160), *C. scarabaeoides* (ICPW 83 and ICPW 278), *P. scariosa* (ICPW 207), C. *platycarpus* (ICPW 68), and *R. aurea* (ICPW 210) compared to 56.7% pupation on the standard artificial diet, ICPL 87, and ICPL 332. Adult emergence was <30 % in the diet with lyophilized pod powder of wild relatives of pigeonpea, except *C. cajanifolius* (ICPW 29) compared to 46.7% with ICPL 87, 40.0% with ICPL 332 , and 53.3% on standard artificial diet (Fig 23). The larval weight was significantly and positively correlated with pupal weight and adult emergence. Whereas, the larval mortality and pupal period were negatively correlated (Table 33).

Relative feeding preference by the third-instar larvae of *H. armigera* towards the leaves and pods of pigeonpea and its wild relatives

Relative feeding preference by the third-instar larvae of *H. armigera* towards the leaves and pods of pigeonpea and its wild relatives studied under no-choice and multi-choice conditions revealed the following results.

Table - 32: Development of *H. armigera* larvae on the artificial diet impregnated with lyophilized pod powder of wild relatives of pigeonpea.

Species	Accession number	Larval period (days)	Pupal period (days)	Pupation (%)	Adult emergence (%)
C. acutifolius	ICPW 1	23.3	11.3	53.3 (46.9)	46.7 (43.1)
C. acutifolius	ICPW 2	26.0	12.3	43.3 (41.2)	33.3 (35.2)
C. albicans	ICPW 13	21.0	11.3	46.7 (43.1)	40.0 (39.1)
C. albicans	ICPW 14	24.0	12.3	50.0 (45.0)	46.7 (43.0)
C. cajanifolius	ICPW 28	20.0	11.0	56.7 (48.8)	36.7 (37.2)
C. cajanifolius	CPW 29	21.3	8.7	40.0 (39.2)	33.3 (35.2)
C.lineatus	ICPW40	23.7	13.7	46.7 (43.1)	40.0 (39.1)
C.lineatus	ICPW 41	26.0	12.3	43.3 (41.2)	23.3 (28.8)
C. sericeus	ICPW 159	28.0	11.3	50.0 (45.0)	46.7 (43.1)
C. sericeus	ICPW 160	30.0	12.3	36.7 (37.2)	26.7 (31.0)
C. platycarpus	ICPW 68	28.0	13.3	33.3 (35.2)	26.7 (31.0)
C. scarabaeoides	ICPW 83	28.3	14.7	30.0 (33.2)	26.7 (31.0)
C. scarabaeoides	ICPW 90	25.7	13.3	43.3 (41.2)	36.7 (37.2)
C. scarabaeoides	ICPW 94	27.0	13.3	50.0 (45.0)	43.3 (41.2)
C. scarabaeoides	ICPW116	24.7	13.3	53.3 (46.9)	46.7 (43.1)
C. scarabaeoides	ICPW 125	23.7	12.3	53.3 (46.9)	46.7 (43.1)
C. scarabaeoides	ICPW 130	28.0	13.0	53.3 (46.9)	40.0 (39.1)
C. scarabaeoides	ICPW 137	25.3	13.7	53.3 (46.9)	34.7 (33.8)
C. scarabaeoides	ICPW 141	27.0	12.0	43.3 (41.2)	36.7 (37.1)
C. scarabaeoides	ICPW 152	25.0	13.3	53.3 (46.9)	40.0 (39.1)
C. scarabaeoides	ICPW278	26.0	12.3	40.0 (39.1)	36.7 (37.1)
C. scarabaeoides	ICPW 280	26.7	14.0	43.3 (41.2)	30.0 (33.0)
C. scarabaeoides	ICPW 281	25.3	13.0	56.7 (48.8)	43.3 (41.1)
D. ferruginea	ICPW 178	25.0	13.3	56.0 (48.5)	40.0 (39.1)
F. bracteata	ICPW 192	24.3	12.0	50.0 (45.0)	36.7 (37.2)
F. stricta	ICPW 202	23.3	12.0	53.3 (46.9)	43.3 (41.2)
P. scariosa	ICPW 207	25.0	14.3	30.0 (33.2)	26.7 (31.0)
R. aurea	ICPW 210	28.7	13.0	30.0 (33.0)	26.7 (30.8)
R. bracteata	ICPW 214	19.0	11.3	53.3 (46.9)	50.0 (45.0)
C. cajan (S)	ICPL 87	15.7	12.0	56.7 (48.8)	46.7 (43.1)
C. cajan (R)	ICPL 332	23.3	11.7	56.7 (48.8)	40.0 (39.1)
Artificial diet		12.7	10.7	56.7 (48.8)	53.3 (46.9)
SE ±		0.48	0.9	4.06 (2.37)	5.46 (3.46)
LSD at 5%		1.338	2.551	11.47 (6.68)	15.63 (9.948)
F-test		0.001	0.023	<0.001 (<0.001)	0.008 (0.061)

Figures in parenthesis are Angular transformed values.
S - Susceptible check. R - Resistant check.

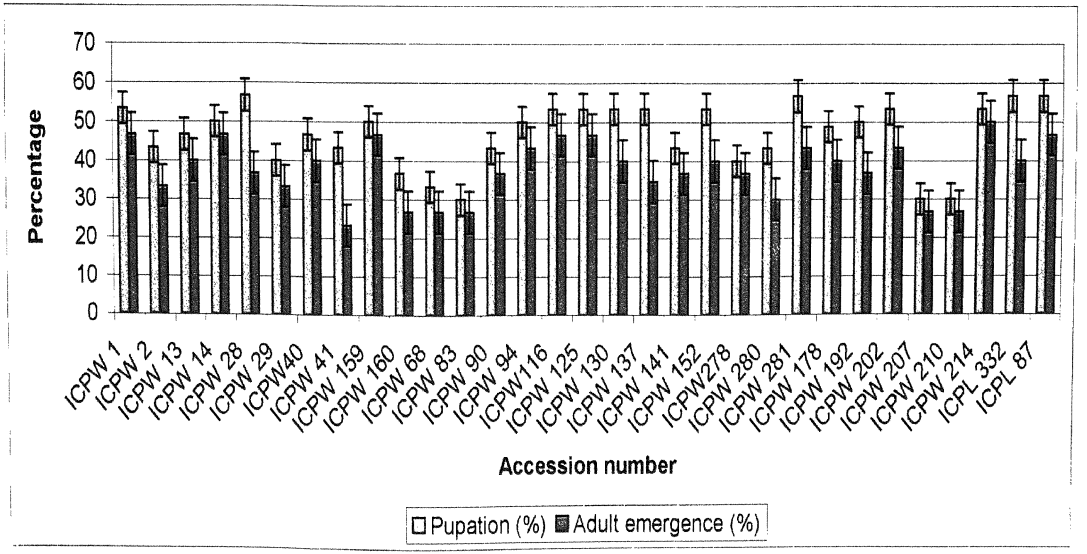


Fig-23 : Pupation and adult emergence of *H. armigera* reared on the artificial diet impregnated with lyophilized pod powder of wild relatives of pigeonpea

Table - 33: Association between different developmental parameters of H. armigera larvae reared on artificial diet impregnated with lyophilized leaf and pod powders of wild relatives of pigeonpea.

		Lyophylized leaf powder							Lyophylized pod powder						
		Larval weight	Pupal weight	Larval Mortality	Larval period	Pupal period	Pupation (%)	Adult emergence (%)	Larval weight	Pupal weight	Larval Mortality	Larval period	Pupal period	Pupation (%)	Adult emergence (%)
Lyophylized leaf powder	Larval weight	1.000													
	Pupal weight	0.218	1.000												
	Larval mortality	-0.115	0.132	1.000											
	Larval period	0.381*	0.170	-0.103	1.000										
	Pupal period	0.457**	-0.055	-0.005	-0.103	1.000									
	Pupation (%)	0.461**	0.025	0.102	-0.393*	-0.392*	1.000								
	Adult emergence (%)	-0.277	-0.102	-0.103	0.069	-0.007	0.553**	1.000							
Lyophylized Pod powder	Larval weight	0.418*	0.066	0.037	-0.457**	-0.073	-0.114	0.233	1.000						
	Pupal weight	0.204	-0.050	0.005	-0.078	0.049	-0.156	-0.012	-0.175	1.000					
	Larval mortality	0.159	0.087	0.363	-0.364*	0.193	-0.071	0.105	-0.564**	-0.386*	1.000				
	Larval period	-0.120	-0.423*	0.136	0.167	0.343*	0.323	-0.076	-0.071	-0.253	-0.030	1.000			
	Pupal period	-0.102	-0.095	0.256	0.071	0.259	0.283	-0.023	-0.051	0.104	-0.185	0.086	1.000		
	Pupation (%)	0.265	-0.104	0.171	0.218	-0.251	-0.080	0.176	-0.116	-0.120	0.066	-0.136	0.077	1.000	
	Adult emergence (%)	0.129	-0.097	0.035	-0.081	-0.025	-0.172	0.137	0.072	0.018	-0.104	0.009	-0.027	0.508**	1.000

*,** Correlation coefficients significant at P = 0.05 and 0.01, respectively.

No-choice leaf feeding assay

Under no-choice conditions, there is no significant variation in the leaf damage between accessions of the same species. At 24 h, the damage caused by third instar larva was low (0.4) in *C. scarabaeoides* (ICPW 83 and ICPW 141), *P. scariosa* (ICPW 202), *R. aurea* (ICPW 210), and was high (3.8) in *C. cajanifolius* (ICPW 28) compared to 3.6 in cultivated pigeonpea variety ICPL 87. However, the damage in *C. albicans* (ICPW 13 (2.8), ICPW 14 (2.6)), *C. lineatus* (ICPW 41 (2.6)), *C. scarabaeoides* (ICPW 90 (2.4)), *D. ferrugenia* (ICPW 178 (2.8), *F. stricta* (ICPW 202 (2.6)) and the cultivated ICPL 332 (2.8) were comparable. At 48 h, similar trend was observed. The damage was low (1.0) in *P. scariosa* (ICPW 202), (1.2) in *C. scarabaeoides* (ICPW 83) and *R. aurrea* compared to the cultivated pigeonpea variety ICPL 87 (6.6) (Table 34).

No-choice pod feeding assay

The pod damage was significantly lower in the pods of wild relatives of pigeonpea except *C. albicans* (ICPW 13) compared to ICPL 87 at 24 h after releasing the larvae in petri dish arena. At 48 h, the accessions of *C. albicans* (ICPW 13 and ICPW 14), and *C. cajanifolius* (ICPW 28) exhibited significantly more pod damage than the cultivated pigeonpea variety ICPL 87 (Table 35).

Multi-choice leaf feeding assay

Under multi-choice conditions bioassay studies were conducted by releasing the third-instar larvae of *H. armigera* on the leaves and pods to know the feeding preference of larvae towards wild relatives of pigeonpea. Thirty-one accessions were divided into five groups. Six accessions were placed in a petri dish arena along with the susceptible check, ICPL 87. Ten larvae were released inside the petri dish arena, and the leaves and pods damaged were scored at 24 and 48 h after initiating the experiment.

The leaf damage (DR) by the third-instar larvae in first group was 2.33 in ICPL 87 - the cultivated susceptible check and 4.17 in ICPL 332 - the resistant check (Table 36). All the accessions tested (ICPW 90, ICPW 116, ICPW 125, ICPW 278, and ICPW 280) suffered significantly less damage than the cultivated pigeonpea. Similar trends were observed at 48 h after initiating the experiment.

Table - 34: Feeding preference by the third-instar larvae of *H. armigera* towards the leaves of wild relatives of pigeonpea under no-choice conditions.

Species	Accession number	Leafdamage rating*	
		24 h	48 h
C acutifolius	ICPW 1	1.7	2.0
C. acutifolius	ICPW 2	1.3	1.8
C. albicans	ICPW 13	2.8	4.8
C. albicans	ICPW 14	2.6	3.6
C. cajanifolius	ICPW 28	3.8	4.6
C. cajanifolius	ICPW 29	2.6	3.6
C. lineatus	ICPW40	2.6	3.8
C. lineatus	ICPW 41	1.6	3.0
C. sericeus	ICPW 159	1.1	1.7
C. sericeus	ICPW 160	0.7	1.1
C. platycarpus	ICPW 68	2.0	4.0
C. scarabaeoides	ICPW 83	3.2	4.0
C. scarabaeoides	ICPW 90	3.4	5.2
C. scarabaeoides	ICPW 94	1.8	2.5
C. scarabaeoides	ICPW116	0.9	2.9
C. scarabaeoides	ICPW 125	1.9	4.0
C. scarabaeoides	ICPW 130	1.6	3.6
C. scarabaeoides	ICPW 137	2.2	4.4
C. scarabaeoides	ICPW 141	0.4	2.6
C. scarabaeoides	ICPW 152	1.8	2.4
C. scarabaeoides	ICPW278	1.8	3.1
C. scarabaeoides	ICPW 280	3.2	6.4
C. scarabaeoides	ICPW 281	2.2	3.5
D. ferruginea	ICPW 178	2.8	4.7
F. bracteata	ICPW 192	1.8	2.8
F. stricta	ICPW 202	2.6	4.1
P. scariosa	ICPW 207	0.4	1.0
R. aurea	ICPW 210	0.4	1.2
R. bracteata	ICPW 214	3.0	4.3
C. cajan (S)	ICPL 87	3.6	6.6
C. cajan (R)	ICPL 332	2.8	4.4
SE ±		0.315	0.514
LSD at 5%		0.88	1.44
F-test		<0.001	<0.001

*Leaf damage rating (1 = <10% leaf area damaged, and 9 = >80% leaf area damaged).
S - Susceptible check. R - Resistant check

Table - 35: Feeding preference by the third-instar larvae of *H. armigera* towards the unwashed pods of wild relatives of pigeonpea.

Species	Accession number	Pod damage rating*	
		24 h	48 h
C. acutifolius	ICPW 1	1.4	3.2
C. acutifolius	ICPW 2	1.2	2.9
C. albicans	ICPW 13	3.1	5.3
C. albicans	ICPW 14	2.3	3.5
C. cajanifolius	ICPW 28	2.6	4.4
C. cajanifolius	ICPW 29	1.0	1.9
C. lineatus	ICPW40	1.4	2.8
C. lineatus	ICPW 41	0.8	1.6
C. sericeus	ICPW 159	0.7	1.3
C. sericeus	ICPW 160	0.8	1.6
C. platycarpus	ICPW 68	1.2	2.0
C. scarabaeoides	ICPW 83	0.4	0.8
C. scarabaeoides	ICPW 90	0.4	0.6
C. scarabaeoides	ICPW 94	1.7	2.0
C. scarabaeoides	ICPW116	0.3	1.3
C. scarabaeoides	ICPW 125	0.8	0.9
C. scarabaeoides	ICPW 130	0.8	1.1
C. scarabaeoides	ICPW 137	0.5	0.8
C. scarabaeoides	ICPW 141	0.8	1.4
C. scarabaeoides	ICPW 152	0.7	1.1
C. scarabaeoides	ICPW278	1.2	1.6
C. scarabaeoides	ICPW 280	0.5	1.5
C. scarabaeoides	ICPW 281	0.8	1.3
D. ferruginea	ICPW 178	1.4	2.4
F. bracteata	ICPW 192	0.4	1.1
F. stricta	ICPW 202	0.4	0.9
P. scariosa	ICPW 207	1.1	1.9
R. aurea	ICPW 210	1.0	1.9
R. bracteata	ICPW 214	2.0	3.2
C. cajan (S)	ICPL 87	2.6	3.3
C. cajan (R)	ICPL 332	2.0	2.9
SE ±		0.25	0.33
LSD at 5%		0.697	0.913
F-test		<0.001	<0.001

*Pod damage rating (1 = <10% pod area damaged, and 9 = >80% pod area damaged).
S - Susceptible check. R - Resistant check.

Table - 36: Feeding preference by the third-instar larvae of *H. armigera* towards the leaves of wild relatives of pigeonpea under multi-choice conditions (set-1).

Species	Accession number	Leaf damage rating*	
		24 h	48 h
C. scarabaeoides	ICPW 90	1.33	3.00
C. scarabaeoides	ICPW 116	0.00	0.50
C. scarabaeoides	ICPW 125	0.33	0.50
C. scarabaeoides	ICPW 278	2.33	3.33
C. scarabaeoides	ICPW 280	1.67	2.33
C. cajan	ICPL 332	1.00	1.67
C. cajan (S)	ICPL 87	2.00	3.00
SE ±		0.398	0.673
LSD at 5%		1.208	2.04
F-test		0.009	0.037

*Leaf damage rating (1 = <10% leaf area damaged, and 9 = >80% leaf area damaged).
S - Susceptible check.

In second group, at 24 h the leaf damage by the third-instar larvae was low (0.33) in *C. scarabaeoides* (ICPW 141) and *P. scariosa* (ICPW 207) and was high (2.33) in *C. cajanifolius* (ICPW 28) and in *F. stricta* (ICPW 202) as compared to a DR of 1.67% in the cultivated ICPL 87. Similar tend was at 48 h, where, significantly low damage was observed in case of *C. scarabaeoides;* ICPW 141 (0.50) ,ICPW 137 (1.17) and ICPW 130 (1.67). *F. stricta* (ICPW 202) with DR 3.00 is preferred as the cultivated pigeonpea, ICPL 87 with DR 3.33. The DR was high (4.00) for *C. cajanifolius* (ICPW 28) (Table 37). In the third group, after 24 h the damage was low in *C. acutifolius* (ICPW 2), *C. scarabaeoides* (ICPW 281 and ICPW 152). Similar trend was observed at 48 h. The leaf damage in *C. platycarpus* at 48 h was comparable (Table 38). In fourth group, theprefered to feed on accessions of *C. acutifolius* (ICPW 1) and *F. bracteata* (ICPW 192), *C. albicans* (ICPW 13) and *C. lineatus* (ICPW 40). However, the high leaf damage was noticed in ICPL 87 both at 24 and 48 h (Table 39). In fifth group, the leaves of *C. sericeus* (ICPW 159) was less preferred both at 24 and 48 h whereas, the leaves of *R. bracteata* were as much preferred as ICPL 87 at 48 h (Table 40).

Table - 37: Feeding preference by the third-instar larvae of *H. armigera* towards the leaves of wild relatives of pigeonpea under multi-choice conditions (set-2)

Species	Accession number	Leafdamage rating*	
		24 h	48 h
C. cajanifolius	ICPW 28	1.67	3.33
C. scarabaeoides	ICPW 130	1.67	1.67
C. scarabaeoides	ICPW 137	0.67	1.17
C. scarabaeoides	ICPW 141	0.33	0.50
F. stricta	ICPW 202	2.33	3.00
P. scariosa	ICPW 207	0.33	1.50
C. cajan (S)	ICPL 87 (S)	3.33	6.00
SE ±		0.33	0.362
LSD at 5%		1.01	1.098
F-test		0.001	<0.001

*Leaf damage rating (1 = <10% leaf area damaged, and 9 = >80% leaf area damaged).

S - Susceptible check.

Table -38: Feeding preference by the third-instar larvae of *H. armigera* towards the leaves of wild relatives of pigeonpea under multi-choice conditions (set-3).

Species	Accession number	Leafdamage rating*	
		24 h	48 h
C. acutifolius	ICPW 2	0.50	1.00
C. cajanifolius	ICPW 29	1.00	2.33
C. platycarpus	ICPW 68	1.67	3.33
C. scarabaeoides	ICPW 152	1.17	1.67
C. scarabaeoides	ICPW 281	0.67	1.67
R. aurea	ICPW 210	1.33	2.00
C. cajan (S)	ICPL 87 (S)	2.00	3.67
SE ±		0.35	0.356
LSD at 5%		1.06	1.08
F-test		0.095	0.001

*Leaf damage rating (1 = <10% leaf area damaged, and 9 = >80% leaf area damaged).

S - Susceptible check.

Table - 39: Feeding preference by the third-instar larvae of *H. armigera* towards the leaves of wild relatives of pigeonpea under multi-choice conditions(set-4).

Species	Accession number	Leafdamage rating*	
		24 h	48 h
C. acutifolius	ICPW 1	1.33	2.00
C. albicans	ICPW 13	1.67	2.67
C. lineatus	ICPW 40	1.67	2.67
C. scarabaeoides	ICPW 83	1.00	2.00
C. sericeus	ICPW 159	0.50	1.33
F. bracteata	ICPW 192	1.33	2.33
C. cajan (S)	ICPL 87 (S)	1.83	3.33
SE ±		0.37	0.418
LSD at 5%		1.10	1.27
F-test		0.238	0.090

*Leaf damage rating (1 = <10% leaf area damaged, and 9 = >80% leaf area damaged).

S - Susceptible check.

Table - 40: Feeding preference by the third-instar larvae of *H. armigera* on the leaves of wild relatives of pigeonpea under multi-choice conditions(set-5).

Species	Accession number	Leafdamage rating*	
		24 h	48 h
C. albicans	ICPW 14	0.67	1.50
C. lineatus	ICPW 41	0.83	1.50
C. scarabaeoides	ICPW 94	1.00	2.00
C. sericeus	ICPW 160	0.50	0.50
D. ferrugenio	ICPW 178	2.67	3.00
R. bracteata	ICPW 214	1.67	4.67
C. cajan (S)	ICPL 87	2.67	3.67
SE ±		0.34	0.63
LSD at 5%		1.03	
F-test		0.001	< 0.001

S - Susceptible check.

Multi-choice pod feeding assay

In the first group, pod damage by the third-instar larvae was 5.33 in ICPL 87, the susceptible check, and 4.17 in ICPL 332, the resistant check. The pod damage at 24 and 48 h in all the accessions of *C. scarabaeoides* (ICPW 90, ICPW 116, ICPW 125, ICPW 278, and ICPW 280) was significantly lower than in the cultivated pigeonpeas (Table 41). In the second experiment, percent pod damage after 24 h, was significantly lower (DR. 0.33 to 0.83) in three accessions of *C. scarabaeoides* (ICPW 130, ICPW 141, and ICPW 137), *F. stricta* and *P. scariosa* as compared to DR of 3.33 in ICPL 87 and 2.83 in *C. cajanifolius* (ICPW 28). Similar trend was observed at 48 h after initiating the experiment (Table 42). In the third group, there was no pod damage after 24 h in *C. scarabaeoides* (ICPW 281) and *R. aurea* (ICPW 210). However, very low pod damage was noticed after 48 h (Table 43). In the fourth group, there was lower feeding on pods of *C. sericeus*, *C. scarabaeoides*, *C. acutifolius*, and *F. bracteata* compared to *C. albicans*, *C. lineatus* and ICPL 87 (Table 44). In fifth group, *R. bracteata* pods were as much preferred as ICPL 87. The pods of *C. albicans* (ICPW 14), *C. lineatus* (ICPW 41), *C. scarabaeoides* (ICPW 94), *C. sericeus* (ICPW 160), and *D. ferruginea* (ICPW 178) were less preferred than those of the cultivated pigeonpea (Table 45).

Table - 41: Feeding preference by the third-instar larvae of *H. armigera* towards the pods of wild relatives of pigeonpea under multi-choice conditions (set-1).

Species	Accession number	Pod damage rating*	
		24 h	48 h
C. scarabaeoides	ICPW 90	0.68	1.00
C. scarabaeoides	ICPW 116	1.50	2.00
C. scarabaeoides	ICPW 125	0.50	0.50
C. scarabaeoides	ICPW 278	0.50	0.83
C. scarabaeoides	ICPW 280	0.83	1.50
C. cajan	ICPL 332	4.17	6.00
C. cajan (S)	ICPL 87	5.33	6.00
SE ±		1.55	1.57
LSD at 5%		2.81	3.83
F-test		0.001	0.001

*Pod damage rating (1 = <10% pod area damaged, and 9 = >80% pod area damaged).
S - Susceptible check.

Table - 42: Feeding preference by the third-instar larvae of *H. armigera* towards the pods of wild relatives of pigeonpea under multi-choice conditions (set-2).

Species	Accession number	Pod damage rating*	
		24 h	48 h
C. cajanifolius	ICPW 28	2.83	6.00
C. scarabaeoides	ICPW 130	0.83	3.33
C. scarabaeoides	ICPW 137	0.83	1.33
C. scarabaeoides	ICPW 141	0.33	1.33
F. stricta	ICPW 202	0.67	2.83
P. scariosa	ICPW 207	0.83	4.33
C. cajan (S)	ICPL 87	3.33	6.00
SE ±		0.66	2.22
LSD at 5%		1.61	NS
F-test		0.001	0.089

*Pod damage rating (1 = <10% pod area damaged, and 9 = >80% pod area damaged).
S - Susceptible check. NS – non-significant

Table - 43: Feeding preference by the third-instar larvae of *H. armigera* towards the pods of wild relatives of pigeonpea under multi-choice conditions (set-3).

Species	Accession number	Pod damage rating*	
		24 h	48 h
C. acutifolius	ICPW 2	1.17	2.33
C. cajanifolius	ICPW 29	1.50	5.33
C. platycarpus	ICPW 68	1.33	4.67
C. scarabaeoides	ICPW 152	0.67	2.50
C. scarabaeoides	ICPW 281	0.00	0.17
R. aurea	ICPW 210	0.00	0.50
C. cajan (S)	ICPL 87	1.50	5.00
SE ±		0.96	2.09
LSD at 5%		NS	NS
F-test		0.272	0.038

*Pod damage rating (1 = <10% pod area damaged, and 9 = >80% pod area damaged).
S - Susceptible check. NS – non-significant

Table - 44: Feeding preference by the third-instar larvae of *H. armigera* towards the pods of wild relatives of pigeonpea under multi-choice conditions (set-4).

Species	Accession number	Pod damage rating*	
		24 h	48 h
C. acutifolius	ICPW 1	0.17	0.33
C. albicans	ICPW 13	1.17	2.00
C. lineatus	ICPW 40	1.17	2.00
C. scarabaeoides	ICPW 83	0.17	0.33
C. sericeus	ICPW 159	0.17	0.33
F. bracteata	ICPW 192	0.33	0.50
C. cajan (S)	ICPL 87	1.50	3.67
SE ±		0.42	0.39
LSD at 5%		NS	0.96
F-test		0.003	0.001

*Pod damage rating (1 = <10% pod area damaged, and 9 = >80% pod area damaged). S - Susceptible check. NS – non-significant

Table - 45: Feeding preference by the third-instar larvae of *H. armigera* towards the pods of wild relatives of pigeonpea under multi-choice conditions (set-5)

Species	Accession number	Pod damage rating*	
		24 h	48 h
C. albicans	ICPW 14	0.50	1.33
C. lineatus	ICPW 41	0.50	0.83
C. scarabaeoides	ICPW 94	0.33	0.33
C. sericeus	ICPW 160	0.33	0.50
D. ferrugenia	ICPW 178	0.67	1.17
R. bracteata	ICPW 214	2.83	4.67
C. cajan (S)	ICPL 87	2.17	4.67
SE ±		0.52	0.63
LSD at 5%		1.27	1.52
F-test		0.001	0.001

*Pod damage rating (1 = <10% pod area damaged, and 9 = >80% pod area damaged). S - Susceptible check.

Role of pod surface chemicals on feeding by the *H. armigera* larvae

The effect of pod surface chemicals on feeding behaviour of *H. armigera* larvae was studied by using the glass fiber discs treated with pod surface extracts under dual-choice conditions and pods under no-choice and dual-choice conditions (Fig 24)

Feeding preference by the third-instar larvae of *H. armigera* towards water, methanol and hexane extracted pods

No-choice conditions

Water washed pods of wild relatives of pigeonpea, (except *C. albicans* (ICPW 13 and ICPW14), *C. cajanifolius* (ICPW 28), and *R. bracteata* (ICPW 214) were significantly less preferred compared to the cultivated pigeonpea varieties, ICPL 87and ICPL 332 (Table- 46).

In the methanol washed pods of wild relatives of pigeonpea suffered low pod damage compared to those of the susceptible check, (ICPL 87) and the resistant check, (ICPL 332). The accessions; ICPW 1 (*C. acutifolius*), ICPW 13 (*C. albicans*), and ICPW 28 (*C. cajanifolius*) showed more pod damage than the cultivated pigeonpea (Table 47).

Hexane-washed pods of *C. acutifolius, C. albicans* and *C. cajanifolius* (ICPW 28) were preferred by the third-instar larvae compared to *C. cajan*. Larval feeding was significantly lower on the pods of *C. scarabaeoides*, *F. stricta*, and *F. bracteata* accessions (Table 48).

Dual-choice conditions

Dual-choice bioassays were carried out by providing the larvae with a choice to choose between the water, methanol, or hexane washed and unwashed pods of the same species/accession. Observations on pod damage in terms of feeding preference by the pest were recorded at 24 and 48 h after releasing the larvae. Significance of differences between the treatments was judged by the paired 't'-test.

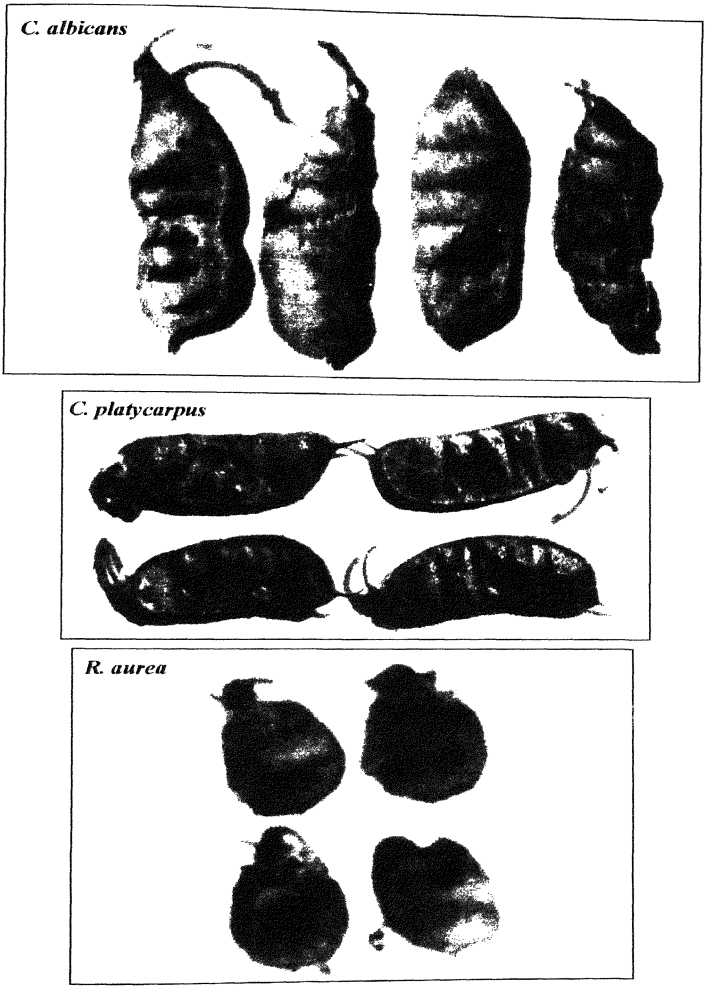


Fig-24 : Feeding preference by the third-instar larvae of *H. armigera* towards water, methanol and hexane, washed and unwashed pods of wild relatives of pigeonpea.

Table- 46: Feeding preference by the third-instar larvae of *H. armigera* on the water washed the pods of wild relatives of pigeonpea

Species	Accession number	Pod damage rating*	
		24 h	48 h
C acutifolius	ICPW 1	1.2	2.9
C. acutifolius	ICPW 2	1.0	2.0
C. albicans	ICPW 13	3.0	4.8
C. albicans	ICPW 14	1.9	3.6
C. cajanifolius	ICPW 28	2.4	4.0
C. cajanifolius	ICPW 29	0.9	1.4
C. lineatus	ICPW40	1.1	2.1
C. lineatus	ICPW 41	1.0	1.7
C. sericeus	ICPW 159	0.6	1.1
C. sericeus	ICPW 160	0.8	1.2
C. platycarpus	ICPW 68	1.4	2.2
C. scarabaeoides	ICPW 83	0.1	0.7
C. scarabaeoides	ICPW 90	0.5	0.9
C. scarabaeoides	ICPW 94	1.2	1.4
C. scarabaeoides	ICPW116	1.0	2.2
C. scarabaeoides	ICPW 125	0.5	0.7
C. scarabaeoides	ICPW 130	0.7	1.1
C. scarabaeoides	ICPW 137	1.3	1.8
C. scarabaeoides	ICPW 141	0.8	1.9
C. scarabaeoides	ICPW 152	1.3	1.9
C. scarabaeoides	ICPW278	0.7	1.0
C. scarabaeoides	ICPW 280	0.4	1.0
C. scarabaeoides	ICPW 281	0.6	0.9
D. ferruginea	ICPW 178	1.2	1.9
F. bracteata	ICPW 192	0.6	1.1
F. stricta	ICPW 202	0.3	0.7
P. scariosa	ICPW 207	1.4	2.0
R. aurea	ICPW 210	0.9	1.3
R. bracteata	ICPW 214	2.0	3.0
C. cajan (S)	ICPL 87	1.9	2.5
C. cajan (R)	ICPL 332	1.7	2.8
SE ±		0.23	0.31
SD at 5%Lssd		0.639	0.870
F-test		<0.001	<0.001

*Pod damage rating (1 = <10% pod area damaged, and 9 = >80% pod area damaged).
S - Susceptible check, R - Resistant check

Table- 47: Feeding preference by the third-instar larvae of *H. armigera* on the methanol washed pods of wild relatives of pigeonpea.

Species	Accession number	Pod damage rating*	
		24 h	48 h
C. acutifolius	ICPW 1	1.1	1.7
C. acutifolius	ICPW 2	0.4	0.7
C. albicans	ICPW 13	1.0	2.0
C. albicans	ICPW 14	0.4	0.9
C. cajanifolius	ICPW 28	1.5	1.9
C. cajanifolius	ICPW 29	0.4	1.0
C. lineatus	ICPW40	0.8	1.4
C. lineatus	ICPW 41	0.6	1.4
C. sericeus	ICPW 159	0.6	0.8
C. sericeus	ICPW 160	0.5	1.1
C. platycarpus	ICPW 68	0.8	1.2
C. scarabaeoides	ICPW 83	0.8	1.0
C. scarabaeoides	ICPW 90	0.4	0.8
C. scarabaeoides	ICPW 94	0.6	0.9
C. scarabaeoides	ICPW116	0.1	0.4
C. scarabaeoides	ICPW 125	0.4	0.5
C. scarabaeoides	ICPW 130	0.2	0.3
C. scarabaeoides	ICPW 137	0.2	0.3
C. scarabaeoides	ICPW 141	0.3	0.6
C. scarabaeoides	ICPW 152	0.2	0.7
C. scarabaeoides	ICPW278	0.5	0.7
C. scarabaeoides	ICPW 280	0.3	0.5
C. scarabaeoides	ICPW 281	0.4	0.4
D. ferruginea	ICPW 178	0.7	1.3
F. bracteata	ICPW 192	0.2	0.8
F. stricta	ICPW 202	0.1	0.4
P. scariosa	ICPW 207	0.4	0.8
R. aurea	ICPW 210	0.5	0.9
R. bracteata	ICPW 214	0.5	0.9
C. cajan (S)	ICPL 87	1.0	1.5
C. cajan (R)	ICPL 332	0.9	1.2
SE ±		0.23	0.30
LSD at 5%		0.654	0.843
F-test		0.007	0.001

*Pod damage rating (1 = <10% pod area damaged, and 9 = >80% pod area damaged).
S - Susceptible check, R - Resistant check

Table- 48: Feeding preference by the third-instar larvae of *H. armigera* on the hexane washed the pods of wild relatives of pigeonpea

Species	Accession number	Pod damage rating*	
		24 h	48 h
C. acutifolius	ICPW 1	2.4	3.7
C. acutifolius	ICPW 2	2.4	3.7
C. albicans	ICPW 13	4.9	6.4
C. albicans	ICPW 14	3.2	4.9
C. cajanifolius	ICPW 28	2.5	4.9
C. cajanifolius	ICPW 29	0.6	1.6
C. lineatus	ICPW40	1.5	2.4
C. lineatus	ICPW 41	1.0	2.1
C. sericeus	ICPW 159	1.2	1.7
C. sericeus	ICPW 160	0.8	1.9
C. platycarpus	ICPW 68	1.2	2.1
C. scarabaeoides	ICPW 83	0.5	1.8
C. scarabaeoides	ICPW 90	0.9	2.2
C. scarabaeoides	ICPW 94	0.4	0.7
C. scarabaeoides	ICPW116	0.8	1.4
C. scarabaeoides	ICPW 125	1.0	1.2
C. scarabaeoides	ICPW 130	0.7	1.2
C. scarabaeoides	ICPW 137	1.0	1.5
C. scarabaeoides	ICPW 141	0.9	1.4
C. scarabaeoides	ICPW 152	1.0	1.6
C. scarabaeoides	ICPW278	1.2	2.0
C. scarabaeoides	ICPW 280	0.8	1.5
C. scarabaeoides	ICPW 281	1.6	2.4
D. ferruginea	ICPW 178	1.8	3.4
F. bracteata	ICPW 192	0.9	1.9
F. stricta	ICPW 202	0.7	1.2
P. scariosa	ICPW 207	1.6	2.0
R. aurea	ICPW 210	0.8	1.6
R. bracteata	ICPW 214	2.4	3.4
C. cajan (S)	ICPL 87	2.6	3.6
C. cajan (R)	ICPL 332	2.3	3.5
SE ±		0.29	0.40
LSD at 5%		0.807	1.125
F-test		<0.001	<0.001

*Pod damage rating (1 = <10% pod area damaged, and 9 = >80% pod area damaged).
S - Susceptible check, R - Resistant check

When the larvae were given a choice between water-washed and unwashed pods, the larvae preferred to feed on the unwashed pods compared to the pods washed with water (Tables 49 & 50). However, the differences were not significant at 24 h after initiating the experiment in case of *C. acutifolius* (ICPW 2), *C. cajanifolius* (ICPW 29), *C. sericeus* (ICPW 159, ICPW160), *C. scarabaeoides* (ICPW 83, ICPW 130, ICPW 137, ICPW 141, ICPW 152, and ICPW 281), *D. ferruginea* (ICPW 178), *P. scariosa* (ICPW 207), *C. platycarpus* (ICPW 68), and *R. aurea* (ICPW 210). At 48 h after initiating the experiment, the differences in larval feeding were not significant in case of *C. acutifolius* (ICPW 2), *C. albicans* (ICPW 13 and ICPW 14), *C. cajanifolius* (ICPW 28 and ICPW 29), *C. sericeus* (ICPW 159 and ICPW 160), *C. scarabaeoides* (ICPW 83, ICPW 94, ICPW 116, ICPW 137, ICPW 141, ICPW 152, ICPW 278, ICPW 280, and ICPW 281), *F. bracteata* (ICPW 192), *C. platycarpus* (ICPW 68), *R. aurea* (ICPW 210), and *C. cajan* (ICPL 32). The pod damage on unwashed pods of ICPL 87 was greater (DR 2.4 and 2.7) than on the pods washed with water (DR 1.4 and 1.8) at 24 and 48 h after initiating the experiment, respectively.

When the larvae were provided a choice to choose between the methanol-washed and unwashed pods, the larvae preferred to feed on the unwashed pods compared to the methanol washed pod of the same accession, both at 24 and 48 h after releasing the larvae (Tables 51 & 52). However, the differences were not significant at 24 h after releasing the larvae in case of *C. cajanifolius* (ICPW 29), *C. scarabaeoides* (ICPW 90, ICPW 125, ICPW 137, ICPW 152, and ICPW 278), and *R. bracteata* (ICPW 214). At 48 h after initiating the experiment, the differences were non-significant only in case of *C. scarabaeoides* (ICPW 125, ICPW 137, ICPW 152, and ICPW 280), and *D. ferruginea* (ICPW 178). In cultivated pigeonpea (ICPL 87), the pod damage rating was 0.5 on the methanol-washed pods compared to 2.1 on the unwashed pods at 24 h, and 1.0 and 2.9 at 48 h after initiating the experiment, respectively.

Table-49 : **Feeding preference by third-instar larvae of *H. armigera* on the water-washed and unwashed pods of wild relatives of pigeonpea under dual-choice conditions at 24h after initiating the experiment**

Species	Accession number	Unwashed pods*	Water washed pods*	t-value	Probability
C. acutifolius	ICPW 1	1.0 ± 0.07	1.3± 0.12	-2.18*	0.036
C. acutifolius	ICPW 2	1.1 ± 0.13	1.1±0.13	0.00	1.000
C. albicans	ICPW 13	2.2 ± 0.14	1.3±0.14	4.62**	<0.001
C. albicans	ICPW 14	1.6 ± 0.13	1.2±0.14	2.08*	0.044
C. cajanifolius	ICPW 28	2.0 ± 0.13	1.5±0.18	2.30*	0.027
C. cajanifolius	ICPW 29	0.7 ± 0.12	0.6±0.09	0.69	0.495
C. lineatus	ICPW40	1.0 ± 0.07	0.8±0.06	2.18*	0.036
C. lineatus	ICPW 41	0.6 ± 0.09	0.9±0.09	-2.47*	0.018
C. sericeus	ICPW 159	0.9 ± 0.09	0.8±0.06	0.97	0.336
C. sericeus	ICPW 160	0.7 ± 0.09	0.7±0.06	0.00	1.000
C. platycarpus	ICPW 68	1.0 ± 0.13	1.1±0.11	-0.59	0.557
C. scarabaeoides	ICPW 83	0.5 ± 0.07	0.5±0.07	0.00	1.000
C. scarabaeoides	ICPW 90	0.6 ± 0.09	0.3±0.06	2.92**	0.006
C. scarabaeoides	ICPW 94	0.5 ± 0.07	0.3±0.06	2.18*	0.036
C. scarabaeoides	ICPW116	0.5 ± 0.07	0.3±0.06	2.18*	0.036
C. scarabaeoides	ICPW 125	0.7 ± 0.06	0.5±0.07	2.18*	0.036
C. scarabaeoides	ICPW 130	0.6 ± 0.13	0.4±0.05	1.41	0.165
C. scarabaeoides	ICPW 137	0.4 ± 0.09	0.3±0.06	0.97	0.336
C. scarabaeoides	ICPW 141	0.7 ± 0.14	0.7±0.12	0.00	1.000
C. scarabaeoides	ICPW 152	0.7 ± 0.14	0.6±0.09	0.62	0.541
C. scarabaeoides	ICPW278	1.0 ± 0. 13	0.7±0.06	2.18*	0.036
C. scarabaeoides	ICPW 280	0.5 ± 0.07	0.7±0.06	-2.18*	0.036
C. scarabaeoides	ICPW 281	1.2 ± 0.17	1.1±0.21	0.37	0.715
D. ferruginea	ICPW 178	0.8 ± 0.06	0.8±0.06	0.00	1.000
F. bracteata	ICPW 192	0.5 ± 0.07	0.3±0.06	2.18*	0.036
F. stricta	ICPW 202	0.4 ± 0.09	0.8±0.09	-3.18**	0.003
P. scariosa	ICPW 207	0.8 ± 0.12	0.6±0.09	1.38	0.176
R. aurea	ICPW 210	0.6 ± 0.09	0.5±0.07	0.89**	0.379
R. bracteata	ICPW 214	1.6 ± 0.13	1.2±0.06	2.76	0.009
C. cajan (S)	ICPL 87	2.4 ± 0.09	1.40±.22	4.19**	<0.001
C. cajan (R)	ICPL 332	1.6 ± 0.13	1.1±0.09	3.15**	0.003

*Pod damage rating (1 = <10% pod area damaged, and 9 = >80% pod area damaged).
S - Susceptible check, R - Resistant check., *,**= t -value significant at P=0.05 and 0.01, respectively.

Table-50 : Feeding preference by third-instar larvae of *H. armigera* on the water- washed and unwashed pods of wild relatives of pigeonpea under dual-choice conditions at 48h after initiating the experiment.

Species	Accession number	Unwashed pods*	Water washed Pods*	t-value	Probability
C. acutifolius	ICPW 1	2.3 ±0.12	1.7 ± 0.16	3.08**	0.004
C. acutifolius	ICPW 2	1.7 ± 0.06	1.9 ± 0.13	-1.38	0.176
C. albicans	ICPW 13	2.2 ± 0.12	3.7 ± 0.09	-10.9**	<0.001
C. albicans	ICPW 14	2.4 ± 0.22	2.8 ± 0.16	-1.47	0.149
C. cajanifolius	ICPW 28	2.8 ± 0.16	2.3 ± 0.22	1.83	0.075
C. cajanifolius	ICPW 29	1.3 ± 0.12	1.1 ± 0.15	1.04	0.304
C. lineatus	ICPW40	1.5 ± 0.07	1.2 ± 0.06	3.27**	0.002
C. lineatus	ICPW 41	0.9 ± 0.11	1.2 ± 0.09	-2.07*	0.046
C. sericeus	ICPW 159	1.2 ± 0.12	1.4 ± 0.09	-1.38	0.176
C. sericeus	ICPW 160	1.2 ± 0.16	1.5 ± 0.19	-1.21	0.232
C. platycarpus	ICPW 68	1.2 ± 0.16	1.4 ± 0.09	-1.13	0.267
C. scarabaeoides	ICPW 83	0.9 ± 0.09	0.7 ± 0.06	1.95	0.059
C. scarabaeoides	ICPW 90	0.9 ± 0.11	0.5 ± 0.07	2.99**	0.005
C. scarabaeoides	ICPW 94	0.8 ± 0.09	0.8 ± 0.09	0.00	1.000
C. scarabaeoides	ICPW116	0.8 ± 0.12	1.0 ± 0.13	-1.16	0.251
C. scarabaeoides	ICPW 125	0.8 ± 0.06	1.1 ± 0.05	-4.14**	<0.001
C. scarabaeoides	ICPW 130	1.1 ± 0.13	0.7 ± 0.09	2.47*	0.018
C. scarabaeoides	ICPW 137	0.9 ± 0.09	0.7 ± 0.06	1.95	0.059
C. scarabaeoides	ICPW 141	1.1 ± 0.22	1.2 ± 0.19	-0.34	0.732
C. scarabaeoides	ICPW 152	1.2 ± 0.16	1.1 ± 0.11	0.52	0.605
C. scarabaeoides	ICPW278	1.4 ± 0.21	1.3 ± 0.09	0.44	0.665
C. scarabaeoides	ICPW 280	1.5 ± 0.13	1.5 ± 0.10	0.00	1.000
C. scarabaeoides	ICPW 281	1.4 ± 0.17	1.3 ± 0.20	0.38	0.704
D. ferruginea	ICPW 178	2.1 ± 0.05	0.9 ± 0.05	18.49**	<0.001
F. bracteata	ICPW 192	0.8 ± 0.12	0.9 ± 0.09	-0.69	0.495
F. stricta	ICPW 202	0.6 ± 0.11	0.9 ± 0.09	-2.12*	0.04
P. scariosa	ICPW 207	1.4 ± 0.11	1.0 ± 0.07	2.99**	0.005
R. aurea	ICPW 210	1.1 ± 0.11	1.0 ± 0.13	0.59**	0.557
R. bracteata	ICPW 214	1.7 ± 0.12	2.1 ± 0.09	-2.76	0.009
C. cajan (S)	ICPL 87	2.7 ± 0.06	1.8 ± 0.24	3.71**	<0.001
C. cajan (R)	ICPL 332	2.1 ± 0.05	1.8 ± 0.19	1.56	0.126

*Pod damage rating (1 = <10% pod area damaged, and 9 = >80% pod area damaged).
S - Susceptible check. R - Resistant check. *,**= t -value significant at P=0.05 and 0.01, respectively.

Table-51 : Feeding preference (at 24h) by third-instar larvae of *H. armigera* on the methanol - washed and unwashed pods of wild relatives of pigeonpea under dual-choice after initiating the experiment

Species	Accession number	Unwashed pods*	Methanol washed pods*	t-value	Probability
C. acutifolius	ICPW 1	1.8 ± 0.16	1 .0 ± 0.19	3.24**	0.003
C. acutifolius	ICPW 2	0.9 ± 0.09	0.4 ± 0.09	4.12**	<0.001
C .albicans	ICPW 13	2.5 ± 0.13	0.7 ± 0.12	10.48**	<0.001
C .albicans	ICPW 14	1.7 ± 0.16	0.6 ± 0.13	5.36**	<0.001
C. cajanifolius	ICPW 28	1.3 ± 0.21	0.7 ± 0.16	2.28*	0.029
C. cajanifolius	ICPW 29	0.7 ± 0.12	0.4 ± 0.13	1.69	0.100
C. lineatus	ICPW40	0.8 ± 0.12	0.4 ± 0.09	2.76**	0.009
C. lineatus	ICPW 41	1.1 ± 0.13	0.2 ± 0.06	6.02**	<0.001
C. sericeus	ICPW 159	1.0 ± 0.07	0.3 ± 0.06	7.63**	<0.001
C. sericeus	ICPW 160	0.8 ± 0.12	0.4 ± 0.09	2.76**	0.009
C. platycarpus	ICPW 68	1.1 ± 0.09	0.7 ± 0.12	2.76**	0.009
C. scarabaeoides	ICPW 83	0.6 ± 0.09	0.3 ± 0.09	2.39*	0.022
C. scarabaeoides	ICPW 90	0.5 ± 0.07	0.4 ± 0.09	0.89	0.379
C. scarabaeoides	ICPW 94	0.8 ± 0.06	0.2 ± 0.06	7.55**	<0.001
C. scarabaeoides	ICPW116	0.7 ± 0.06	0.4 ± 0.09	2.92**	0.006
C. scarabaeoides	ICPW 125	0.5 ± 0.07	0.4 ± 0.09	0.89	0.379
C. scarabaeoides	ICPW 130	0.7 ± 0.06	0.3 ± 0.09	3.72**	<0.001
C. scarabaeoides	ICPW 137	0.5 ± 0.07	0.4 ± 0.09	0.89	0.379
C. scarabaeoides	ICPW 141	0.6 ± 0.09	0.3 ± 0.09	2.39*	0.022
C. scarabaeoides	ICPW 152	0.5 ± 0.07	0.5 ± 0.10	0.00	1.000
C. scarabaeoides	ICPW278	0.5 ± 0.07	0.4 ± 0.13	0.66	0.515
C. scarabaeoides	ICPW 280	0.6 ± 0.09	0.3 ± 0.09	2.39*	0.022
C. scarabaeoides	ICPW 281	1.0 ± 0.13	0.2 ± 0.06	5.81**	<0.001
D. ferruginea	ICPW 178	0.7 ± 0.06	0.5 ± 0.07	2.18*	0.036
F. bracteata	ICPW 192	0.9 ± 0.09	0.2 ± 0.06	6.82**	<0.001
F. stricta	ICPW 202	0.5 ± 0.07	0.2 ± 0.06	3.27**	0.002
P. scariosa	ICPW 207	0.7 ± 0.12	0.1 ± 0.05	4.77**	<0.001
R. aurea	ICPW 210	1.1 ± 0.11	0.3 ± 0.06	6.37**	<0.001
R. bracteata	ICPW 214	0.9 ± 0.13	0.9 ± 0.09	0.00	1.000
C. cajan (S)	ICPL 87	2.1± 0.09	0.5 ± 0.13	10.51**	<0.001
C. cajan (R)	ICPL 332	1.9 ± 0.09	0.5 ± 0.07	12.46**	<0.001

*Pod damage rating (1 = <10% pod area damaged, and 9 = >80% pod area damaged).
S - Susceptible check. R - Resistant check. *,**= t -value significant at P=0.05 and 0.01, respectively.

Table-52 : Feeding preference by third-instar larvae of *H. armigera* on the methanol- washed and un-washed pods of wild relatives of pigeonpea under dual-choice at 48h after initiating the experiment

Species	Accession number	Unwashed pods*	Methanol washed pods*	t-value	Probability
C. acutifolius	ICPW 1	3.0 ± 0.21	1.0 ± 0.19	7.12**	< 0.001
C. acutifolius	ICPW 2	2.0 ± 0.16	0.5 ± 0.07	8.44**	< 0.001
C. albicans	ICPW 13	3.5 ± 0.25	0.9 ± 0.13	9.13**	< 0.001
C. albicans	ICPW 14	3.7 ± 0.20	0.9 ± 0.13	11.64**	< 0.001
C. cajanifolius	ICPW 28	2.4 ± 0.23	1.1 ± 0.13	4.82**	< 0.001
C. cajanifolius	ICPW 29	1.1 ± 0.18	0.6 ± 0.11	2.32*	0.026
C.lineatus	ICPW40	1.6 ± 0.15	0.5 ± 0.07	6.52**	< 0.001
C.lineatus	ICPW 41	1.6 ± 0.20	0.7 ± 0.06	4.39**	< 0.001
C. sericeus	ICPW 159	1.5 ± 0.10	0.5 ± 0.13	6.16**	< 0.001
C. sericeus	ICPW 160	1.4± 0.11	0.7 ± 0.12	4.32**	< 0.001
C. platycarpus	ICPW 68	1.5 ± 0.10	0.6 ± 0.11	5.94**	< 0.001
C. scarabaeoides	ICPW 83	1.0 ± 0.10	0.3 ± 0.09	5.09**	< 0.001
C. scarabaeoides	ICPW 90	0.2 ± 0.06	0.6 ± 0.09	-3.9**	< 0.001
C. scarabaeoides	ICPW 94	1.2 ± 0.06	0.4 ± 0.09	7.8**	< 0.001
C. scarabaeoides	ICPW116	1.0 ± 0.07	0.5 ± 0.07	4.87**	< 0.001
C. scarabaeoides	ICPW 125	0.6 ± 0.11	0.4 ± 0.09	1.41	0.165
C. scarabaeoides	ICPW 130	1.2 ± 0.09	0.4 ± 0.09	6.37**	< 0.001
C scarabaeoides	ICPW 137	0.8 ± 0.12	0.5 ± 0.10	1.93	0.061
C. scarabaeoides	ICPW 141	1.0 ± 0.15	0.4 ± 0.09	3.56**	0.001
C. scarabaeoides	ICPW 152	1.0 ± 0.13	1.1 ± 0.11	-0.59	0.557
C. scarabaeoides	ICPW278	1.4 ± 0.05	0.6 ± 0.17	4.58**	< 0.001
C. scarabaeoides	ICPW 280	1.2 ± 0.16	1.3 ± 0.14	-0.48	0.633
C. scarabaeoides	ICPW 281	1.3 ± 0.12	0.4 ± 0.09	6.2**	< 0.001
D. ferruginea	ICPW 178	1.0 ± 0.07	0.9 ± 0.05	1.16	0.251
F. bracteata	ICPW 192	1.1 ± 0.13	0.4 ± 0.05	4.95**	< 0.001
F. stricta	ICPW 202	0.8 ± 0.09	0.2 ± 0.06	5.58**	< 0.001
P. scariosa	ICPW 207	1.3 ± 0.14	0.2 ± 0.06	7.4**	< 0.001
R. aurea	ICPW 210	1.7 ± 0.06	0.4 ± 0.08	13.15**	< 0.001
R. bracteata	ICPW 214	1.7 ± 0.20	1.2 ± 0.09	2.27*	0.029
C. cajan (S)	ICPL 87	2.9 ± 0.18	1.0 ± 0.16	7.76**	<0.001
C. cajan (R)	ICPL 332	2.5 ± 0.10	0.9 ± 0.15	8.72**	<0.001

*Pod damage rating (1 = <10% pod area damaged, and 9 = >80% pod area damaged).
S - Susceptible check. R - Resistant check. *,**= t -value significant at P=0.05 and 0.01, respectively.

When the larvae were provided with a choice to choose between the unwashed and hexane washed pods. The larvae preferred to feed on the hexane washed pods than on the unwashed pods, indicating that hexane might have removed some of the antifeedant compounds from the pod surface (Tables 53 & 54). Differences in pod damage were non-significant in case of *C. cajanifolius* (ICPW 28), *C. scarabaeoides* (ICPW 90, ICPW 94, ICPW 116, and ICPW 281), *D. ferruginea* (ICPW 178), *F. bracteata* (ICPW 192), *P. scariosa* (ICPW 207), *R. bracteata* (ICPW 214), and *C. cajan* (ICPL 332) at 24 h after initiating the experiment. At 48 h after initiating the experiment, the differences in pod feeding were non-significant only in case of *C. scarabaeoides* (ICPW 90 and ICPW 281), and *D. ferruginea* (ICPW 178). Pod damage rating at 48 h after initiating the experiment in the unwashed pods of ICPL 87 was 2.0 compared to 3.2 in the pods washed with hexane.

Feeding preference by different instars of *H. armigera* towards a pod surface extract treated and un treated glass fiber discs

This assay was carried out by using 3rd, 4th and 5th instar larvae of *H. armigera* towards methanol and hexane pod surface extracts treated and untreated glass fiber discs. The larvae preferred to feed on the discs treated with methanol extract than on the control discs (Table 55 & Fig 25). The larvae consumed more area in discs treated with pod surface extract of ICPL 87 as compared to the discs treated with the pod surface extracts of ICPL 332 and ICPW 83 extracts. The disc area consumed by the fifth-instar larva was more compared to third and fourth-instar larvae (Table 55). In case of hexane extract treated discs, though the larvae preferred to feed on the control discs than on the treated discs the differences were not significant (Table 56).

Table- 53: Feeding preference by third-instar larvae of *H. armigera* on the hexane-washed and unwashed pods of wild relatives of pigeonpea under dual-choice conditions at 24h after initiating the experiment

Species	Accession number	Unwashed pods*	Hexane washed Pods*	t-value	Probability
C. acutifolius	ICPW 1	1.0 ±0.13	2.3 ± 0.14	-6.98**	<0.001
C. acutifolius	ICPW 2	1.1 ± 0.13	2.5 ± 0.24	-5.09**	<0.001
C. albicans	ICPW 13	0.6 ± 0.05	1.4 ± 0.13	-5.66**	<0.001
C. albicans	ICPW 14	0.9 ± 0.09	2.0 ± 0.15	-6.52**	<0.001
C. cajanifolius	ICPW 28	0.5 ± 0.07	0.6 ± 0.05	-1.16	0.251
C. cajanifolius	ICPW 29	1.2 ± 0.12	2.3 ± 0.22	-4.34**	<0.001
C. lineatus	ICPW40	0.6 ± 0.09	0.9 ± 0.05	-3.08**	0.004
C. lineatus	ICPW 41	0.6 ± 0.09	1.0 ± 0.15	-2.37*	0.023
C. sericeus	ICPW 159	0.5 ± 0.07	1.1 ± 0.09	-5.34**	<0.001
C. sericeus	ICPW 160	0.6 ± 0.09	0.9 ± 0.09	-2.47*	0.018
C. platycarpus	ICPW 68	0.6 ± 0.09	0.9 ± 0.11	-2.12*	0.04
C. scarabaeoides	ICPW 83	0.4 ± 0.09	0.8 ± 0.12	-2.76**	0.009
C. scarabaeoides	ICPW 90	0.6 ± 0.09	0.7 ± 0.06	-0.97	0.336
C. scarabaeoides	ICPW 94	0.6 ± 0.09	0.6 ± 0.09	0.00	1.000
C. scarabaeoides	ICPW116	0.6 ± 0.13	0.8 ± 0.06	-1.38	0.176
C. scarabaeoides	ICPW 125	0.7 ± 0.12	1.0 ± 0.07	-2.18*	0.036
C. scarabaeoides	ICPW 130	0.4 ± 0.09	0.7 ± 0.06	-2.92**	0.006
C. scarabaeoides	ICPW 137	0.7 ± 0.06	1.4 ± 0.09	-6.82**	<0.001
C. scarabaeoides	ICPW 141	0.5 ± 0.07	0.8 ± 0.12	-2.18*	0.036
C. scarabaeoides	ICPW 152	0.5 ± 0.07	0.9 ± 0.11	-2.99**	0.005
C. scarabaeoides	ICPW278	0.5 ± 0.07	1.0 ± 0.13	-3.45**	0.001
C. scarabaeoides	ICPW 280	0.4 ± 0.09	0.9 ± 0.13	-3.15**	0.003
C. scarabaeoides	ICPW 281	0.6 ± 0.09	0.8 ± 0.12	-1.38	0.176
D. ferruginea	ICPW 178	1.4 ± 0.13	1.1 ± 0.18	1.32	0.194
F. bracteata	ICPW 192	0.7 ± 0.09	0.6 ± 0.09	0.80	0.431
F. stricta	ICPW 202	0.5 ± 0.07	1.2 ± 0.12	-5.09**	<0.001
P. scariosa	ICPW 207	0.6 ± 0.09	0.8 ± 0.06	-1.95	0.059
R. aurea	ICPW 210	0.6 ± 0.09	0.9 ± 0.09	-2.47*	0.018
R. bracteata	ICPW 214	1.2 ± 0.12	1.2 ± 0.06	0.00	1.000
C. cajan (S)	ICPL 87	1.6 ± 0.13	2.0 ± 0.15	-2.03*	0.050
C. cajan (R)	ICPL 332	1.5 ± 0.16	1.8 ± 0.24	-1.05	0.302

*Pod damage rating (1 = <10% pod area damaged, and 9 = >80% pod area damaged).
S - Susceptible check, R - Resistant check. *,**= t -value significant at P=0.05 and 0.01, respectively.

Table-54 : Feeding preference by third-instar larvae of *H. armigera* on hexane-washed and unwashed pods of wild relatives of pigeonpea under dual-choice at 48h after initiating the experiment.

Species	Accession number	Unwashed pods*	Hexane washed Pods*	t-value	Probability
C. acutifolius	ICPW 1	1.4 ± 0.17	4.1 ± 0.21	-10.2**	<0.001
C. acutifolius	ICPW 2	1.3 ± 0.12	4.5 ± 0.26	-11.17**	<0.001
C. albicans	ICPW 13	1.0 ± 0.07	3.0 ± 0.24	-7.96**	< 0.001
C. albicans	ICPW 14	1.1 ± 0.13	3.0 ± 0.22	-7.44**	< 0.001
C. cajanifolius	ICPW 28	0.6 ± 0.05	1.2 ± 0.12	-4.77**	< 0.001
C. cajanifolius	ICPW 29	1.3 ± 0.12	1.8 ± 0.16	-9.87**	< 0.001
C. lineatus	ICPW40	0.8 ± 0.06	3.8 ± 0.22	-6.04**	< 0.001
C. lineatus	ICPW 41	1.0 ± 0.10	1.8 ± 0.09	-5.81**	< 0.001
C. sericeus	ICPW 159	0.9 ± 0.09	1.7 ± 0.17	-4.17**	< 0.001
C. sericeus	ICPW 160	0.6 ± 0.09	1.5 ± 0.13	-5.91**	< 0.001
C. platycarpus	ICPW 68	0.7 ± 0.09	1.8 ± 0.09	-8.48**	< 0.001
C. scarabaeoides	ICPW 83	0.6 ± 0.09	0.9 ± 0.05	-3.08**	0.004
C. scarabaeoides	ICPW 90	0.8 ± 0.09	1.0 ± 0.10	-1.45	0.154
C. scarabaeoides	ICPW 94	0.8 ± 0.14	1.3 ± 0.12	-2.77**	0.009
C. scarabaeoides	ICPW116	0.9 ± 0.09	1.3 ± 0.12	-2.76**	0.009
C. scarabaeoides	ICPW 125	0.5 ± 0.13	0.9 ± 0.05	-2.99**	0.005
C. scarabaeoides	ICPW 130	0.4 ± 0.09	0 7 ± 0.06	-2.92**	0.006
C. scarabaeoides	ICPW 137	0.8 ± 0.09	1.6 ± 0.15	-4.50**	< 0.001
C. scarabaeoides	ICPW 141	0.7 ± 0.06	1.3 ± 0.22	-2.59**	0.014
C. scarabaeoides	ICPW 152	0.8 ± 0.12	2.0 ± 0.10	-7.71**	< 0.001
C. scarabaeoides	ICPW278	0.5 ± 0.13	1.5 ± 0.16	-4.87**	< 0.001
C. scarabaeoides	ICPW 280	0.8 ± 0.12	1.6 ± 0.23	-3.06**	0.004
C. scarabaeoides	ICPW 281	2.0 ± 0.10	1.8 ± 0.16	1.07	0.290
D. ferruginea	ICPW 178	0.9 ± 0.11	1.0 ± 0.15	-0.54	0.589
F. bracteata	ICPW 192	0.7 ± 0.09	1.5± 0.07	-6.84**	< 0.001
F. stricta	ICPW 202	0.7 ± 0.06	1.4 ± 0.05	-9.65**	< 0.001
P. scariosa	ICPW 207	1.0 ± 0.07	1.2 ± 0 06	-2.18*	0.036
R. aurea	ICPW 210	0.8 ± 0.09	1.6 ± 0.09	-6.37**	< 0.001
R. bracteata	ICPW 214	1.4 ± 0.13	2.2 ± 0.12	-4.50**	< 0.001
C. cajan (S)	ICPL 87	2.0 ± 0.00	3.2 ± 0.21	-5.08**	<0.001
C. cajan (R)	ICPL 332	1.8 ± 0.12	2.5 ± 0.15	-3.76**	< 0.001

* Pod damage rating (1 = <10% pod area damaged, and 9 = >80% pod area damaged).
S - Susceptible check, R - Resistant check. *,**= t -value significant at P=0.05 and 0.01, respectively.

Table - 55: Feeding preference by different instars of *H. armigera* when provided with a choice between control and methanol pod surface extracts of different species of pigeonpea.

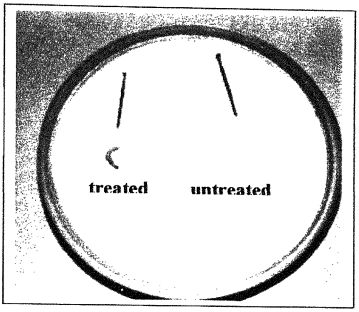
Species	Accession number	Instar	Control	Methanol extract	t- value	Probability
C. scarabaeoides	ICPW 83	3rd	0.010 ±0.003	0.015 ± 0.005	-1.41	0.178
C. cajan	ICPL 332	3rd	0.005 ±0.004	0.008 ± 0.002	-0.90	0.394
C. cajan	ICPL 87	3rd	0.008 ± 0.003	0.016 ±0.003	-1.08	0.309
C. scarabaeoides	ICPW 83	4th	0.009±0.003	0.013±0.008	-1.65	0.651
C. cajan	ICPL 332	4th	0.010±0.006	0.009±0.005	0.11	0.903
C. cajan	ICPL 87	4th	0.044±0.008	0.130±0.006	-3.73*	0.500
C. scarabaeoides	ICPW 83	5th	0.005±0.002	0.033±0.025	-1.16	0.286
C. cajan	ICPL 332	5th	0.081±0.029	0.080±0.040	0.02	0.984
C. cajan	ICPL 87	5th	0.018 ±0.010	0.129± 0.037	3.17*	0.015

Table - 56: Feeding preference by different instars of *H. armigera* when provided with a choice between control and hexane pod surface extracts of different species of pigeonpea.

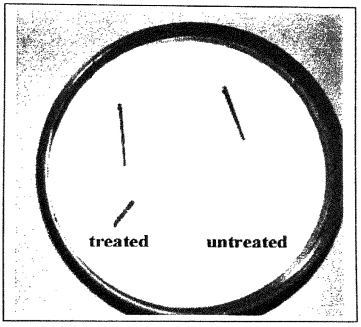
Species	Accession number	Instar	Control disc	Hexane extract	t- value	Probability
C. *scarabaeoides*	ICPW 83	3rd	0.014 ±0.003	0.010±0.003	1.08	0.412
C. *cajan*	ICPL 332	3rd	0.025 ±0.007	0.010±0.003	1.91	0.089
C. *cajan*	ICPL 87	3rd	0.012 ±0.004	0.007±0.002	1.10	0.279
C. *scarabaeoides*	ICPW 83	4th	0.010±0.006	0.009±0.005	0.11	0.903
C. *cajan*	ICPL 332	4th	0.015±0.007	0.014±0.009	0.09	0.932
C. *cajan*	ICPL 87	4th	0.069±0.010	0.071±0.011	-0.13	0.897
C. scarabaeoides	ICPW 83	5th	0.037±0.027	0.014±0.007	1.46	0.178
C. *cajan*	ICPL 332	5th	0.092±0.032	0.025±0.02	-1.81	0.071
C. *cajan*	ICPL 87	5th	0.106±0.045	0.36±0.295	0.83	0.416

3rd instar larvae on :

C. scarabaeoides
(ICPW 83)



C. cajan
(ICPL 87)



C. cajan
(ICPL 332)

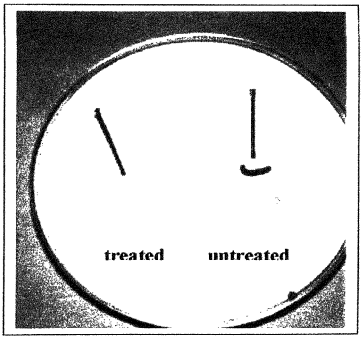
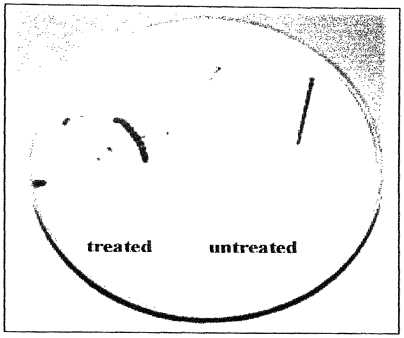


Fig-25 : Feeding preference by different instar larvae of *H. armigera* towards methanol extracted pod surface chemicals, treated and un treated glass fiber discs.

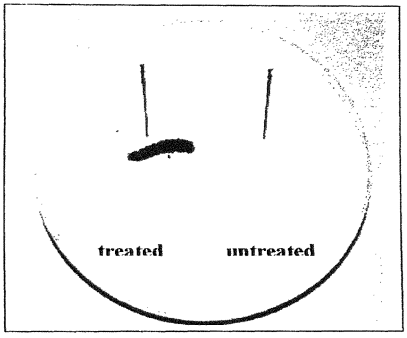
Contd.,

4th instar larvae on :

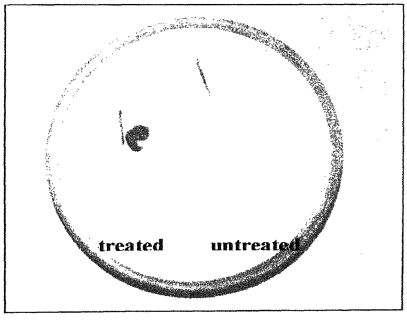
C. scarabaeoides
(ICPW 83)



C. cajan
(ICPL 87)



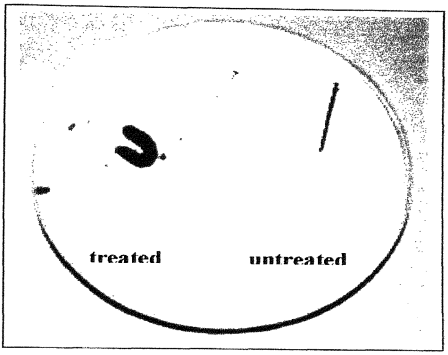
C. cajan
(ICPL 332)



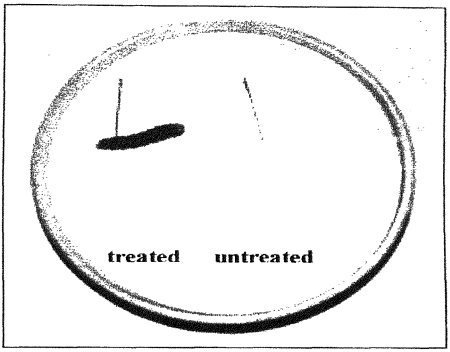
Contd.,

5th instar larvae on :

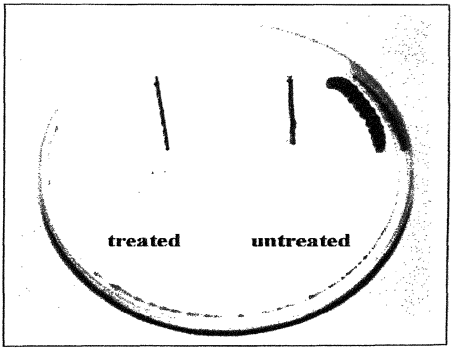
C. scarabaeoides
(ICPW 83)



C. cajan
(ICPL 87)



C. cajan
(ICPL 332)



Feeding preference by third-instar larvae of *H. armigera* towards glass fiber discs treated with methanol extracted pod surface chemicals

When given a choice between the methanol extract treated glass fiber disc and an untreated disc, the larvae preferred to feed on the discs treated with pod surface chemicals of *C. acutifolius C. scarabaeiodes* (ICPW 83), *D. ferruginea, R. bracteata, F. stricta* and *C. cajan*. However, the differences were non-significant between the control discs and the discs treated with pod surface chemicals of *C. albicans, C. sericeus, F. bracteata* and *R. aurea* (Table 57).

Table - 57 : Feeding preference by the third-instar larvae of *H. armigera* when provided with a choice between control disc and a disc treated with methanol extract of different species of wild relatives of pigeonpea.

Species	Accession number	Control disc	Methanol disc	t -value	Probability
C. acutifolius	ICPW 2	0.013 ±0.002	0.029 ±0.004	-3.48	0.040
C. albicans	ICPW 14	0.016 ±0.003	0.011±0.003	1.14	0.270
C. cajanifolius	ICPW 28	0.005 ±0.002	0.017±0.004	-2.79	0.016
C. lineatus	ICPW 41	0.015 ±0.004	0.013 ±0.003	0.38	0.707
C. sericeus	ICPW 160	0.011 ±0.002	0.010 ±0.002	0.32	0.754
C. platycarpus	ICPW 68	0.007 ±0.003	0.010 ±0.003	-0.82	0.424
C. scarabaeiodes	ICPW 83	0.010 ±0.003	0.016 ±0.003	-1.41	0.178
C. scarabaeiodes	ICPW 125	0.014 ±0.003	0.010 ±0.004	0.80	0.434
D. ferruginea	ICPW 178	0.007 ±0.003	0.013 ±0.003	-1.33	0.199
F. bracteata	ICPW 192	0.010 ±0.003	0.009 ±0.003	0.23	0.820
F. stricta	ICPW 202	0.005 ±0.003	0.010 ±0.003	-1.77	0.259
P. scariosa	ICPW 207	0.013 ±0.003	0.005 ±0.002	2.14	0.048
R. aurea	ICPW 210	0.008 ±0.003	0.005 ±0.002	0.82	0.425
R. bracteata	ICPW 214	0.006 ±0.002	0.012 ±0.003	-1.64	0.119

Biochemical composition of leaves and pods of wild relatives of pigeonpea

Total soluble sugars

There were significant differences in total soluble sugars in the leaves of wild relatives of pigeonpea (Table 58). The amounts >5% of soluble sugars were in the accessions of *C. acutifolius* (ICPW 1, and ICPW 2), *C. albicans* (ICPW 13), *C. scarabaeoides* (ICPW 130, ICPW 137, ICPW 280, and ICPW 281), *C. cajanifolius* (ICPW 28), and *P. scariosa* (ICPW 207). The total soluble sugars less than 5.62% were observed in the leaves of ICPL 87. In the case of pods, the total sugar content was >5% in *C. albicans* (ICPW 13 and ICPW 14), and *R. bracteata* (ICPW 214). Less than 2.5% sugar content was recorded in the pods of *C. cajanifolius* (ICPW 28 and ICPW 29), *C. lineatus* (ICW 40), *C. sericeus* (ICPW 159), *C. scarabaeoides* (except ICPW 125, ICPW 130, ICPW 278), *R. aurea* (ICPW 210), and *C. platycarpus* (ICPW 68). The level of sugar content recorded in all the wild accessions was less than that of the cultivated pigeonpea variety, ICPL 87 (7.12 %).

Total polyphenols

The amounts of polyphenols were significantly greater in the leaves of wild relatives of pigeonpea compared to 82.5 mg/g in the cultivated pigeonpea varieties, ICPL 87 and 115 mg/g in ICPL 332 (except ICPW 13 of *C. albicans*, ICPW 28 of *C. cajanifolius*, ICPW 159 of *C. serecius*, ICPW 116 of *C. scarabaeoides*, ICPW 192 of *R. bracteata* , ICPW 210 of *R. aurea* and ICPW 68 of *C. platycarpus*) . High amounts (>150 mg) of polyphenols were observed in *C. scarabaeoides* (ICPW 94, ICPW 125, ICPW 137, and ICPW 281), and *F. stricta* (ICPW 202) (Table 59). The amounts of polyphenols recorded in the pods of all the accessions were more than the amounts of polyphenol 43 mg/g recorded in the cultivated ICPL 87 (except ICPW 280 of amount of *C. scarabaeoides* and ICPW 192 of *F. bracteata*) and 56 mg/g recorded in ICPL 332 (except ICPW 125 , ICPW 152 of *C. scarabaeoides*, ICPW 210 of *R. aurea* and ICPW 68 of *C. platycarpus*). The amounts of polyphenols were lower both in the leaves and pods of ICPL 87 as compared to ICPL 332.

Table - 58 : Total soluble sugars in leaves and pods of wild relatives of pigeonpea.

Species	Accession number	Soluble sugars (%)	
		Leaves	Pods
C. acutifolius	ICPW 1	5.25	1.68
C. acutifolius	ICPW 2	5.12	2.62
C. albicans	ICPW 13	5.12	5.25
C. albicans	ICPW 14	4.25	5.12
C. cajanifolius	ICPW 28	5.37	2.14
C. cajanifolius	ICPW 29	2.22	1.20
C. lineatus	ICPW 40	4.12	1.50
C. lineatus	ICPW 41	3.44	3.37
C. sericeus	ICPW 159	4.68	2.32
C. sericeus	ICPW 160	4.87	4.50
C. platycarpus	ICPW 68	3.87	0.71
C. scarabaeoides	ICPW 83	2.25	1.83
C. scarabaeoides	ICPW 90	3.12	1.15
C. scarabaeoides	ICPW 94	3.87	1.05
C. scarabaeoides	ICPW116	4.50	1.34
C. scarabaeoides	ICPW 125	4.62	4.00
C. scarabaeoides	ICPW 130	5.25	3.31
C. scarabaeoides	ICPW 137	5.25	1.99
C. scarabaeoides	ICPW 141	3.35	0.86
C. scarabaeoides	ICPW 152	4.12	1.81
C. scarabaeoides	ICPW278	4.50	3.87
C. scarabaeoides	ICPW 280	5.37	1.91
C. scarabaeoides	ICPW 281	5.25	0.45
D. ferruginea	ICPW 178	4.00	4.87
F. bracteata	ICPW 192	2.21	3.50
F. stricta	ICPW 202	2.28	4.50
P. scariosa	ICPW 207	5.12	3.68
R. aurea	ICPW 210	2.12	1.47
R. bracteata	ICPW 214	3.25	5.62
C. cajan (S)	ICPL 87	5.62	7.12
C. cajan (R)	ICPL 332	4.87	3.00
SE ±		0.18	0.10
LSD at 5%		0.522	0.282
F-test		<0.001	<0.001

S - Susceptible check. R - Resistant check.

Table - 59 : Amount of polyphenols in leaves and pods of wild relatives of pigeonpea.

Species	Accession number	Polyphenols (mg/g)	
		Leaves	Pods
C. acutifolius	ICPW 1	115.0	236.7
C. acutifolius	ICPW 2	130.0	270.0
C. albicans	ICPW 13	101.7	135.0
C. albicans	ICPW 14	127.9	173.3
C. cajanifolius	ICPW 28	83.7	100.0
C. cajanifolius	ICPW 29	103.3	110.0
C. lineatus	ICPW 40	133.8	80.0
C. lineatus	ICPW 41	145.3	110.0
C. sericeus	ICPW 159	104.2	145.0
C. sericeus	ICPW 160	147.5	173.3
C. platycarpus	ICPW 68	105.0	55.3
C. scarabaeoides	ICPW 83	123.0	118.3
C. scarabaeoides	ICPW 90	144.3	110.0
C. scarabaeoides	ICPW 94	156.7	110.0
C. scarabaeoides	ICPW116	113.3	80.0
C. scarabaeoides	ICPW 125	177.4	52.7
C. scarabaeoides	ICPW 130	143.3	80.0
C. scarabaeoides	ICPW 137	175.0	100.0
C. scarabaeoides	ICPW 141	127.5	65.0
C. scarabaeoides	ICPW 152	130.0	46.7
C. scarabaeoides	ICPW278	127.5	66.7
C. scarabaeoides	ICPW 280	142.5	35.0
C. scarabaeoides	ICPW 281	162.5	67.0
D. ferruginea	ICPW 178	129.2	110.0
F. bracteata	ICPW 192	92.5	35.0
F. stricta	ICPW 202	160.0	123.0
P. scariosa	ICPW 207	141.3	82.0
R. aurea	ICPW 210	112.5	44.3
R bracteata	ICPW 214	110.0	73.7
C. cajan (S)	ICPL 87	82.5	43.0
C. cajan (R)	ICPL 332	115.0	56.0
SE ±		1.70	4.95
LSD at 5%		4.93	13.99
F-test		<0.001	<0.001

S - Susceptible check. R - Resistant check.

Tannins

The amounts of tannins in the leaves were significantly greater than those on pods. Tannins in leaves of *C. cajanifolius* (ICPW 29) were quite low(0.32%), and high amount (18.36%) and(13.15%) of tannins were observed in ICPW 40 and ICPW 41 of *C. lineatus* compared to that of ICPL 332 (0.08%) and ICPL 87 (0.88 %) of *C. cajan* (Table 60). In the pods, higher amounts of tannins were observed in ICPW 14 (77.1 %), followed by ICPW 13 (61.0%) of *C. albicans* as compared to ICPL 87 (4.9%) of *C. cajan.*

Proteins

Protein content in the leaves of wild relatives of pigeonpea was lower than in the susceptible check, ICPL 87 (3.66%) except in the accessions of *C. sericeus* and *R. bracteata*. Protein content in the leaves of wild relatives of pigeonpea was significantly lower in *C. scarabaeoides* [ICPW 130 (0.62%), ICPW 280 (0.79%) and ICPW 94 (0.81%)] accessions. Protein content was quite high in the leaves of *C. sericeus* (ICPW 159 (3.90%) and ICPW 160 (3.68%)), and in *R. bracteata* (ICPW 214 (4.41%)) (Table 61). The accessions of *C. acutifolius, C. cajanifolius, C. scarabaeoides, F. stricta,* and *R. bracteata* had more proteins in the pods compared to the cultivated pigeonpea. Protein content was low in the pods of C. *albicans* [ICPW 13 (0.78%) and ICPW 14 (0.95%)], and *R. aurea* [ICPW 210 (1.14%)]. Whereas, the protein content was significantly high in the pods of *C. scarabaeoides* [ICPW 83 (4.17%) and ICPW 281 (4.17%)], compared to ICPL 87 (1.94%) and ICPL 332 (1.98%). The percentage of soluble proteins in the pods of all the accessions of *C. scarabaeoides* was significantly higher than the percentage soluble proteins in their leaves (Table 61).

Table - 60 : Amount of tannins in leaves and pods of wild relatives of pigeonpea.

Species	Accession number	Tannins	
		Leaves	Pods
C. acutifolius	ICPW 1	8.60	26.9
C. acutifolius	ICPW 2	5.79	22.5
C. albicans	ICPW 13	3.24	61.0
C. albicans	ICPW 14	1.21	77.1
C. cajanifolius	ICPW 28	1.37	5.8
C. cajanifolius	ICPW 29	0.32	7.9
C. lineatus	ICPW 40	18.36	3.2
C. lineatus	ICPW 41	13.15	4.6
C. sericeus	ICPW 159	3.27	14.6
C. sericeus	ICPW 160	2.93	19.9
C. platycarpus	ICPW 68	7.57	3.7
C. scarabaeoides	ICPW 83	3.36	4.3
C. scarabaeoides	ICPW 90	7.71	4.2
C. scarabaeoides	ICPW 94	12.42	4.3
C. scarabaeoides	ICPW116	5.34	1.4
C. scarabaeoides	ICPW 125	12.62	1.9
C. scarabaeoides	ICPW 130	11.53	1.9
C. scarabaeoides	ICPW 137	10.47	3.0
C. scarabaeoides	ICPW 141	7.13	2.7
C. scarabaeoides	ICPW 152	3.50	3.8
C. scarabaeoides	ICPW278	11.23	3.7
C. scarabaeoides	ICPW 280	10.21	2.7
C. scarabaeoides	ICPW 281	12..8	2.7
D. ferruginea	ICPW 178	5.99	4.6
F. bracteata	ICPW 192	6.50	2.3
F. stricta	ICPW 202	6.52	26.0
P. scariosa	ICPW 207	10.92	12.8
R. aurea	ICPW 210	6.79	1.2
R. bracteata	ICPW 214	2.15	4.9
C. cajan (S)	ICPL 87	0.88	4.9
C. cajan (R)	ICPL 332	0.08	17.9
SE ±		8.22	3.08
F-test		<0.001	<0.001
LSD at 5%		0.119	0.267

S - Susceptible check. R - Resistant check.

Table - 61: Amount of total soluble proteins in leaves and pods of wild relatives of pigeonpea.

Species	Accession number	Protein (%)	
		Leaves	Pods
C. acutifolius	ICPW 1	3.44	2.19
C. acutifolius	ICPW 2	2.28	2.47
C. albicans	ICPW 13	3.51	0.78
C. albicans	ICPW 14	2.81	0.95
C. cajanifolius	ICPW 28	2.19	3.31
C. cajanifolius	ICPW 29	3.62	3.20
C. lineatus	ICPW 40	2.00	1.81
C. lineatus	ICPW 41	2.01	1.93
C. sericeus	ICPW 159	3.90	1.62
C. sericeus	ICPW 160	3.68	1.56
C. platycarpus	ICPW 68	2.41	1.65
C. scarabaeoides	ICPW 83	2.59	4.17
C. scarabaeoides	ICPW 90	2.35	2.95
C. scarabaeoides	ICPW 94	0.81	3.08
C. scarabaeoides	ICPW116	2.64	3.69
C. scarabaeoides	ICPW 125	1.64	2.67
C. scarabaeoides	ICPW 130	0.62	3.60
C. scarabaeoides	ICPW 137	1.89	2.97
C. scarabaeoides	ICPW 141	1.89	2.82
C. scarabaeoides	ICPW 152	1.67	2.80
C. scarabaeoides	ICPW278	1.69	3.49
C. scarabaeoides	ICPW 280	0.79	2.97
C. scarabaeoides	ICPW 281	1.12	4.17
D. ferruginea	ICPW 178	2.22	1.65
F. bracteata	ICPW 192	1.39	1.87
F. stricta	ICPW 202	3.23	2.09
P. scariosa	ICPW 207	1.67	1.82
R. aurea	ICPW 210	2.68	1.14
R. bracteata	ICPW 214	4.41	2.25
C. cajan (S)	ICPL 87	3.66	1.94
C .cajan (R)	ICPL 332	2.86	1.98
SE $\pm$		0.205	0.496
LSD at 5%		0.508	1.402
F-test		<0.001	<0.001

S - Susceptible check. R - Resistant check.

HPLC profiles of pod surface extracts

The HPLC profiles of the pod surface extracts revealed considerable variation in their composition in different wild relatives of pigeonpea. The total number of peaks observed in the methanol solvent extracts (Fig 26) was more compared to the number of peaks in the hexane extract (Fig 27), except incase ICPW2, ICPW 160, ICPW 83, ICPW178, ICPW 192, and ICPW207 (Table 62).

Table – 62: Total number of peaks in methanol and hexane pod surface extracts of different wild relatives of pigeonpea

Species	Accession number	Number of peaks	
		Methanol extract	Hexane extract
C. acutifolius	ICPW 2	10	12
C. albicans	ICPW 14	15	12
C. cajanifolius	ICPW 28	15	11
C. lineatus	ICPW 41	14	12
C. sericeus	ICPW 160	10	12
C. platycarpus	ICPW 68	19	12
C. scarabaeoides	ICPW 83	11	14
C. scarabaeoides	ICPW 125	18	8
D. ferruginea	ICPW 178	13	17
F. bracteata	ICPW 192	14	10
F. stricta	ICPW 202	17	12
P. scariosa	ICPW 207	8	14
R. aurea	ICPW 210	17	8
R. bracteata	ICPW 214	22	11
C. cajan (S)	ICPL 87	18	13
C .cajan (R)	ICPL 332	19	18

Methanol extracts

Maximum number of peaks (22) were recorded in methanol extract of *D. ferrugenia* (ICPW 214) and lowest (8) in *P. scariosa* (ICPW 207) compared to 19 peaks in ICPL 332 and 18 peaks in ICPL 87 (Table 63).

Of the 18 peaks present in ICPL 87, $peak_1$ was present in ICPL 332, and *C. scarabaeoides* (ICPW 83), and *R. bracteata* (ICPW 214), while it was absent in rest of the wild relatives of pigeonpea. $Peak_2$ was observed in ICPL 332, *C. scarabaeoides* (ICPW 83, and ICPW 125), *F. bracteata* (ICPW 192), and *R. bracteata* (ICPW 214), while Peak 3 was present in all the wild accessions, except *C. cajanifolius* (ICPW 28), *C. sericeus* (ICPW 160), *D. ferrugenia* (ICPW 178), and *P. scariosa* (ICPW 207). $Peak_4$ was observed in ICPL 332 and in *C. scarabaeoides* (ICPW 83), *F. stricta* (ICPW 202), *P. scariosa* (ICPW 207), *R. aurea* (ICPW 210) and *C. albicans* (ICPW 14). $Peak_5$ was observed in ICPL 332 and ICPW 214, but was absent in rest of the wild relatives of pigeonpea. Peak 6 was observed in ICPL 332, *C. lineatus* (ICPW 41), *C. scarabaeoides* (ICPW 83 and ICPW 125), *F. bracteata* (ICPW 192), *F. stricta* (ICPW 202), *P. scariosa* (ICPW 207), and *R. bracteata* (ICPW 214). $Peak_7$ was observed in all the genotypes tested, except in ICPW 83, ICPW 192, ICPW 207, and ICPW 210. $Peak_8$ was present in *C. albicans* (ICPW 14), *C. scarabaeoides* (ICPW 125), ICPW 192, ICPW 210, and *R. bracteata* (ICPW 214), while $Peak_9$ was present in all the test genotypes, except ICPW 2, ICPW 28, ICPW 41, ICPW 160, ICPW 125, ICPW 178, and ICPW 210. $Peak_{10}$ was observed in ICPW 83, ICPW 178, ICPW 202, and ICPW 214. $Peak_{11}$ was present in all the genotypes tested, except in ICPW 83, and ICPW 207. $Peak_{12}$ was observed in all the genotypes, while $Peak_{13}$ was observed in ICPL 332, ICPW 2, ICPW 14, and ICPW 178. $Peak_{14}$ was also observed in all the genotypes, except ICPW 2, ICPW 28, ICPW 83, ICPW 178, ICPW 202, and ICPW 207. $Peak_{15}$ was not observed in ICPW 14, ICPW 28, ICPW 83, ICPW 202, and ICPW 207. $Peak_{16}$ was no observed in any of the accessions. $Peak_{17}$ was observed only in ICPW 214, while $Peak_{18}$ was observed in ICPL 332, ICPW 14, ICPW 125, ICPW 192, ICPW 202, ICPW 207 and ICPW 210.

The peaks with more than 5% area of the total were considered as the major peaks, and their relative distribution in different species presented an interesting picture. The peak at retention time (rt) 2.6 was present in all the wild relatives of pigeonpea (*C. acutifolius, C. albicans, C. cajanifolius, C. sericeus, C. platycarpus, C. scarabaeoides, D. ferrugenia, F. bracteata, P. scariosa R. aurea, R. bracteata*), except in both the cultivated checks. The peak at rt 13.5 was observed only in the resistant genotypes to *H. armigera*. Its maximum area was in ICPW 207 (56.36%), followed by ICPW 214 (21.47%). The peak at rt 10.2 was observed in all the wild accessions, except ICPW 28, ICPW 83, ICPW 202, and ICPW 207. The peak at rt 12.2 was observed only in the resistant wild relatives of pigeonpea (ICPW 14, ICPW 83, ICPW 192 and ICPW 207), and very low amounts were observed in ICPL 332 and ICPL 87. The peak at rt 21.2 was observed in ICPW 2, ICPW 41, ICPW 160, ICPW 178, ICPW 210 and ICPW 214, but was of very low intensity in ICPW 14, ICPW 28, ICPW 68, ICPW 125, and ICPW 202. The compound at peak 9.9 was either present in minor quantities or completely absent in the wild relatives of pigeonpea, but was present in significant amounts in ICPL 332 (5.18%). The peak at 3.16 was observed in ICPL 332 (19.1%), but was completely absent in all the wild relatives of pigeonpea, and in very small amounts in ICPL 87. The compound at rt 34.89 was present in significant amounts in ICPW 41 (23.56%) and ICPW 2 (8.15%), but was absent in rest of the genotypes. The peak at rt 14.987 was observed in significant amounts in ICPW 214 (6.99%), ICPW 202, and ICPW 160 (11.19%). The peak at rt 30.59 was present only in ICPW 68 (18.22%). The compounds at rt 17.4, 24.1, 25.7 and 27.5 were present in significant amounts in both ICPL 332 and ICPL 87, but were absent, or present in very small quantities, in the wild relatives. The presence of these particular peaks in the cultivated species might be responsible for their susceptibility to *H. armigera*. The peak at rt 30.5 was observed only in ICPW 68, and was absent in the rest of wild relatives, and cultivated pigeonpea, ICPL 332. It is interesting to note that *H. armigera* larvae showed more preference towards the pod surface chemicals extracted in methanol.

Table - 63: HPLC finger prints of methanol extract of pod surface of wild relatives of pigeonpea.

Species	Accession number	Pk #	Retention time	Area	Area (%)
C. acutifolius	**ICPW 2**	1	2.667	291214	3.45
		2	10.325	590978	7.00
		3	11.168	63340	0.75
		4	11.712	129011	1.53
		5	21.173	700772	8.30
		6	23.200	299910	3.55
		7	23.893	195337	2.31
		8	24.480	3109914	36.85
		9	27.061	2370707	28.09
		10	34.859	687745	8.15
		Totals		**3438928**	**100.00**
C. albicans	**ICPW 14**	1	2.901	9002600	20.18
		2	5.323	88168	0.20
		3	6.368	32852	0.07
		4	10.208	21777783	48.83
		5	12.320	4566530	10.24
		6	15.573	162047	0.36
		7	16.363	1370129	3.07
		8	20.256	283591	0.64
		9	20.661	50397	0.11
		10	21.227	472648	1.06
		11	23.691	165649	0.37
		12	24.373	649562	1.46
		13	27.787	580390	1.30
		14	31.584	3560628	7.98
		15	34.955	1840513	4.13
		Totals		**44603487**	**100.00**
C.cajanifolius	**ICPW 28**	1	2.656	15342749	24.27
		2	8.853	1037403	1.64
		3	11.595	1421210	2.25
		4	12.224	2358984	3.73
		5	14.592	670673	1.06
		6	15.509	1187866	1.88
		7	16.341	21027443	33.26
		8	17.941	2595012	4.10
		9	21.237	559763	0.89
		10	22.357	534621	0.85
		11	23.744	960275	1.52
		12	24.213	472035	0.75
		13	25.269	2968774	4.70
		14	26.955	9934663	15.71
		15	35.296	2152656	3.40
		Totals		**63224127**	**100.00**

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Species	Accession number	Pk #	Retention time	Area	Area (%)
C. lineatus	**ICPW 41**	1	2.677	911046	23.71
		2	10.155	158617	4.13
		3	11.371	16588	0.43
		4	11.680	6404	0.17
		5	13.493	93196	2.43
		6	14.880	76458	1.99
		7	16.491	31572	0.82
		8	21.131	780967	20.33
		9	22.293	308660	8.03
		10	23.136	100064	2.60
		11	25.429	311410	8.11
		12	25.696	141983	3.70
		13	34.891	905169	23.56
		Totals		**3842134**	**100.00**
C.serecius	**ICPW 160**	1	2.411	626156	1.81
		2	8.267	92503	0.27
		3	10.197	22301003	64.55
		4	14.272	3865286	11.19
		5	18.155	786162	2.28
		6	21.397	1774181	5.14
		7	22.443	1441467	4.17
		8	23.317	310080	0.90
		9	25.355	68960	0.20
		10	27.531	3282065	9.50
		Totals		**34547863**	**100.00**
C. platycarpus	**ICPW 68**	1	2.453	1214777	1.68
		2	2.709	2033447	2.82
		3	3.392	119447	0.17
		4	7.115	119580	0.17
		5	7.936	158648	0.22
		6	9.120	799199	1.11
		7	10.400	1519181	2.10
		8	11. 925	426011I	0.59
		9	13.611	1 805102	2.50
		10	20.149	336490	0.47
		11	21.355	5477649	7.59
		12	22.645	140704	0.19
		13	23.840	941295	1.30
		14	24.587	30363966	42.06
		15	25.685	13154088	18.22
		16	26.325	240567	0.33
		17	27.339	30363966	42.06
		18	30.592	13154088	18.22
		19	35.147	240567	0.33
		Totals		**72184333**	**100.00**

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Species	Accession number	Pk #	Retention time	Area	Area (%)
C. scarabaeoides	**ICPW 83**	1	2.880	27923	0.31
		2	3.147	317113	3.52
		3	6.101	58533	0.31
		4	6.763	22165	3.52
		5	8.320	32594	0.31
		6	9.493	802930	3.52
		7	11.221	1549131	0.31
		8	12.064	2822132	3.52
		9	13.248	656979	0.31
		10	17.376	2640491	3.52
		11	19.029	87392	0.31
		Totals		**9017383**	**100.00**
C. scarabaeoides	**ICPW 125**	1	2.667	8176023	20.57
		2	5.365	138879	0.35
		3	6.347	124910	0.31
		4	7.104	25203	0.06
		5	7.616	19644	0.05
		6	9.291	1026664	2.58
		7	10.475	4626538	11.64
		8	11.307	17015353	42.81
		9	13.344	1436347	3.61
		10	14.816	88035	0.22
		11	15.765	521699	1.31
		12	16.587	3574331	8.99
		13	21.216	1395646	3.51
		14	22.357	374236	0.94
		15	23.200	580510	1.46
		16	25.205	154696	0.39
		17	27.232	178779	0.45
		18	35.232	286329	0.72
		Totals		**39743822**	**100.00**
D. ferruginea	**ICPW 178**	1	2.912	2275378	8.63
		2	9.099	282497	1.07
		3	10.379	655951	2.49
		4	14.027	162712	0.62
		5	16.725	347033	1.32
		6	18.325	431112	1.64
		7	19.797	65314	0.25
		8	21.707	9838804	37.32
		9	22.389	752609	2.85
		10	23.371	9393847	35.63
		11	24.640	1239624	4.70
		12	27.369	26964 7	1.02
		13	34.923	650912	2.47
		Totals		**26365440**	**100.00**

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Species	Accession number	Pk #	Retention time	Area	Area (%)
F. bracteata	**ICPW 192**	1	2.869	13083222	32.33
		2	5.280	372872	0.92
		3	7.861	615821	1.52
		4	8.939	182500	0.45
		5	11.051	3383213	8.36
		6	12.224	8197470	20.26
		7	13.472	541543	1.34
		8	15.552	830854	2.05
		9	17.429	2420111	5.98
		10	23.936	1817671	4.49
		11	25.717	1680283	4.15
		12	27.851	422098	1.04
		13	29.120	571104	1.41
		14	35.104	6351165	15.69
		Totals		**40469927**	**100.00**
F. stricta	**ICPW 202**	1	2.923	3435487	19.06
		2	8.064	994332	5.52
		3	9.568	280879	1.56
		4	11.829	362992	2.01
		5	12.299	341651	1.90
		6	13.568	1146371	6.36
		7	13.941	294256	1.63
		8	14.933	1512371	8.39
		9	17.792	1000419	5.55
		10	19.989	1636293	9.08
		11	21.013	110085	0.61
		12	22.432	294536	1.63
		13	23.072	67338	0.37
		14	23.765	2255876	12.52
		15	26.123	3159741	17.53
		16	31.008	238677	1.32
		17	35.051	891531	4.95
		Totals		**18022835**	**100.00**
P. scariosa	**ICPW 207**	1	2.699	533232	4.33
		2	12.267	2074028	16.82
		3	13.547	6947926	56.36
		4	17.173	177697	1.44
		5	20.960	1115020	9.04
		6	22.176	538854	4.37
		7	23.072	157550	1.28
		8	35.019	783213	6.35
		Totals		**12327520**	**100.00**

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Species	Accession number	Pk #	Retention time	Area	Area (%)
R. aurea	**ICPW 210**	1	2.645	696031	6.08
		2	5.365	144878	1.27
		3	8.821	170625	1.49
		4	10.453	3249138	28.37
		5	11.264	1212421	10.59
		6	12.320	233808	2.04
		7	15.691	330173	2.88
		8	16.267	1365335	11.92
		9	18.144	169412	1.48
		10	21.152	1821895	15.91
		11	22.272	542679	4.74
		12	23.125	242424	2.12
		13	23.851	825868	7.21
		14	25.163	149169	1.30
		15	27 .328	113036	0.99
		16	32.384	65720	0.57
		17	35.211	118574	1.04
		Totals		**11451186**	**100.00**
R. bracteata	**ICPW 214**	1	2.645	3167816	14.46
		2	3.413	171558	0.78
		3	4.661	145665	0.67
		4	7.061	476630	2.18
		5	9.024	787624	3.60
		6	10.283	1883821	8.60
		7	11.211	115516	0.53
		8	11.925	71419	0.33
		9	12.363	651406	2.97
		10	12.821	1 19120	0.54
		11	13.589	4703216	21.47
		12	14.453	953682	4.35
		13	14.987	1530426	6.99
		14	15.605	1657217	7.57
		15	17.728	1095710	5.00
		16	19.701	41163	0.19
		17	21.216	1341297	6.12
		18	22.336	338706	1.55
		19	23.168	435900	1.99
		20	25.419	654611	2.99
		21	27.477	537508	2.45
		22	33.035	1023345	4.67
		Totals		**21903356**	**100.00**

Contd.,

Species	Accession number	Pk #	Retention time	Area	Area (%)
C. cajan	**ICPW 332**	1	3.168	1637761	19.21
		2	4.459	78289	0.92
		3	6.133	184870	2.17
		4	9.909	441488	5.18
		5	10.635	8983	0.11
		6	11.125	324685	3.81
		7	12.171	61850	0.73
		8	12.480	52465	0.62
		9	13.184	513953	6.03
		10	14.613	27289	0.32
		11	15.392	27603	0.32
		12	17.504	586681	6.88
		13	18.891	105591	1.24
		14	22.795	73358	0.86
		15	23.019	11371	0.13
		16	24.181	211 1426	24.76
		17	25.845	650500	7.63
		18	27.595	1517243	17.79
		19	35.371	111797	1.31
		Totals		**8527203**	**100.00**
C. cajan	**ICPL 87**	1	3.157	276546	0.86
		2	9.899	174622	0.55
		3	11.200	275346	0.86
		4	12.117	54507	0.17
		5	12.683	24362	0.08
		6	13.301	350633	1.10
		7	14.699	42966	0.13
		8	15.712	451182	1.41
		9	17.493	8091104	25.31
		10	19.040	1092944	3.42
		11	21.205	606704	1.90
		12	23.051	424213	1.33
		13	24.192	4341035	13.58
		14	25.78	4459033	13.95
		15	27.200	10379638	32.46
		16	30.965	348160	1.09
		17	33.260	376189	1.18
		18	35.627	203204	0.64
		Totals		**31972388**	**100.00**

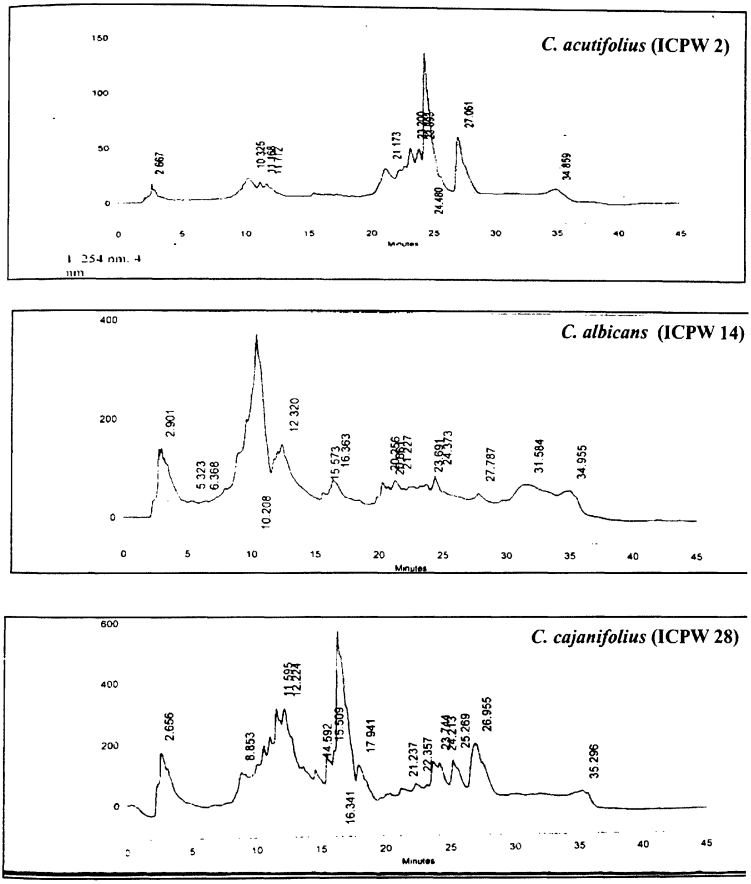
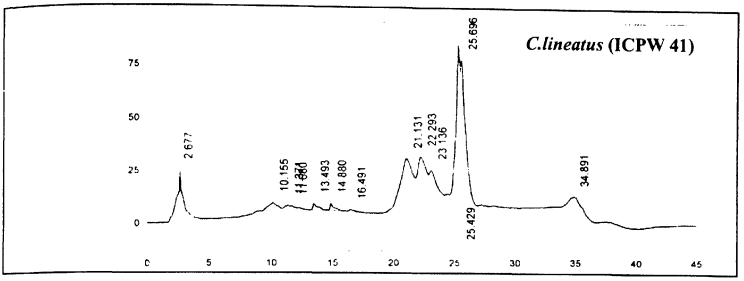
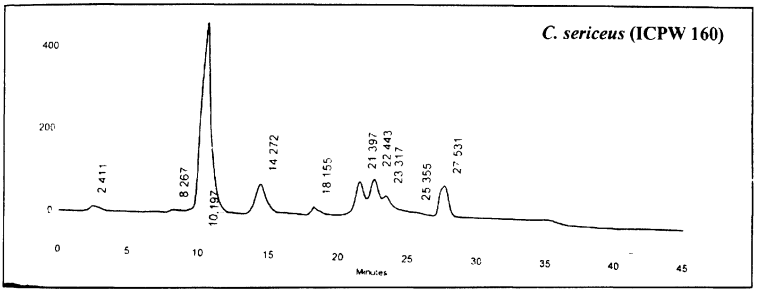
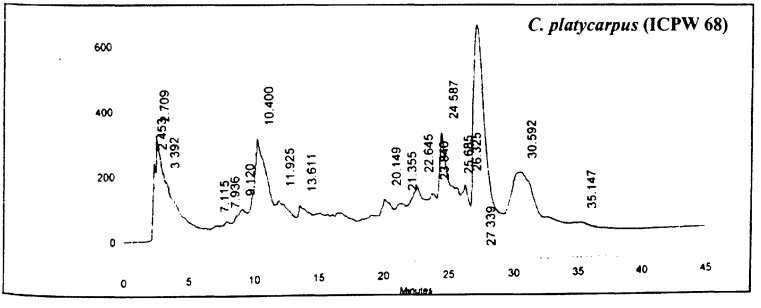


Fig – 26 : HPLC profiles of methanol extract of pod surface of wild relatives of pigeonpea

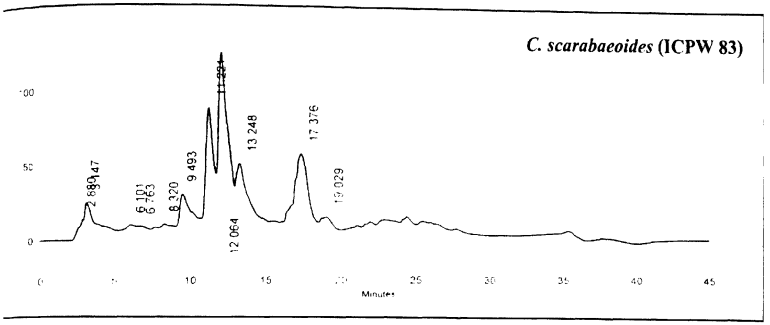
Contd..

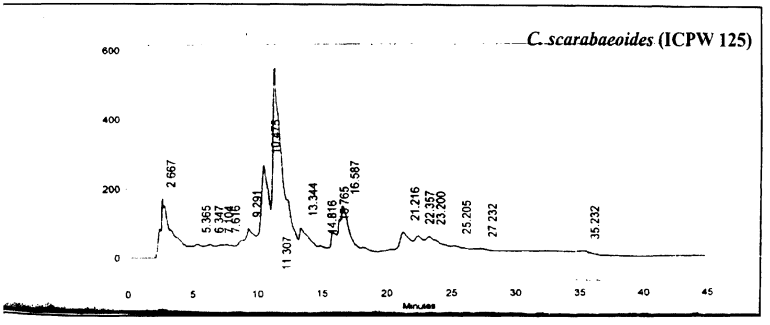

C.lineatus (ICPW 41)
75
50
25
0
2.677
10.155
13.493
14.880
16.491
21.131
22.293
23.136
25.696
25.429
34.891
0
5
10
15
20
25
30
35
40
45

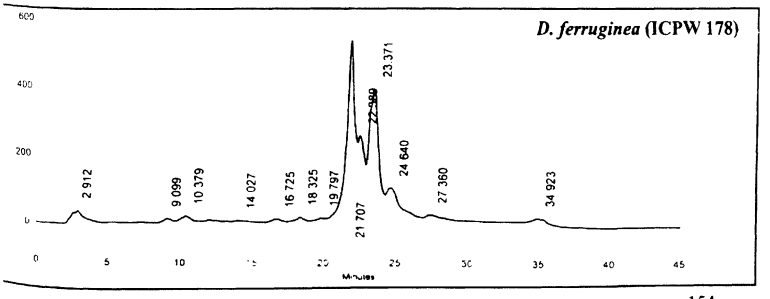

C. sericeus (ICPW 160)
400
200
0
2.411
8.267
10.192
14.272
18.155
21.397
22.443
23.317
25.355
27.531
0
5
10
15
20
25
30
35
40
45
Minutes

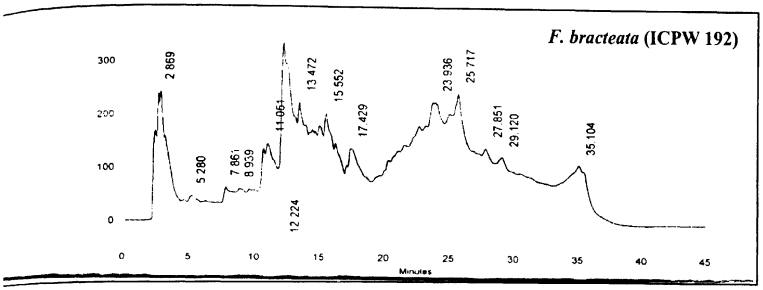

C. platycarpus (ICPW 68)
600
400
200
0
2.709
3.392
7.115
7.936
9.120
10.400
11.925
13.611
20.149
21.355
22.645
24.587
25.685
26.325
27.339
30.592
35.147
0
5
10
15
20
25
30
35
40
45
Minutes

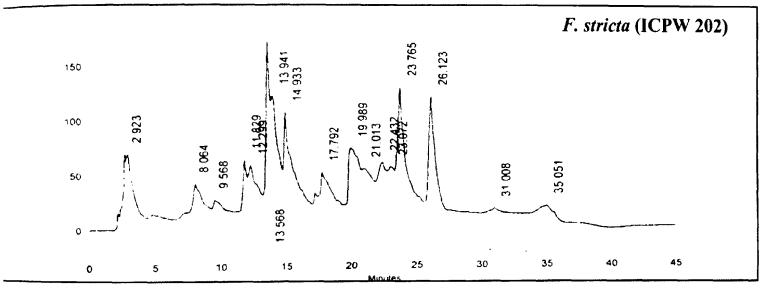
Contd..

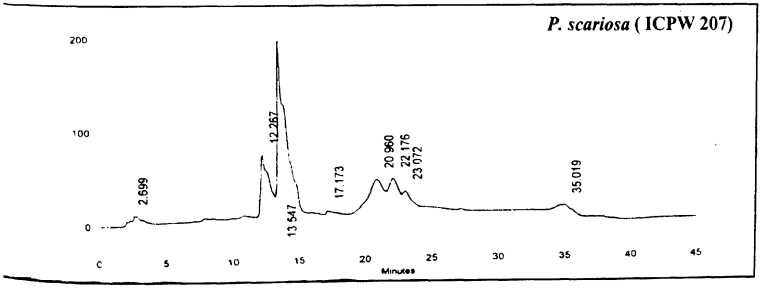

C. scarabaeoides (ICPW 83)
2 689
6 101
6 763
8 320
9 493
11 824
12 064
13 248
17 376
19 029
Minutes


C. scarabaeoides (ICPW 125)
2 667
5 365
6 347
7 014
7 618
8 291
10 475
11 307
13 344
14 818
15 765
16 587
21 216
22 357
23 200
25 205
27 232
35 232
Minutes

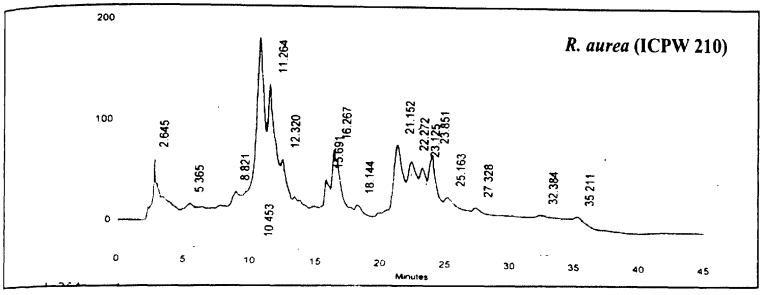

D. ferruginea (ICPW 178)
2 912
9 099
10 379
14 027
16 725
18 325
19 797
21 707
23 371
24 640
27 360
34 923
Minutes

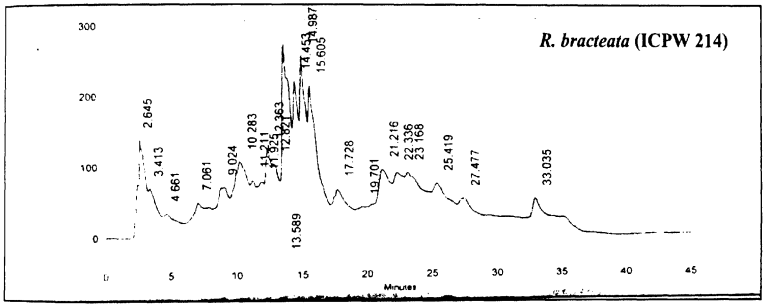

F. bracteata (ICPW 192)
300
200
100
0
2.869
5.280
7.861
8.939
11.061
12.224
13.472
15.552
17.429
23.936
25.717
27.851
29.120
35.104
0
5
10
15
20
25
30
35
40
45
Minutes

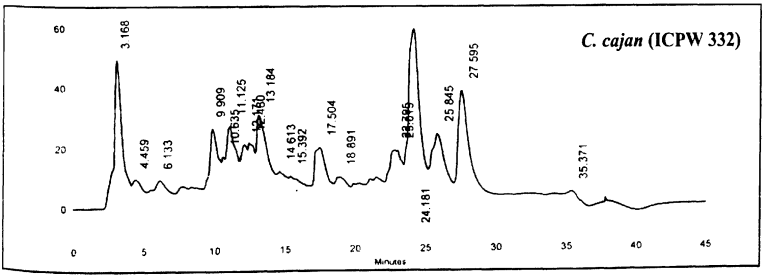

F. stricta (ICPW 202)
150
100
50
0
2.923
8.064
9.568
13.568
13.941
14.933
17.792
19.989
21.013
23.765
26.123
31.008
35.051
0
5
10
15
20
25
30
35
40
45
Minutes


P. scariosa (ICPW 207)
200
100
0
2.699
12.267
13.547
17.173
20.960
22.176
23.072
35.019
0
5
10
15
20
25
30
35
40
45
Minutes

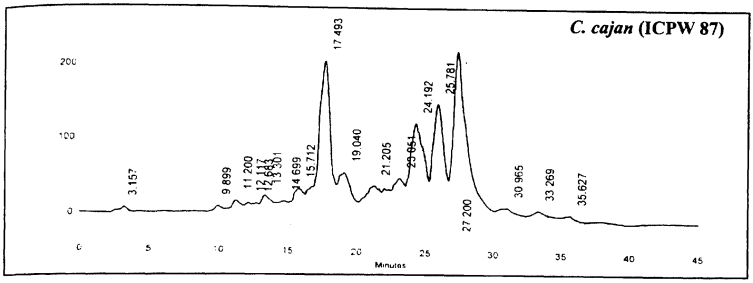
Contd.,


R. aurea (ICPW 210)
200
100
0
2.645
5.365
8.821
10.453
11.264
12.320
15.691
16.267
18.144
21.152
22.272
23.163
23.851
25.163
27.328
32.384
35.211
0
5
10
15
20
25
30
35
40
45
Minutes


R. bracteata (ICPW 214)
300
200
100
0
2.645
3.413
4.661
7.061
9.024
10.283
11.125
11.613
12.363
12.821
14.453
14.987
15.605
13.589
17.728
19.701
21.216
22.336
23.168
25.419
27.477
33.035
0
5
10
15
20
25
30
35
40
45
Minutes


C. cajan (ICPW 332)
60
40
20
0
3.168
4.459
6.133
9.909
10.635
11.125
12.180
13.184
14.613
15.392
17.504
18.891
23.395
23.895
24.181
25.845
27.595
35.371
0
5
10
15
20
25
30
35
40
45
Minutes

Contd.,



Hexane extracts

Highest number of peaks (18) was observed in ICPW 332 of *C. cajan,* and lowest (8) in *C. scarabaeoides* (ICPW 125), and *R. aurea* (ICPW 210). A total of 13 peaks were observed in the susceptible check, ICPL 87 (Table 64).

$Peak_1$ at rt 2.6 was observed in all the wild relatives and ICPL 332 of the cultivated pigeonpea (except in *R. aurea* (ICPW 210)). $Peak_2$ at rt 5.3 was present only in *P. scariosa* (ICPW 207). $Peak_3$ at rt 12.3 was observed only in cultivated pigeonpea, ICPL 332 (0.47) and ICPW 214 (2.42) of wild relatives, while $peak_4$ at rt 14.6 was observed in ICPL 332, ICPW 28, ICPW 41, ICPW 160, and ICPW 178. $Peak_5$ at rt 15.5 was observed in ICPL 332, *C. cajanifolius* (ICPW 28*)* and *D. ferruginea* (ICPW 178). $Peak_6$ at rt 16.3 was observed in ICPL 332, *C. acutifolius* (ICPW 2*), C. albicans* (ICPW 14), *C. cajanifolius* (ICPW 28*), C. sericeus* (ICPW 160), *C. platycarpus* (ICPW 68), *C. scarabaeoides* (ICPW 83), *D. ferruginea* (ICPW 178), and *F. bracteata* (ICPW 192*)*. $Peak_7$ at rt 17.8 was observed in ICPL 332 and in 3 wild species [*C. cajanifolius* (ICPW 28*), C. sericeus* (ICPW 41), and *F. stricta* (ICPW 202)]. $Peak_8$ at rt 20.16 was present in ICPL 332 and in all the wild relatives of pigeonpea (expect ICPW 28, ICPW 41, ICPW 68, ICPW 192). $Peak_9$ at rt 24.21 was observed in ICPL 332, ICPW 14, ICPW28, ICPW 41, ICPW 160, ICPW 178 and ICPW 202. $Peak_{10}$ at rt 25.3 was observed in ICPL 332, ICPW 2, ICPW 14, ICPW 41, ICPW 68, ICPW 83, ICPW 192 and ICPW 214. $Peak_{11}$ at rt 28.38 was observed in ICPL 332, ICPLW 178 and ICPW 192, while $Peak_{12}$ at rt 32.03 was observed only in ICPW 214. $Peak_{13}$ at rt 35.3 was observed in ICPL 332 and all the

Table - 64: HPLC finger prints of hexane extract of pod surface of wild relatives of pigeonpea.

Species	Accession number	Pk #	Retention time	Area	Area (%)
C. acutifolius	ICPW 2	1	2.699	55415	3.11
		2	6.709	20741	1.16
		3	16.331	15538	0.87
		4	18.091	16556	0.93
		5	20.341	181785	10.21
		6	22.176	103735	5.82
		7	23.232	35337	1.98
		8	25.163	588812	33.06
		9	27.008	33941	1.91
		10	34.848	625588	35.13
		11	35.531	45916	2.58
		12	36.661	57520	3.23
		Totals		**1780884**	**100.00**
C. albicans	ICPW 14	1	2.667	53725	0.26
		2	9.952	81489	0.40
		3	16.192	993715	4.84
		4	19.957	1376143	6.71
		5	20.885	899188	4.38
		6	22.411	1113471	5.43
		7	23.339	751789	3.66
		8	24.053	992409	4.84
		9	25.141	6906729	33.66
		10	27 .488	2661430	12.97
		11	29.344	1022541	4.98
		12	35.200	3667973	17.87
		Totals		**20520602**	**100.00**
C. cajanifolius	ICPW 28	1	2.315	771796	1.72
		2	8.267	183595	0.41
		3	11.691	24646759	54.82
		4	14.485	577127	1.28
		5	15.445	1343187	2.99
		6	16.277	8931204	19.87
		7	17.771	1157700	2.58
		8	24.811	2192761	4.88
		9	26.123	3122444	6.95
		10	29.909	660010	1.47
		11	34.859	1370553	3.05
		Totals		**44957136**	**100.00**

Contd.,

Species	Accession number	Pk #	Retention time	Area	Area (%)
C. lineatus	ICPW 41	1	2.709	52514	0.25
		2	7.669	34813	0.17
		3	14.709	48670	0.24
		4	17.696	83400	0.41
		5	18.208	47958	0.23
		6	21.23 7	172834	0.84
		7	22.357	1211256	5.87
		8	23.776	42427	0.21
		9	24.117	91235	0.44
		10	25.291	18279996	88.53
		11	27.989	273639	1.33
		12	34.859	307291	1.49
		Totals		**20648033**	**100.00**
C. serecius	ICPW 160	1	2.688	55183	2.34
		2	6.709	17496	0.74
		3	14.539	8344	0.35
		4	16.245	21224	0.90
		5	20.437	123584	5.25
		6	22.379	78480	3.33
		7	23.029	41714	1.77
		8	24.181	1122127	47.63
		9	26.432	451737	19.18
		10	30.133	81895	3.48
		11	34.859	304451	12.92
		12	36.683	49533	2.10
		Totals		**2355768**	**100.00**
C. platycarpus	ICPW 68	1	2.677	58295	0.27
		2	6.603	42791	0.19
		3	16.235	177952	0.81
		4	18.752	5053106	23.00
		5	19.253	2430228	11.06
		6	19.744	2588949	11.78
		7	21.685	1249941	5.69
		8	23.424	2708086	12.33
		9	25.131	3479082	15.84
		10	26.133	1599768	7.28
		11	30.336	853943	3.89
		12	35.221	1727844	7.86
		Totals		**21969985**	**100.00**

Contd.,

Species	Accession number	Pk #	Retention Time	Area	Area (%)
C. scarabaeoides	**ICPW 83**	1	2.709	55561	2.37
		2	6.688	14191	0.60
		3	7.755	6986	0.30
		4	8.341	14723	0.63
		5	11.061	137463	5.86
		6	13.707	23844	1.02
		7	16.320	173071	7.37
		8	20.501	134246	5.72
		9	22.336	812752	34.62
		10	25.056	88659	3.78
		11	26.880	323648	13.79
		12	34.869	444940	18.95
		13	35.531	52404	2.23
		14	36.789	65165	2.78
		Totals		**2347653**	**100.00**
C. scarabaeoides	**ICPW 125**	1	2.613	54637	1.34
		2	6.709	23965	0.59
		3	20.437	164634	4.03
		4	23.211	1452600	35.56
		5	26.677	504824	12.36
		6	27.669	313848	7.68
		7	29.472	118002	2.89
		8	35.296	1452653	35.56
		Totals		**4085163**	**100.00**
D. ferruginea	ICPW 178	1	2.699	51618	2.26
		2	6.688	12657	0.56
		3	10.997	6648	0.29
		4	14.069	42152	1.85
		5	15.477	15613	0.69
		6	16.320	9409	0.41
		7	18.197	14377	0.63
		8	20.448	133926	5.88
		9	21.205	197499	8.67
		10	22.059	107811	4.73
		11	23.456	231069	10.14
		12	24.363	500657	21.97
		13	27.040	375948	16.50
		14	28.949	129663	5.69
		15	34.869	321041	14.09
		16	35.531	58288	2.56
		17	36.832	70771	3.11
		Totals		**2279147**	**100.00**

Contd.,

Species	Accession number	Pk #	Retention time	Area	Area (%)
F. bracteata	**ICPW 192**	1	2.688	54392	0.47
		2	13.877	47618	0.41
		3	16.181	400652	3.44
		4	23.509	968378	8.32
		5	25.429	8329669	71.58
		6	26.923	359015	3.09
		7	28.800	26516	0.23
		8	29.952	153684	1.32
		9	30.944	1285	0.01
		10	35.264	1295048	11.13
		Totals		**11636257**	**100.00**
F. stricta	**ICPW 202**	1	2.645	54836	3.23
		2	6.656	106243	6.26
		3	17.024	10173	0.60
		4	17.579	21216	1.25
		5	19.584	238097	14.02
		6	20.427	35123	2.07
		7	22.261	271491	15.99
		8	23.125	59842	3.52
		9	26.187	487961	28.74
		10	26.944	135462	7.98
		11	30.485	60057	3.54
		12	35.232	217385	12.80
		Totals		**1697886**	**100.00**
P. scariosa	**ICPW 207**	1	2.635	81930	4.71
		2	5.376	4150	0.24
		3	7.968	46526	2.68
		4	11.221	15723	0.90
		5	12.341	40346	2.32
		6	13.632	101608	5.85
		7	18.261	23275	1.34
		8	20.384	335173	19.28
		9	21.365	251338	14.46
		10	23.531	241356	13.88
		11	24.651	60835	3.50
		12	27.040	118060	6.79
		13	30.581	29885	1.72
		14	35.360	388144	22.33
		Totals		**1738349**	**100.00**

Contd.,

Species	Accession number	Pk #	Retention time	Area	Area (%)
R. aurea	**ICPW 210**	1	6.635	22036	0.37
		2	18.603	218713	3.69
		3	20.992	1228291	20.75
		4	22.283	1399555	23.64
		5	23 .296	1254245	21.19
		6	26.923	227393	3.84
		7	30.016	680916	11.50
		8	35.243	888053	15.00
		Totals		**5919202**	**100.00**
R. bracteata	**ICPW 214**	1	2.667	79415	0.73
		2	12.768	262156	2.42
		3	13.397	117413	1.08
		4	19.317	218855	2.02
		5	20.256	67594	0.62
		6	23.488	911345	8.42
		7	25.141	1836534	16.96
		8	26.293	858799	7.93
		9	27.189	2180068	20.13
		10	32.363	3895048	35.97
		11	33.717	400487	3.70
		Totals		**10827714**	**100.00**
C. cajan	**ICPL 87**	1	2.741	76203	0.56
		2	5.397	1621 32	1.20
		3	12.405	142052	1.05
		4	14.635	6623 85	4.90
		5	15.573	1190470	8.81
		6	16.384	6953558	51.45
		7	17.856	385744	2.85
		8	20.160	355355	2.63
		9	24.213	6623 85	4.75
		10	25.387	1198835	8.87
		11	28.384	1413611	10.46
		12	32.032	184186	1.36
		13	35.360	147942	1.09
		Totals		**13514166**	**100.00**

Contd..

Species	Accession number	Pk #	Retention time	Area	Area (%)
C. cajan	**ICPL 332**	1	2.059	9252	0.08
		2	2.656	79139	0.68
		3	12.203	53949	0.47
		4	14.517	159748	1.38
		5	15.488	328129	2.83
		6	16.299	3554445	30.71
		7	17.803	199700	1.73
		8	18.336	80329	0.69
		9	20.288	149258	1.29
		10	21.195	245884	2.12
		11	22.347	520429	4.50
		12	23.552	322129	2.78
		13	24.107	368525	3.18
		14	25.408	1768661	15.28
		15	27.040	2356535	20.36
		16	28.384	1004690	8.68
		17	30.400	170555	1.47
		18	35.253	203903	1.76
		Totals		**11575260**	**100.00**

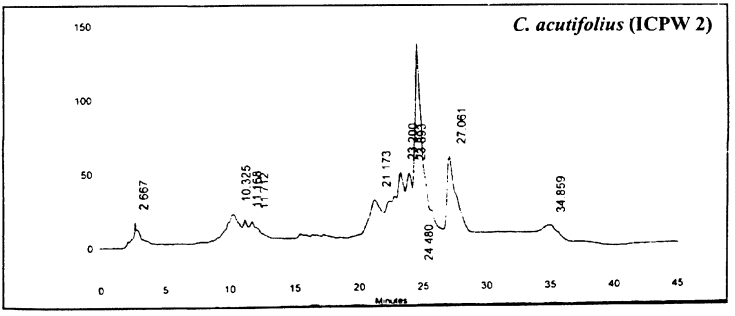


Fig – 27 : HPLC finger prints of hexane extract of pod surface of wild relatives of pigeonpea.

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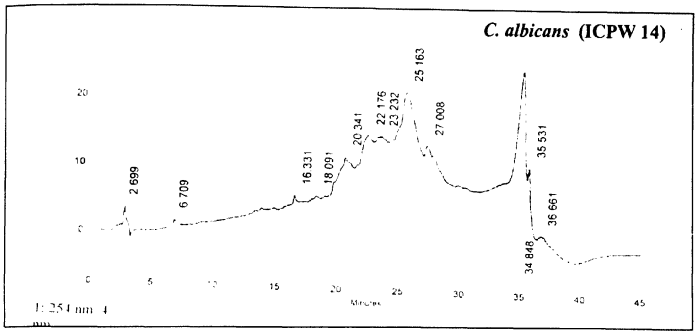
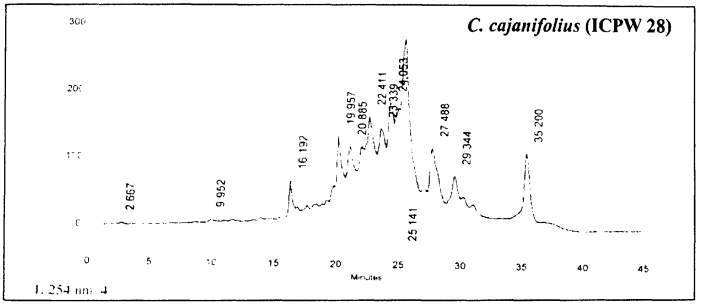
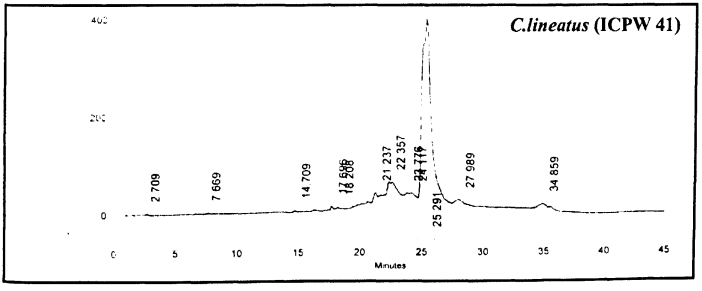
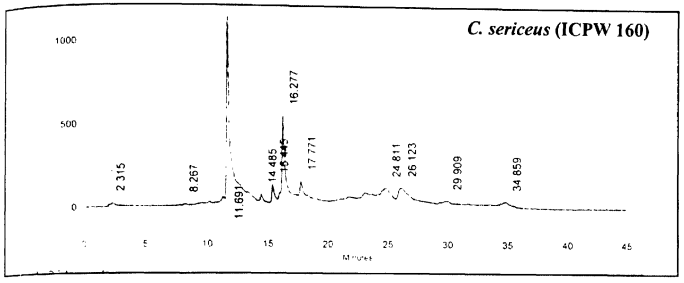
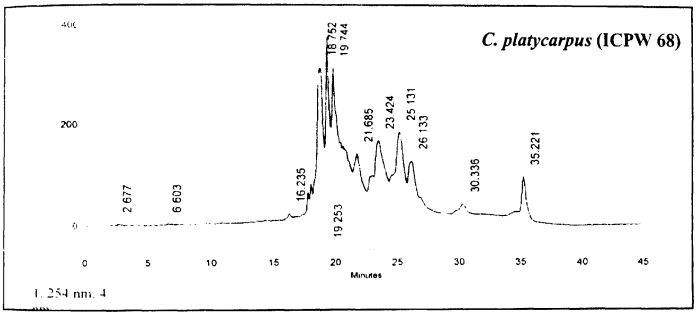

C. albicans (ICPW 14)
2 699
6 709
16 331
18 091
20 341
22 176
23 232
25 163
27 008
35 531
36 661
34 848
Minutes

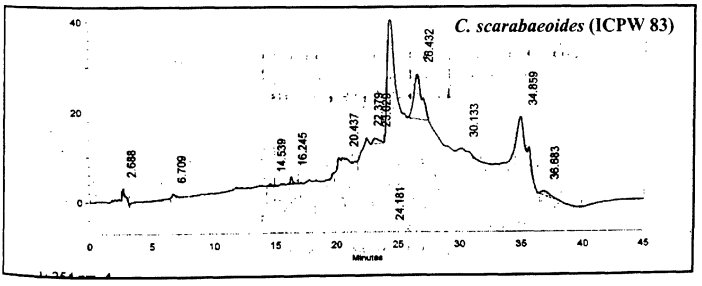

C. cajanifolius (ICPW 28)
2 667
9 952
16 197
19 957
20 885
22 411
23 339
24 953
25 141
27 488
29 344
35 200
Minutes


C.lineatus (ICPW 41)
2 709
7 669
14 709
21 237
22 357
25 291
27 989
34 659
Minutes

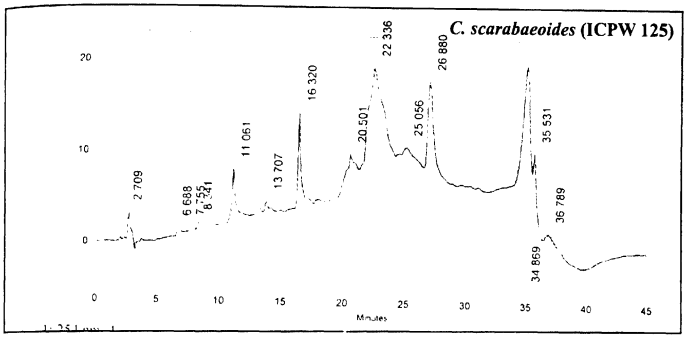
Contd..

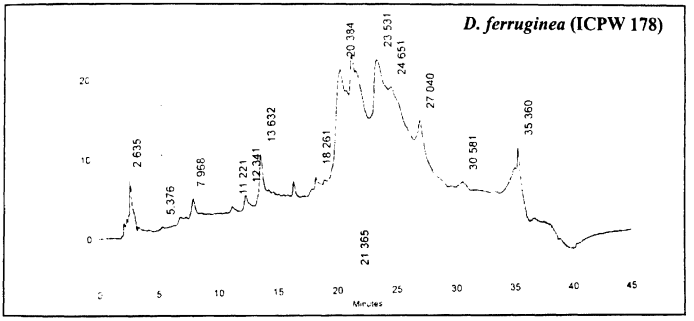

C. sericeus (ICPW 160)
1000
500
0
2.315
8.267
11.691
14.485
16.277
17.771
24.811
26.123
29.909
34.859
0
5
10
15
20
25
30
35
40
45
Minutes

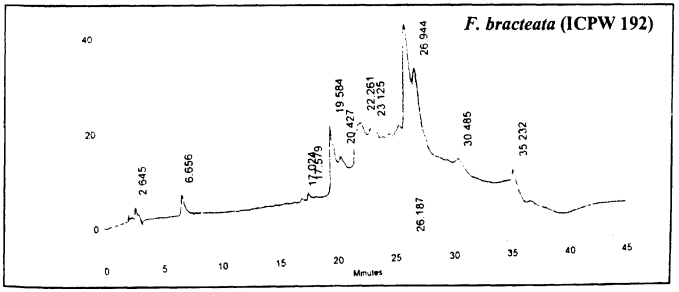

C. platycarpus (ICPW 68)
400
200
0
2.677
6.603
16.235
18.752
19.744
19.253
21.685
23.424
25.131
26.133
30.336
35.221
0
5
10
15
20
25
30
35
40
45
Minutes


C. scarabaeoides (ICPW 83)
40
20
0
2.688
6.709
14.539
16.245
20.437
22.379
23.869
24.181
28.432
30.133
34.859
36.883
0
5
10
15
20
25
30
35
40
45
Minutes

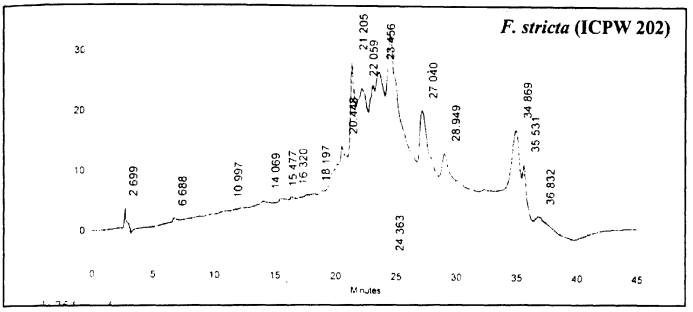
Contd..

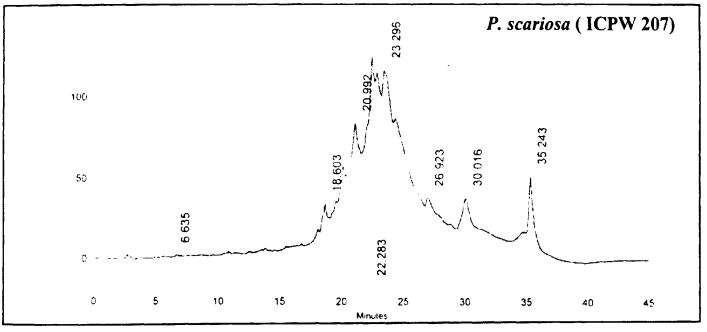

C. scarabaeoides (ICPW 125)
2 709
6 688
7 155
8 341
11 061
13 707
16 320
20 501
22 336
25 056
26 880
34 869
35 531
36 789
Minutes

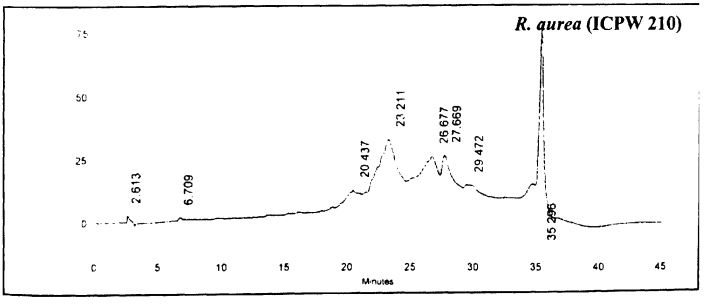

D. ferruginea (ICPW 178)
2 635
5 376
7 968
11 221
12 341
13 632
18 261
20 394
21 365
23 531
24 651
27 040
30 581
35 360
Minutes


F. bracteata (ICPW 192)
2 645
6 656
17 029
17 579
19 584
20 427
22 261
23 125
26 187
26 944
30 485
35 232
Minutes

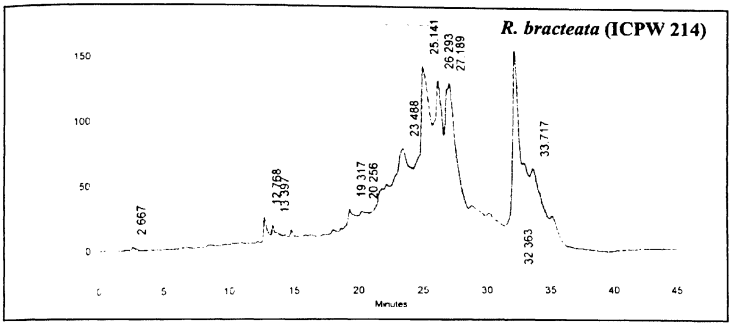
Contd..

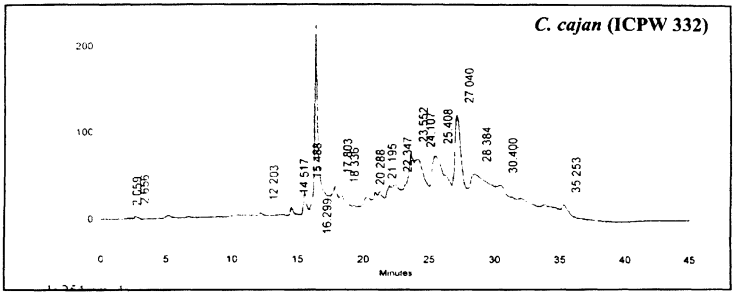

F. stricta (ICPW 202)
2 699
6 688
10 997
14 069
15 477
16 320
18 197
20 448
21 205
22 059
23 456
24 363
27 040
28 949
34 869
35 531
36 832
Minutes

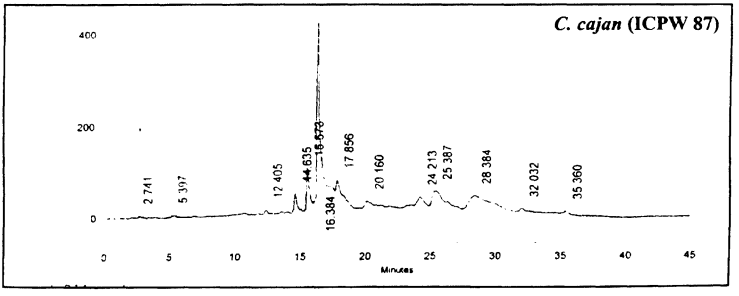

P. scariosa (ICPW 207)
6 635
18 603
20 992
23 296
22 283
26 923
30 016
35 243
Minutes


R. aurea (ICPW 210)
2 613
6 709
20 437
21 211
26 677
27 669
29 472
35 295
Minutes

Contd..


R. bracteata (ICPW 214)
150
100
50
0
2 667
12 768
13 397
19 317
20 256
23 488
25 141
26 293
27 189
32 363
33 717
0
5
10
15
20
25
30
35
40
45
Minutes


C. cajan (ICPW 332)
200
100
0
2 659
2 855
12 203
14 517
15 488
16 299
17 803
18 336
20 288
21 195
22 347
24 102
24 552
25 408
27 040
28 384
30 400
35 253
0
5
10
15
20
25
30
35
40
45
Minutes


C. cajan (ICPW 87)
400
200
0
2 741
5 397
12 405
14 635
15 673
16 384
17 856
20 160
24 213
25 387
28 384
32 032
35 360
0
5
10
15
20
25
30
35
40
45
Minutes

wild relatives except, ICPW 28, ICPW 41,ICPW 160, and ICPW 210. The peaks at rt 6.6 , 7.7, 8.2, 9.9 , 10.9, 11.6, 13.6, , 19.3, 26.1,29.3, 32.03, 33.71, 34.85 and 36.6 were observed only in wild relatives of pigeonpea.The peak at rt 2.05, was observed only in ICPL 332.

The compound at rt 20.3 was present in significant amounts in ICPW 2 (10.21), ICPW 160 (5.25%), ICPW 83 (5.72%), ICPW 178 (5.88%), ICPW 207 (19.28%), and ICPW 210 (20.77%). The peak at rt 22.1 was observed in ICPW 2 (5.82%), ICPW 14 (5.43%), ICPW 41 (5.87%), ICPW 83 (34.62%), ICPW 202 (15.99%), and ICPW 210 (23.64%). The peak at rt 25.1 was important in ICPW 2 (33.06%), ICPW 14 (33.66%), ICPW 41 (88.53%), ICPW 68 (15.81%), ICPW 192 (71.58%), ICPW 214 (16.93%), ICPL 332 (15.28%), and ICPL 87 (8.87%). The compound at rt 34.84 was present in ICPW 2 (35.13%), ICPW 160 (12.92%), ICPW 83 (16.95%), and ICPW 178 (14.09%). The peak at rt 11.69 was important in ICPW 28 (54.82%) and ICPW 83 (5.86). Compounds at rt 15.53 (8.81%) and 28.38 (10.46%) present in ICPL 87, were not observed in the wild accessions. The peak at rt 16.38 (51.45%) was significant only in ICPW 28 (19.87%), ICPW 83(7.37%), and in ICPL 332 (30.71%). Most of the major peaks found in the wild accessions were absent in the cultivated pigeonpea varieties ICPL 87 and ICPL 332. The cultivated checks, ICPL 87 and ICPL 332, had similar peak patterns.

Discussion

DISCUSSION

Helicoverpa armigera is the most damaging pest of pigeonpea and chickpea, the two of the most important legume crops for resource poor farmers in South Asia. It has become difficult to control this pest because of development of resistance to conventional insecticides (Armes *et al.*, 1992). Interaction between a plant and an insect is highly complex, and requires a deeper understanding of insect behavior. Wild relatives of pigeonpea are highly resistant to *H. armigera* (Sharma *et al.*, 2001). Identification of factors associated with resistance will be useful in developing varieties with increasing levels of stable resistance to the pest and diversifying the basis of resistance to this pest.

Screening for resistance to *H. armigera*

Plant resistance to insects is the result of interaction between the physico-chemical characteristics of the host plant and the insect. Physico-chemical characteristics of the host plant help the insects in colonization of the plants or deter or harm them. Plant traits also influence the activity and abundance of the natural enemies of insect herbivores. Physical plant characters that influence the effectiveness of insect natural enemies include non-glandular trichomes, surface waxes, size and shape of plant organs and protective structures. Plant shape and size provide a physical refuge to the prey or interferes with foraging activities of a natural enemy. The impact of predators and parasitoids on *H. armigera* is relatively low in pigeonpea as their activity is hindered by trichomes and their exudates on pigeonpea leaves, buds, and pods (Shanower *et al.*, 1999; Romeis *et al.*, 1999).

The main objective of this research was to test the hypotheses as to how physico-chemical characteristics of the host plant influence the food selection behaviour of *H. armigera*. Wild relatives of pigeonpea are the useful sources of resistance to *H. armigera* (Shanower *et al.*, 1997; Romeis *et al.*, 1999). Evaluation of 29 accessions of wild relatives of pigeonpea along with two cultivated varieties (resistant and susceptible checks), showed significant variation in their resistance to *H. armigera*. Oviposition non

preference was an important component of resistance to *H. armigera* in ICPW 137, ICPW 152, ICPW 94, and ICPW 130 of *C. scarabaeoides*. The larval numbers were lower in ICPW 94, ICPW 137 and ICPW 152 (0.00) of *C. scarabaeoides*, followed by ICPW 210 (0.30) of *R. aurea*, and ICPW 68 (0.87) of *C. platycarpus* as compared to ICPL 87 (8.40) of *C. cajan*. Damage by the *H. armigera* in the tagged inflorescences of early-duration wild relatives of pigeonpea ranged from 0.0% in *C. scarabaeoides* (ICPW 137) to 4.12% in *C. platycarpus* (ICPW 68) compared to 83.83% damage in the pods of susceptible ICPL 87 of *C. cajan* (Table 12). In the medium-duration accessions, egg laying was quite high on ICPW 28, and the total pods in the tagged inflorescences were low compared to ICPL 87, suggesting that *C cajanifolius* was as susceptible to pod borer damage as *C. cajan*, while the accessions belonging to C. *scarabaeoides* and *C. sericeus* were highly resistant (Table 14). Oviposition was high on *R. bracteata* (ICPW 214) and low on *C. acutifolius* (ICPW 1). The number of larvae were also low on *C. acutifolius* (ICPW 2 and ICPW 1), *C. albicans* (ICPW 14). Pod damage was also low in *C. albicans* (Table 16). Similar trends in egg laying and pod borer damage have earlier been reported by Sharma (2001). There was considerable variation in the percentage of healthy pods in *C. scarabaeoides* accessions, suggesting that it is important to evaluate the available accessions for resistance to insect pests before selecting a particular species for use in breeding for resistance to *H. armigera*. Accessions belonging to *R. aurea, C. scarabaeoides, C. sericeus,* and *C. acutifolius*, and *F. bracteata* showed high levels of resistance to *H. armigera,* while the accessions belonging to *C. cajanifolius* were as susceptible to *H. armigera* as the susceptible pigeonpea genotype, ICPL 87. Among the cultivated pigeonpea genotypes, ICPL 332 (the resistant check) was consistently less damaged than ICPL 87 (Table 14). The interactions of insects with the crop plants are quite complex, and it is important to evaluate a range of accessions for resistance to the target insects before considering their utilization as sources of resistance in a crop improvement program.

Factors associated with resistance to *Helicoverpa armigera*

Trichomes

The most common morphological trait in pigeonpea and its wild relatives is the presence of trichomes (Peter *et al.,* 1995). Trichomes are associated with resistance to insect herbivores such as leafhoppers and lepidopteron insects. Glandular trichomes act as a resistance mechanism owing to the compounds exuded by them (Ranger and However, 2001; Frelichowski and Juvik, 2001), and the trichome density (Valverde 2001, Gurr and MacGrath, 2001). However, this theory is not always true. Chu *et al.,* (2000) showed that white fly infestation is high in cotton genotypes having a high density of trichomes. The types of trichomes, their orientation, density, and length have been correlated with reduced insect damage in several crops (Jeffer, 1986; David and Easwaramoorthy, 1988; Peter *et al.,* 1995). In order to study the role of morphological differences in trichomes in resistance to *H. armigera,* scanning electron micrographs of all the genotypes were taken. Four types of trichomes viz; type A, type B, type C, and type D were identified on the pod surfaces of pigeonpea and its wild relatives (Fig 9). The trichomes showed significant differences in their density on different genotypes (Fig 10). Genotypic differences and environmental factors affect the growth and development of trichomes (Southwood, 1986). Variation in the form and function of trichomes, within the same species, are frequently associated with plant resistance to insect attack (Southwood, 1986)

Helicoverpa armigera lays more than 80% of its eggs on pods and calyxes (Romeis, 1997), and hence the distribution and density of trichomes on these structures is quite important. The density of trichomes differed significantly among calyxes and pods of pigeonpea and its wild relatives. On calyxes, the trichomes; type A, type B, type C, and type D varied significantly in density and distribution among the species. However, the variation within a species was not large. The density of type A trichomes was very low in accessions of *C. scarabaeoides, C. albicans,* and *R. aurea.* There was no significant variation in density of type A trichomes in *C. acutifolius, C. cajanifolius,*

C. lineatus, and on cultivated pigeonpea variety ICPL 87 (Table 8). Type A, type B, type C and type D trichomes were observed on the pods of all the wild relatives of pigeonpea except the type A trichomes in *C. sericeus* and *C. scarabaeooides*. Trichomes were present in greater density towards the edges than in the middle areas of pods. Similar observations have been made by Romeis and Shanower (1996). Density of type A trichomes was high on the pods of *R. bracteata* and *C. platycarpus* (Table 9). A significant positive correlation was observed between the number of eggs laid and the density of type A trichomes on calyxes. Hartleib and Rembold, (1996) suggested that glandular trichomes act as attractants to the adult moths.

The number of type B trichomes on calyxes was significantly lower compared to other types of trichomes in all the wild relatives of pigeonpea, and these trichomes were completely absent in calyxes of *C. scarabaeoides*, except in ICPW 152 (Table 8). In pods, there were significant differences in density of type B trichomes within the species. Significantly high numbers of type B trichomes were observed on the pods of *C. lineatus, C. albicans,* and *C. cajanifolius* as compared to those on the cultivated ICPL 87 (Table 9). The function of type B trichomes is not well known. However, Bisen and Sheldrake (1981) suggested that they are a source of characteristic fragrance in pigeonpea. The fragnance in pods of *C. lineatus* might be due to the presence of high number of type B trichomes. The secretions in the type B trichomes are liberated only when the cell wall is ruptured. This could be caused by a chewing by the insects, such as *H. armigera* or by abiotic factors such as high temperatures or low air humidity (Ascensao *et al.,* 1985). Bisen and Sheldrake (1981) considered, type E trichome to be a developmental stage of type B.

The density of nonglandular trichomes; type C and type D was quite high on calyxes and pods of the wild relatives of pigeonpea (Tables 8 & 9). The nonglandular type C trichomes were higher on the pods of *C. scarabaeoides* than on other species (Table 9). High larval mortality on these accessions might be due to the pubescence of type C trichomes on the pods. Trichome density has a negative impact on larval growth and suvival (Valverde *et al.*, 2001; Gurr and Mac Grath, 2001; John Peter, 1995).

Exudates from glandular trichomes in pigeonpea contain factors that act as phago stimulants towards the *H. armigera* larvae (Green *et al.,* 2002 and 2003*).* The non-glandular trichomes, which are present at much higher densities on wild relatives of pigeonpea than on the cultivated pigeonpea, might act as a physical barrier to feeding by the *H. armigera* larvae. Comparisons made among the four types of trichomes on pigeonpea genotypes and its wild relatives have shown their role as morphological traits associated with resistance to *H. armigera.*

Antexenosis mechanism of resistance to *H.armigera*

Resistance in wild relatives of pigeonpea is primarily due to antixenosis, expressed as oviposition non-preference by the *H. armigera* females. Antixenosis, which focuses on non-preference by the ovipositing female, has the potential to reduce the selection pressure for evaluation of new biotypes. The no-choice, dual-choice and multi-choice cage tests conducted to quantify the antixenosis mechanism of resistance to *H. armigera* revealed significant differences in number of eggs laid on different species and within the accessions of same species (Tables 17,18 & 19). Female moths preferred to lay eggs on reproductive structures (flowers and pods) as compared to vegetative parts (leaves). Similar observations were reported by Romeis (1997).

Under no-choice conditions, there was considerable variation in oviposition preference of the female moths on different accessions of the same species. *Cajanus albicans* (ICPW 13) and *C. scarabaeoides* (ICPW 90, ICPW 94 ICPW 116, and ICPW 137) were non-preferred for oviposition (<100 eggs per female) compared to the cultivated pigeonpea (334 eggs per female) (Table 17 & Fig 11). Presence of high density of nonglandular trichomes might be one of the reasons for their non-preference. The accessions, *C. acutifolius* (ICPW 2), *C. cajanifolius* (ICPW 28 and ICPW 29), *C. lineatus* (ICPW 40), *D. ferruginea* (ICPW 178), and *F. bracteata* (ICPW 192) with high density of glandular trichomes, were preferred as a substrate for oviposition (236 to 425 eggs per female) (Table 17). Female moths laid more eggs on accessions with glandular trichomes

as compared to the accessions with nonglandular trichomes. Under dual-choice conditions, the moths preferred to lay eggs on the cultivated pigeonpea compared to the wild species (Table 18 & Fig 12). Under multi-choice conditions, the moths preferred to oviposit on ICPW 13, ICPW 14, ICPW 159, ICPW 90, ICPW 125, ICPW 137, ICPW 178, and ICPW 207 (Tables 19 & Fig 13). Similar results were recorded under no-choice conditions.

Antibiosis

Growth and development of *H. armigera* larvae on leaves, flowers and pods

The antibiosis mechanism of resistance to *H. armigera* was measured in terms of reduced body weights, mortality, and prolongation of larval period. Antibiosis to *H. armigera* varied significantly among the wild relatives of pigeonpea. The results showed significant variation in development and survival of *H. armigera* larvae reared on leaves, flowers, and pods of different species of wild relatives of pigeonpea. Lower larval weights and longer developmental periods were observed in larvae reared on leaves compared to those reared on flowers and pods of wild relatives of pigeonpea (Tables 20 – 25). Similar results have earlier been reported by Sison and Shanower (1994). Srivastava and Srivastava (1990) reported that the pupae of larvae reared on chickpea pods were heavier and developed more quickly than the larvae reared on chickpea leaves. This variation might be due to physical (Peter and Shanower, 1996) and nutritive differences in plant parts (Shanower *et al.*, 1997).

In the present studies, the laboratory assays indicated that there was a gradual increase in mortality of *H.armigera* larvae fed on the leaves of wild relatives of pigeonpea. The larvae of *H. armigera* suffered upto 76% mortality when reared on the leaves of wild relatives of pigeonpea compared to 50% mortality on the pigeonpea variety, ICPL 87 (Table 21). Thus antibiosis is an important mechanism of resistance against *H. armigera* in wild relatives of pigeonpea. The mean developmental time for *H. armigera* larvae grown on the wild relatives of pigeonpea was relatively longer compared to the larvae

reared on the cultivated pigeonpeas. Prolonged larval duration also indicates antibiosis as a component of ressitance in wild relatives of pigeonpea. Mortality of early instars and prolonged development are good indicators of antibiotic mechanisms of resistance against insect pests (Painter, 1951; Dahms, 1972; Slansky, 1982). The larval mortality was high on some of the wild relatives of pigeonpea (Table 21), and very few larvae survived to the pupal or adult stages (Fig 14). Dodia *et al.*, (1996) observed adverse affects on the development of larvae reared on the wild relatives of pigeonpea and their F_1s as compared to the larvae reared on the cultivated pigeonpea. The mortality on pods may also be due to biophysical factors such as seed coat thickness and /or toughness, and presence of pod surface chemicals, which act as antifeedants.

Differences in the nutritional quality of different plant parts may also account for the variations observed in the growth and survival of *H. armigera* larvae. A significant and positive correlation was observed between the larval and pupal weights in the larvae reared on leaves, flowers, and pods, while a significant and negative correlation was observed between the larval weights and the larval developmental periods in larvae reared on the leaves and pods (Table 26).

Growth and development of *H. armigera* larvae on artificial diet impregnated with lyophilized leaves and pod powders.

Antibiosis mechanism of resistance in wild relatives of pigeonpea was also confirmed by rearing the larvae on artificial diet impregnated with different amounts of lyophilized leaf and pod powders. Singh and Rembold (1988) reported differential survival rates and the developmental periods of *H. armigera* larvae on diets containing powdered seed materials of chickpea, soyabean, or maize. Proportionate increase in inhibition of larval growth and mortality was observed with an increase in concentrations of lyophilized leaf and pod powders of wild relatives of pigeonpea in the artificial diet (Tables 27 & 30).

Larval and pupal weights, and larval survival rates were greater in larvae reared on diets containing lyophilized leaf and pod powders (Tables 28 & 31) compared to the larvae reared on the intact leaves, flowers, and pods (Tables 20,21,23 & 24). This may be due to the availability of more nutrients in the artificial diet. Larval growth was slower on diets containing the lyophilized leaf and pod powders compared to the standard artificial diet (Tables 28 & 31). Similar observations were made by Yoshida and Shanower (2000), who indicated that the presence of growth inhibitors in the leaf and pod powder might result in the reduced larval survival and slow growth of the larvae.

There were significant differences in larval developmental period, larval weight, and mortality of the larvae reared on diets with lyophilized leaf and pod powders of wild relatives of pigeonpea as compared to the larvae reared on diets with leaf and pod powders of cultivated pigeonpeas (Tables 28,29,31 & 32). Yoshida and Shanower, (2000) reported slow growth rates of *H. armigera* on artificial diets containing *C. scarabaeoides* pod powder than on the diets containing *C. cajan* pod powder. These differences may be due to the presence of antifeedant or growth inhibiting compounds in the wild relatives of pigeonpea. The levels of resistance to *H. armigera* observed in the artificial diets impregnated with lyophilized leaves or pods were slightly different than those observed on the intact plant parts (Figs 14, 15, 18 & 22). Physical factors such as trichomes and pod wall toughness might be some of the factors contributing to host plant resistance to *H. armigera* in intact leaves and pods.

Relative feeding preference by the third-instar larvae of *H. armigera* on leaves and pods of wild relatives pigeonpea under no-choice and multi-choice conditions

The relative feeding preference of *H. armigera* larvae towards different plant parts (leaves and pods) and towards the pod surface chemicals was studied using bioassays under laboratory conditions. There were significant differences in leaf and pod damage among wild relatives of pigeonpea.

Under no-choice conditions, the differences in larval feeding preference were not apparent among the wild relatives of pigeonpea (Table 34). Similar results were observed under multi-choice conditions as well, where the larvae preferred to feed on the leaves of the cultivated pigeonpea as compared to those of the wild relatives (Tables 36 – 40). The biochemical composition of the leaves might be responsible for their acceptance or rejection as food by the *H. armigera* larvae.

Under no-choice conditions, the third-instar larvae of *H. armigera* showed less feeding preference towards the wild relatives of pigeonpea, where the percentage damage was low compared to that on the pods of cultivated pigeonpea variety, ICPL 87 (Table 35). In pod-choice experiments, the larvae of *H. armigera* are able to distinguish between different species of *Cajanus*. The larvae preferred to feed on the pods of ICPL 87 as compared to those of its wild relatives (Tables 41- 45). Shanower *et al.*, (1997) observed that *H. armigera* larvae spent more time feeding on pods of *C. cajan* than on *C. scarabaeoides*. Sharma *et al.*, (2001) and Green *et al.*, (2002b, 2003) reported that several chemicals occur on the pod surface of cultivated pigeonpea, which were absent in the pods of wild relatives. The presence of dense non-glandular trichomes might be one of the reasons for preference of pigeonpea as food by the *H. armigera* larvae. Sharma *et al.*, (2001) reported that first and second-instars preferred pods of ICPL 87 to both ICPW 83 with trichomes and ICPW 83 from which the trichomes had been removed. However, more larvae were observed on ICPW 83 pods without trichomes than on the intact ICPW 83 pods.

Role of pod surface chemicals on feeding by the third-instar larvae of *H. armigera*

The effects of chemicals present on the pod-surface on the food preference by the *H. armigera* larvae was studied by presenting the larvae with a choice between pods that had been surface-extracted in water, hexane, or methanol and un-extracted pods. Under no-choice conditions, pods from the wild relatives were less preferred by the *H. armigera*

larvae compared to the pods of cultivated pigeonpea varieties, ICPL 87and ICPL 332 when the pods were washed with water (Table 46). When the pods were washed with hexane, the larvae preferred to feed on the pods of *C. acutifolius, C. albicans* and *C. cajanifolius* (ICPW 28) as compared to the pods of *C. cajan* (Table 48). In the methanol washed pods, the larvae preferred the pods of ICPW 1 (*C. acutifolius*), ICPW 13 (*C. albicans*), and ICPW 28 (*C. cajanifolius*) as compared to the pods of cultivated pigeonpea (Table 47). When the larvae were provided with a choice to choose between the unwashed pods and the hexane washed pods, the larvae preferred to feed on the hexane washed pods indicating that hexane must have removed some of the antifeedant compounds from the pod surface (Tables 51 & 52). Once these compounds are removed through the extraction, the larvae preferred to feed on the pods of wild relatives of pigeonpea. Similar results were reported by Green *et al.,* (2002 a). When the larvae were provided with a choice between the methanol-washed and unwashed pods, the larvae preferred to feed on the unwashed pods compared to the methanol washed pods of the same accession indicating that the phagostimulant compounds were extracted into the methanol (Tables 53 & 54). These compounds may be responsible for preference of pods as food by the *H. armigera* larvae in cultivated pigeonpea.

Feeding preference by different instars of *H. armigera* towards a pod surface extract treated and un treated glass fiber discs

The effect of pod surface chemicals of wild relatives of pigeonpea on feeding preference of *H. armigera* larvae was evaluated under laboratory conditions by glass fiber disc bioassay method. The feeding preferences of third, fourth, and fifth–instar larvae were similar towards the glass fiber discs treated with pod surface extracts (Tables 55 & 56; Fig 24). Among the two solvents used (methanol and hexane), the larvae preferred to feed on the methanol extract treated glass fiber discs. Methanol extract of ICPL 87 stimulated feeding by the third, fourth, and fifth-instar larvae of *H. armigera*. The disc area consumed by the fifth–instars was more than the fourth and third-instars in both the solvents (Tables 55 & 56). This may be due to changes in the nutritional

requirements between the instars. Older larvae have increased appetite (Raubenheimer and Barton-Browne, 2000), and need more protein (Simpson *et al.,* 1988). In a dual-choice bioassay, the data showed that the larvae of *H. armigera* were able to perceive the methanol extract of the pod surfaces, as they consumed more of the glass fiber discs impregnated with methanol extract than the control discs (Table 57). The preference of larvae towards methanol extract treated discs might be due to the presence of phago-stimulants in the methanol extract. The third, fourth, and fifth-instar larvae of *H. armigera* preferred more to feed on the methanol extract of the pod surfaces of C. *cajan* (ICPL 87) as compared to that of *C. scarabaeoides* (ICPW 83) (Table 55). The differences in pod surface might be one of the reasons for differential response to pod surface extracts of different species. Sharma *et al.*, (2001), Green *et al.*, (2002b) reported similar observations. Larvae preferred to consume control discs than the discs treated with hexane extract which suggest that hexane extracts had some anti-feedant compounds. The amounts of phago-stimulants and anti-feedants on the pod surface play an important role in food selection by the larvae of *H. armigeral.*

A complete understanding of the nature and number of compounds present on the pod surface of wild relatives of pigeonpea would facilitate the selection of wild relatives of pigeonpea with different mechanisms of resistance to *H. armigera.* Although, methanol extracts stimulated the feeding by *H. armigera* larvae, it may also contain phenolics that deter feeding, or compounds that have no effect on the food selection behavior of *H. armigera* larvae. Hence, further studies are necessary to isolate the compounds and study their effect on food selection by *H. armigera* larvae.

Biochemical basis of resistance to *H. armigera*

The biochemical constituents present in the cells and tissues of the host have been reported to exert profound influence on biology of insect pests in various ways (Painter 1951, 1958; Beck 1965). However, the biochemical nature of antibiosis mechanism of resistance in wild relatives of pigeonpea towards the larvae of *H. armigera* is not fully

understood. Therefore, one of the major aspects of the present study was to estimate the amounts of sugars, tannins, phenols, and proteins, and their association with host plant resistance to *H. armigera*.

Sugars

There were marked differences in the amounts of soluble sugars among the wild relatives of pigeonpea. The amounts of total sugars were high in the cultivated pigeonpeas compared to that in the wild relatives (Table 58). Macfoy *et al.,*(1983) recorded high concentrations of sugars and amino acids in the susceptible cowpea cultivar Vita-1 to *Maruca testulais*. The results obtained in the present study are also in agreement with the above findings. Sharma *et al.*, (1993) reported slower larval development on the midge- resistant sorghum cultivars with lower amounts of sugars.

Polyphenol

Low amounts of polyphenols in the cultivated pigeonpea pods might be the one of the reasons for their high susceptibility to *H. armigera*. Low amount of phenols in pigeonpea flowers favored more damage by *M. testulais* (Ganapathi, 1996). High amounts of polyphenols were recorded in resistant and late-maturing wild relatives of pigeonpea as compared to the cultivated pigeonpeas (Table 59). Mukerji *et al., (*1993); Sahoo and Patnaik (2003), reported similar observations in pigeonpea.

Tannins

Tannins in plants have been considered as insect growth inhibitors for several years, owing to their presumed binding to the proteins to form insoluble digestion-inhibiting complexes (Smith 1989). However Martin *et al.,* (1987) indicated that there is little evidence to suggest that tannins inhibit insect digestion. The observed effects of tannins appear more likely to be due to their action as feeding deterrents. A correlation between tannin content of grain and midge resistance in sorghum has been suggested by Santos and Carom (1974) and Sharma *et al.*,(1990a). In the present studies, considerable variation was recorded in the tannin content in the leaves and pods of wild relatives of

pigeonpea The accessions of *C acutifolius C albicans C sericeus F stricta,* and *P scariosa* had high amounts of tannins in their pods compared to that in the cultivated pigeonpea (Table 60)

Proteins

The protein content of commonly grown pigeonpea cultivars ranges between 17 9 to 24 3 g/100 g for whole grain, and between 21 1 to 28 1 g/100 g for split seed (Salunkhe et al 1986) In the present study, the percentage of soluble proteins were significantly high in the pods of *C scarabaeoides* compared to those of ICPL 87 The accessions of *C acutifolius, C cajanifolius C scarabaeoides, F stricta,* and *R bracteata* also had high amounts of soluble protein in pods compared to that of the pigeonpea (Table 61) Wild species of pigeonpea have been found to be a promising source of high-protein, and several high-protein genotypes with a protein content as high as 32 5% have been developed (Singh *et al* 1990)

The present studies indicated that high levels of resistance to *H armigera* in wild relatives of pigeonpea might be due to lower amounts of sugars and high amounts of tannins, polyphenols, and proteins However, further studies are necessary to understand the role of sugars, tannins, polyphenols, and proteins in host plant resistance to *H armigera*

HPLC profiles of Flavonoids

Flavonoids and isoflavonoids are known to confer resistance against insect attack in several plant species (Hedin and Waage, 1986, Grayer *et al*, 1992) Flavonoids in soybean contribute to genotypic resistance against plant pathogens (Keen *et al*, 1972, Keen and Paxton, 1975, Ingham *et al*, 1981, Ebel, 1986) and insects (Chiang *et al*, 1986, Khan *et al*, 1986, Sharma and Norris, 1990) There were substantial chemical differences between the accessions of wild relatives of pigeonpea (table 62) Similar observations have been made by Green *et al*, (2001) High performance liquid chromatography data showed that there were qualitative and quantitative differences in

the compounds present on the pod surfaces of different accessions of wild relatives of pigeonpea (Tables 63 & 64; Figs 25 & 26). The total number of peaks observed in the methanol solvent extracts was more compared to the number of peaks in the hexane extract in all the accessions, except in ICPW2, ICPW 160, ICPW 83, ICPW 178, ICPW 192, and ICPW 207 (Figs 25 & 26). These differences in the pod surface chemicals might influence the host selection behavior of *H. armigera* larvae. It would be necessary to compare the biological activity of different compounds towards *H. armigera* to confirm if quantitative differences in pod surface compounds affect the larval feeding on different wild relatives of pigeonpea.

Summary

SUMMARY

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is an important pulse crop of the semi-arid tropics being cultivated in India, Kenya, Tanzania, Uganda, and Malawi in Eastern Africa, and Dominican Republic and Puerto Rico in Central America. India accounts for 85 to 90% of the world's area under pigeonpea cultivation. It is a multipurpose crop, with major source of proteins. The yield potential of pigeonpea is 2.5 to 3.0 t ha^{-1}. The productivity of cultivated pigeonpea continues to be constrained by various biotic and abiotic stresses. Insects are the most important biotic constraint to pigeonpea production worldwide, causing losses of more than US $ 1000 million every year. More than 200 species of insects feed on pigeonpea, of which *Helicoverpa armigera, Maruca vitrata, Melanagromyza obtusa, Clavigralla* spp., *Nezara viridula* and *Callosobruchus* spp. are the most important (Lateef and Reed, 1990). Of these, legume podborer, *Helicoverpa armigera*, is the most destructive and notorious pest of the field crops (Lateef and Reed, 1990). Losses due to this pest in pigeonpea have been estimated as US$ 317 million and possibly over US$ 2 billion on different crops worldwide annually (Sharma, 2001). Traditional control measures generally rely on chemical insecticides, which may have a negative impact on the environment and also cause the insecticidal resistance to the pest. An estimate of over US$ 1 billion is spent on insecticides to control this pest. Currently, it is the most difficult species to control because of emergence of resistance to most of the commercially available insecticides. Biological methods of insect pest control will help sustain the environment and reduce input costs

To overcome these losses, farmers resort to excessive use of pesticides. Continuous use of insecticides and chemicals has led to the insecticide resistance in this pest, which resulted in several crop failures. Therefore, host plant resistance is the preferred alternative in the management of this pest. Understanding the mechanisms of resistance and identification of resistance sources and traits are some of the important steps involved in all the host plant resistance programs. Plants exhibit enormous variation in the level of resistance to insects. Plants exhibit resistance to insect pests through two

mechanisms. The first is often referred to as non-preference resistance. The plant has characteristics that impair the insect's ability to use the host plant for egg laying, food or shelter. The characteristics of the host plant can be either chemical (the plant contains a noxious compound that repels the insect) or physical (the plant leaf has long hairs, the trichomes, that prevent egg laying or feeding). The second type of resistance is termed *antibiosis*. With this type of resistance, the insect's metabolic processes are affected as a result of feeding on a resistant plant. Insects feeding on plants with this type of resistance may experience reduced growth rates, smaller adults with reduced numbers of eggs, a shortened lifespan, physical deformities, or even death.

Wild species of *Cajanus* have been identified as potentially valuable source of germplasm for improving the levels of resistance in pigeonpea against insect pests (Pundir and Singh, 1987; Sharma *et al.*, 2001). High levels of resistance are available in the wild relatives of pigeonpea such as *Cajanus scarabaeoides, C. sericeus* and *C. acutifolius*, which can be used as sources of resistance in the breeding programme for the development of cultivars with resistance to *H. armigera* (Sharma *et al.*, 2001).

With this in view, the present investigation was undertaken to evaluate the wild relatives of pigeonpea for resistance to *H. armigera*, identification of physico-chemical factors associated with resistance to *H. armigera* and to characterize the sources of resistance for different resistance mechanisms such as oviposition non-preference, and antibiosis.

In the present investigation, 29 accessions of wild relatives of pigeonpea and two cultivated pigeonpea varieties were screened in the field under multi-choice conditions to evaluate their relative resistance/susceptibility to *H. armigera*. Distinct differences were observed in all the tested genotypes for days to flowering and maturity, leaf area, pod length and width, number of locules per pod, number of seeds per pod, and 100- seed weight. Oviposition non-preference was an important component of resistance to *H. armigera* in *C. scarabaeoides* accessions where the number of eggs laid by the insect were quite low or completely absent. The larval abundance was also low on the *C. scarabaeoides* accessions both in the short duration and medium duration varieties.

Damage by the *H. armigera* in the tagged inflorescences of early-duration wild relatives of pigeonpea ranged from 0.0% in *C. scarabaeoides* (ICPW 137) to 4.12% in *C. platycarpus* (ICPW 68) compared to 83.83% damage in the pods of ICPL 87 of *C. cajan,* the cultivated check. In the medium-duration accessions, egg laying was quite high on *C. cajanifolius* (ICPW 28), and the total number of pods in the tagged inflorescences were low compared to ICPL 87, suggesting that *C. cajanifolius* was as susceptible to pod borer damage as *C. cajan*, while the accessions belonging to C. *scarabaeoides* and *C. sericeus* were highly resistant. In long duration varieties oviposition was high on *R. bracteata* (ICPW 214) and low on *C. acutifolius* (ICPW 1). The number of larvae were low on *C. acutifolius* (ICPW 2 and ICPW 1), *C. albicans* (ICPW 14). Pod damage was also low in *C. albicans*. Variation in the percentage of healthy pods in *C. scarabaeoides* accessions, suggest that it is important to evaluate the available accessions for resistance to insect pests before selecting a particular species for use in breeding for resistance to *H. armigera*. Accessions belonging to *R. aurea, C. scarabaeoides, C. sericeus, C. acutifolius*, and *F. bracteata* showed high levels of resistance to *H. armigera,* while the accessions belonging to *C. cajanifolius* were as susceptible as the susceptible check, ICPL 87. Among the cultivated pigeonpea genotypes, ICPL 332 (the resistant check) was consistently less damaged than ICPL 87.

Trichomes are epidermal appendages of diverse form and structure present on the leaf, stem, flower (calyx) and pod surfaces of many plant types. The most common resistance mechanism conferred by the morphological structures is the presence of trichomes. To understand the morphological differences in trichomes and their density and distribution, the flowers and pods of wild relatives of pigeonpea were examined under a Zeiss Stereomicroscope (Carl Zeiss, Inc., Thornwood, NY) at a magnification of 32X with an ocular measuring grid and also scanned under Electron microscope. Four types of trichomes viz; type A, type B, type C, and type D were identified on the flower (calyx) and pod surfaces of pigeonpea and its wild relatives. Type A and type B were glandular trichomes whereas, type C and type D were non-glandular trichomes. The type A trichome had a long tubular neck with 4 to 8 cells, and an enlarged base with 6 to 10

cells. It secretes clear exudates visible as droplets at the top and along the shaft of the trichome. Type B trichome is a sac like structure containing yellow, oily substance. The secretions in the type B trichomes are liberated only when the cell wall is ruptured. Type C and D trichomes were unsegmented and nonglandular. The type C trichome was short and type D trichome was 4 to 11 times longer than type C trichome.

The trichomes showed significant differences in their density on different genotypes. The density of trichomes was significantly high on pods compared to the calyxes. Trichomes were present in greater density towards the edges than in the middle areas of pods. Type A, type B, type C and type D trichomes were observed on the calyxes and pods of all the wild relatives of pigeonpea (except the type B trichomes in calyxes of *C. scarabaeoides,* and type A trichomes in pods of *C. sericeus* and *C. scarabaeooides*). The density of type C trichomes was very high on the pods in all the accessions of *C. scarabaeoides*. Density of type D trichome was significantly higher on the pods of *C. sericeus*. A significant and positive correlation was observed between the number of eggs laid, larval abundance, pod damage and the density of type A trichomes on calyxes and pods, while there was a significant and negative correlation between the number of eggs laid, larval abundance, pod damage, and the density of type C and type D trichomes on calyx and pods. Type B trichomes showed no association with egg laying, larval abundance, and pod damage. This gives a clear indication that the secretions of type A trichomes are acting as insect attractants and type C and type D trichomes are acting as deterrents and contributing towards resistance against *H. armigera*. Therefore development of cultivars with nonglandular trichomes will be helpful in reducing the pest damage.

Under laboratory conditions, we evaluated the wild relatives of pigeonpea for their resistance to *H. armigera* by studying the antexenosis and antibiosis mechanisms of resistance. Antixenosis (non-preference) for oviposition was studied under no-choice, dual- choice and multi-choice conditions.

In the no-choice test, the moths were confined to inflorescences of the same species/genotype in a wooden cage (36 x 36 x 30 cm), whereas, in dual choice conditions the moths were offered a choice between the susceptible check, ICPL 87 and the test variety, while under multi-choice conditions, the inflorescences of all the 29 test varieties, along with the susceptible and resistant checks, were tested by keeping in a large cage (80 x 70 x 60 cm). A considerable variation was found in oviposition preference between the species and also within a species. All the accessions of *C. scarabaeoides* were least preferred for oviposition. It is observed that the accessions with high density of type C trichomes were less preferred for oviposition by *H. armiger* a female, and the accessions with high density of type A trichomes were highly preferred.

All the wild species were less attractive to egg-laying by *H. armigera* in the field and in a laboratory experiment thus coinferming the antixenosis mechanism of resistance in wild relatives of pigeonpea.

The antibiosis mechanism of resistance to *H. armigera* was measured in terms of reduced body weights, mortality, and prolongation of larval period by rearing larvae on the leaves, flowers and pods, and also on the artificial diet impregnated with lyophilized leaf and pod powders. Significant differences were observed in the larval and pupal weights in the insects reared on the leaves of wild relatives of pigeonpea. The larval and pupal weights on the wild species were significantly lower than those on the cultivated pigeonpeas. At same time higher larval mortality was observed on the wild relatives of pigeonpea compared to the cultivated pigeonpea. Lower pupation and adult emergence were recorded in the larvae reared on the leaves of *C. scarabaeoides* compared to the cultivated pigeonpeas. . Lower larval weights and longer developmental periods were observed in the larvae reared on leaves compared to those reared on flowers and pods of wild relatives of pigeonpea. . The mean developmental time for *H. armigera* larvae grown on the wild relatives of pigeonpea was relatively longer compared to the larvae reared on the cultivated pigeonpeas. Prolonged larval duration also indicates antibiosis mechanism of resistance in wild relatives of pigeonpea.

The larval and pupal weights, and larval survival rates were greater in larvae reared on the artificial diets containing lyophilized leaf and pod powders compared to the larvae reared on the intact leaves, flowers, and pods. This may be due to the availability of more nutrients in the artificial diet. There were significant differences in the larval developmental period, larval weight, and mortality of the larvae reared on the artificial diets impregnated with lyophilized leaf and pod powders of wild relatives of pigeonpea as compared to the larvae reared on the diets with leaf and pod powders of cultivated pigeonpeas. The levels of resistance to *H. armigera* observed in the artificial diets impregnated with lyophilized leaves or pods were slightly different than those observed on the intact plant parts. Physical factors such as trichomes and pod wall toughness might be some of the factors contributing to host plant resistance to *H. armigera* in intact leaves and pods.

Relative feeding preference by the third-instar larvae of *H. armigera* towards the leaves and pods of pigeonpea and its wild relatives was studied under no choice and multi-choice conditions. The differences in larval feeding preference were not apparent among the wild relatives of pigeonpea under no-choice and multi-choice conditions. The larvae preferred to feed on the leaves of the cultivated pigeonpea as compared to those of the wild relatives. The biochemical composition of the leaves might be responsible for their acceptance or rejection as food by the *H. armigera* larvae. In case of pods the larvae of *H. armigera* showed less feeding preference towards the wild relatives of pigeonpea, where the percentage damage was low compared to that on the pods of cultivated pigeonpea variety, ICPL 87. In pod-choice experiments, the larvae of *H. armigera* are able to distinguish between different species of *Cajanus*. The larvae preferred to feed on the pods of ICPL 87 as compared to those of its wild relatives.

The effect of pod surface chemicals of pigeonpea and its wild relatives on feeding behavior of *H. armigera* larvae was studied by observing the feeding preference of larvae towards the pods after extracting the surface chemicals extracted pods and the glass fiber discs treated with pod surface chemicals. Under no-choice conditions, pods of the wild relatives were less preferred by the *H. armigera* larvae compared to the pods of cultivated

pigeonpea varieties, ICPL 87and ICPL 332, when the pods were washed with water. When the pods were washed with hexane, the larvae preferred to feed on the pods of *C. acutifolius, C. albicans* and *C. cajanifolius* (ICPW 28) as compared to the pods of *C. cajan.* In the methanol washed pods, the larvae preferred the pods of ICPW 1 (*C. acutifolius*), ICPW 13 (*C. albicans*), and ICPW 28 (*C. cajanifolius*) as compared to the pods of cultivated pigeonpeas. When the larvae were provided with a choice to choose between the unwashed pods and the hexane washed pods, the larvae preferred to feed on the hexane washed pods indicating that hexane must have removed some of the antifeedant compounds from the pod surface. Once these compounds are removed through the extraction, the larvae preferred to feed on the pods of wild relatives of pigeonpea. When the larvae were provided a choice between the methanol-washed and unwashed pods, the larvae preferred to feed on the unwashed pods compared to the methanol washed pod of the same accession indicating that the phagostimulant compounds were extracted into the methanol. These compounds may be responsible for preference of pods as food by the *H. armigera* larvae in the cultivated pigeonpeas. When the pod surface chemicals were tested for their preference by different instars of *H. armigera* larvae, the third-instar, fourth-instar and fifth–instar larvae showed similar preference towards the glass fiber discs treated with pod surface extracts. Among the two solvents used (methanol and hexane), the larvae preferred to feed on the methanol extract treated glass fiber discs. Methanol extract of ICPL 87 stimulated feeding by the third-instar, fourth-instar and fifth-instar larvae of *H. armigera*. The disc area consumed by the fifth–instars was more than the fourth-instar and third-instar in both the solvents. This may be due to changes in the nutritional requirements between the instars. The larvae preferred to consume control discs than the discs treated with hexane extract suggesting that the hexane extracts had some anti-feedant compounds. The amounts of phago-stimulants/attractants and anti-feedants/deterrents on the pod surface play an important role in food selection by the larvae of *H. armigera.*

Biochemical composition in the leaves and pods of wild relatives of pigeonpea was studied by estimating the amounts of total soluble sugars, poly phenols, tannins and

proteins, and also the flavonoid profiles through HPLC technique. There were marked differences in the amounts of soluble sugars among the wild relatives of pigeonpea. The amounts of total sugars were high in the cultivated pigeonpeas compared to the wild relatives. High amounts of polyphenols were recorded in the resistant and late-maturing wild relatives of pigeonpea as compared to the cultivated pigeonpeas. Considerable variation was recorded in the tannin content in the leaves and pods of wild relatives of pigeonpea. The percentage of soluble proteins was significantly high in the pods of *C. scarabaeoides* compared to of ICPL 87. Wild species of pigeonpea have been found to be a promising source of high-protein. The present studies indicated that high levels of resistance to *H. armigera* in wild relatives of pigeonpea might be due to lower amounts of sugars and high content of tannins, polyphenols, and proteins. However, further studies are necessary to understand the type of sugars, tannins, polyphenols, and proteins conferring host plant resistance to *H. armigera.*

The HPLC profiles revealed substantial differences in the pod surface chemicals of wild relatives of pigeonpea. The HPLC profiles of the pod surface extracts showed more number of peaks in the methanol solvent extracts compared to the peaks in the hexane solvent in all the wild accessions except ICPW2, ICPW 160, ICPW 83, ICPW178, ICPW 192 and ICPW207. Some of the compounds in methanol extract were in significant amounts in both ICPL 332 and ICPL 87, but they were either totally absent or present in very small quantities in the wild relatives. The presence of these particular compounds, in the cultivated species, might be responsible for their susceptibility to *H. armigera.* Most of the major peaks observed in hexane extracts of wild relatives of pigeonpea were absent in the cultivated pigeonpea ICPL 87 and ICPL 332, indicate the presence of compounds acting as phago-deterrents in the wild relatives of pigeonpea. However, the isolation of the compounds and their bioassay will provide a clear picture of their mode of action.

An overview of the results shows that antixenosis and antibiosis mechanisms of resistance are playing a key role in conferring resistance against *H. armigera.* The morphological (trichomes) and chemical (pod surface chemicals) constituents present in

the wild relatives of pigeonpea were found to be responsible for the above two types of resistance against *H. armigera*. The interactions between the morphological traits of genotypes and *H. armigera* revealed that the wild accessions; *C. acutifolius C. albicans C. scarabaeoides* (ICPW 83, ICPW 90, ICPW 94, ICPW 116, ICPW 125, ICPW 130 and ICPW 137), *P. scariosa* and *R. aurea*, with high density of non-glandular trichomes, were least preferred for oviposition, and the pod damage by *H. armigera* was also very low. Whereas, the accessions; *C. cajanifolius* (ICPW 28 & ICPW 29), and *R. bracteata* (ICPW 214), with glandular trichomes were highly preferred for oviposition and for feeding. Further, the pod damage was maximum in these accessions indicating that these accessions are as susceptible to pod borer as the cultivated *C. cajan*

The oviposition studies conducted under no-choice, dual-choice and multi- choice conditions also revealed that the accessions of wild relatives were highly non-preferred to oviposition by the *H. armigera* females compared to the cultivated pigeonpeas. The accessions of *C. scarabaeoides, C. acutifolius, C. albicans, P. scariosa* and *R. aurea* were less preferred compared to other wild relatives and the cultivated pigeonpea.

The trichomes are important morphological structures in conferring resistance to these wild accessions. Four types of trichomes; type A, type B, type C and type D, were observed on the calyxes and pods of the wild relatives of pigeonpea. Of the four trichomes, type A and type B were found to be glandular, and type C and type D were glandular in nature. The variation in their structure and density are responsible for the variation in the levels of resistance in these wild accessions. In the present investigation, it is found that the secretions of glandular trichomes, type A and type B, on the pods of wild accessions might be acting as attractants to the insect and thus causing the accessions as susceptible. Whereas, the high density of non-glandular trichomes, type C and type D, on the pods might be acting as deterrents to the insect and causing the moths to exhibit non-preference for oviposition.

The data recorded on the growth and development of larvae reared on leaves, flowers and pods, and their lyophilized powders exhibited the antibiosis mechanism of resistance against *H. armigera* larvae. The antibiosis mechanism of resistance expressed

in terms of reduced larval and pupal weights, prolonged developmental periods and non-preference of the accessions as food, observed in bioassay studies might be due to the presence of chemicals within and on the pod wall surface of wild relatives of pigeonpea.

The biochemical studies have revealed that the accessions of wild relatives of pigeonpea; *C. scarabaeoides, C. albicans, C. serecius, P. scariosa,* and *R. aurea* with low amount of sugars, and high amounts of tannins, polyphenols and proteins suffered low pod damage. The glass fiber disc bioassay of pod surface chemicals also showed a significant role in influencing the feeding preference of *H. armigera* larvae. The HPLC data showed that there are qualitative and quantitative differences in the compounds present on the pod surface of different accessions of wild relatives of pigeonpea. Some of these compounds might be acting as phagostimulants, while some other as phago-deterrents. It can be concluded from the present investigation that the wild accessions of *C. scarabaeoides, C. albicans, C. serecius, P. scariosa,* and *R. aurea* were found to be more resistant against *H. armigera* and hence, they can be used in the breeding programs for the development of resistant pigeonpea varieties.

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Zalucki, M.P., Murray, D.A.H., Gregg, P.C., Fitt, G.P., Twine, P.H. & Jones, C. 1994 Ecology of *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) in the inland of Australia: larval sampling and host plant relationships during winter and spring. Australian Journal of Zoology **42:** 329- 346.

Clarifications on the comments made by the examiners

Comment

Whether the bibliography is sufficient and relevant.

The examiner himself has commended stating that "The bibliography is adequate and relevant to the problem".

Comment

The following are the examiners' comments about the missing references

- Whether all the references cited in the Text are incorporated in the Bibliography and vice versa

- The following references cited in the Text are missing in the Reference section

  Bhatnagar *et al.*, 1982; ICRISAT, 1993; Khan and Saxena, 1986; Salunkhe *et al.*, 1986; Sharma *et al.*, 1990; Yencho and Tingey, 1994

  Green *et al.*, 2001; Sharma and Nooris, 1990 ; Stevenson *et al.*, 2001

  Lateef and Reed, 1992 Santhakumari *et al.*, 1979

The following references are included under the chapter References (Bibliography)

Bhatnagar, V.S., Lateef, S. S., Sithanantham, S., Pawar, C. S. and Reed, W. 1982. Research on *Heliothis* at ICRISAT. In : Proceedings of the International Workshop on *Heliothis* Management (eds. W. Reed, and V. Kumble). International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India. pp 385-396.

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Khan, Z.R. and Saxena, R.C.1986. Effect of stream distillate extracts of resistant and susceptible rice cultivars on behaviour of *Sogatella furcifera* (Homoptera: Delphacidae). J. Econ. Entomol. 70: 928-935.

Salunkhe , D.K., Chavan, J.K and Kadam, S.S. 1986. Pigeonpea as an important food source. CRC Critical Reviews in Food Science and Nutrition. 23 (2) : 103-145.

Sharma, H.C., Leuschner, K. and Vidyasagar, P. 1990. Factors influencing by the sorghum midge, *Contarinia sorghicola* Coq. Annals of Applied Biology. 116 : 431-439.

Yencho, G.C. and Tingey, W.M. 1994. Glandular trichomes of *Solanum berthaultii* alter host preference of the Colorado potato beetle, *Leptinotarsa decemllineata*. Entomologia Experimentalis *et* Applicata. 70:217-225.

In the following references the YEARS are changed

From	To
Green *et al.*, 2001	Green *et al.*, 2003
Sharma H.C. and Norris, D.M.1990	Sharma H.C. and Norris, D.M.1991
Stevenson *et al.*, 2001	Stevenson *et al.*, 2002

In the following reference the year 1992 is corrected as 1990 in text on Page No. 184 Para No.1 and Lines 11&13 and included in the Bibliography chapter as:

Lateef, S.S. and Reed, W. 1990. Insect Pests of Pigeonpea. In: S.R. Singh (ed.) Insect Pests of Tropical Food Legumes. John Wiley & Sons, New York. pp 193-242.

In the following reference the name of the author Snathakumari is corrected as Santhakumari as:

Santhakumari, M., Reddy., C.S., Reddy, A.R.C. and DA, V.S. 1979. CAN behavior in grain legume. Naturwissenscaften 66 : 554

Comment

The reference cited as Nene *et al.*, 1990 in the Text is given as Nene and Sheila, 1990 under reference.

The reference cited as Nene and Sheila, 1990 in the Bibliography is the correct one and hence, "Nene *et al.*, 1990" is corrected as "Nene and Sheila, 1990" in the text on Page No. 1, Para No. 2 and Line No. 9

Comment

The reference of Smith 1989 is given twice under bibliography.

This reference is repeated in the chapter references and hence, deleted once.

Comment

Some references viz; Eherlich & Raven,1964; Krips *et al.*, 1999; Price *et al.*, 1978; Duffer(y),1986; Gomez and Gomez,1984; Manjunath *et al.*, 1989; Mathews, 1989; Navasero / Navasero and Ramaswamy, 1991; Parsons, 1940; Peter and Shanower,1996; Ranger and Hower,2001; and Stevenson *et al.*, 2002. These references may either be cited under text or deleted from the bibliography.

All the above mentioned references are not missing in the text as stated by the examiners. They were very much mentioned in the text on different pages as stated below. Hence, these references need not either be cited again under the text or deleted from the bibliography.

Scientist	Year	Page No.	Paragraph No.	Line No.
Duffey	**1986**	**18**	**3**	**5**
Gomez and Gomez	**1984**	**52**	**3**	**3**
Manjunath *et al.*,	**1989**	**10**	**2**	**3**
Mathews	**1989**	**2**	**1**	**3**
Navasero and Ramaswamy	**1991**	**19**	**4**	**2**
Parson	**1940**	**13**	**2**	**5**
Peter and Shanower	**1996**	**175**	**2**	**11**
Ranger and Hower	**2001**	**172**	**1**	**4/5**
Stevenson *et al.*,	**2002**	**4**	**1**	**9**
Eherlich and Raven	**1964**	**12**	**3**	**2**
Krips *et al.*,	**1999**	**17**	**3**	**8**
Price *et al.*,	**1978**	**49**	**1**	**2**

Comment

Spell check of the names of the authors 1) Rieley, and 2) Pearson / Parson in the text and bibliography.

The spelling of the names of the authors is corrected as 1) Riley and 2) Parson in the text on page Nos. 8 and 13 respectively.

Comment

It is mentioned as "Laxmipathy and Srigiriraju, 2000 in the reference section, but in text it is as Laxmipathy, 2000".

Laxmipathy Srigiriraju is the name of the single author only but not Laxmipathy and Srigiriraju as stated by the examiner. Hence, Laxmipathy, 2000 mentioned in the text and Laxmipathy, Srigiriraju. 2000 mentioned in the bibliography are correct.

Comment

In the text, % symbol would have been given for the values in the parenthesis and in the text it should be in words.

It is correct to mention "%" symbol after the values both in the parenthesis and also in the text and hence, the word "percentage" need not be mentioned in the text.

Comment

In the reference cited, uniformity may be followed for citing the journal names.

The different journals have different set of rules while writing the names of journals in the Bibliography. Hence, uniformity could not be followed for citing the journals names but instead the pattern suggested by different journals was used.

Further, the spelling and other mistakes indicated in the text by the examiners were corrected.