REVIEW ARTICLE Legume Research- An International Journal, Volume 47 Issue 11: 1827-1834 (November 2024)

Legumes: Source of Bioactive Compounds and Their Potential Use in Legume Crops Improvement: A Review

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10.18805/LR-4945

ABSTRACT

The bioactive phytochemicals are the most important bioactive compounds against insect herbivores. These phytochemicals produced by many legumes and react herbivore attacks. Therefore, keeping in mind the said facts, this review discusses the potential use of bioactive compounds like Proteinase inhibitors, trypsin inhibitors, lectins, vicillins (7S storage proteins), phenolics, amino acids, sugars and α-amylase inhibitors in legume plants as most important weapons that confer resistance against herbivorous insects. These inhibitors inhibiting proteases present in the larval gut and has insecticidal potential against insect pests. This review demonstrates the potential of natural phytochemicals from legume plants and or artificially diet incorporate to inhibit the development of pathogens and insect pests. Thus, legumes can be used to produce phytochemicals as bio-pesticides and minimize or avoid the use of agrochemicals in crop protection. This ability making them interesting bioactive for next generation of sustainable pesticides for more sustainable agriculture.

Key words: Bioactive compounds, Legume crops, Potential use.

Legumes are the most important staple food for humans and they are also referred to as vegetable protein, vegetable meat, poor man's food and so on (Boye *et al*., 2010; Sparvoli *et al*., 2016). The presence of high nutritional properties such as protein (17-50%), slow digestion carbohydrates (0.4-55%), dietary fibre (3-15%) and low in fat (0.8-6.6%) determines the importance of legume crops. In addition to their nutritional value, legumes are high in bioactive compounds such as proteinase inhibitors, enzymes, storage proteins, lectins, amylase inhibitors, chitinases, β-1, 3-glucanases, polyphenols, phytosterols and resistant starch (Macedo *et al*., 2016; Mauricio *et al*., 2000; Oliva *et al*., 2010; Oddepally *et al*., 2013; Shewry *et al*., 1995). Legumes face biotic and abiotic stressors that have a substantial impact on their yield, potentially resulting in considerable significant economic losses and defining the food production limit range (Jat *et al*., 2017; Jat *et al*., 2021a). Herbivorous insect, viruses, bacteria, fungi and nematodes are among the most important biotic factors (Jat *et al*., 2021b; Atkinson and Urwin, 2012; Franke *et al*., 2018). *Fusarium oxysporum*, *F. solani*, *F. udum* and *F. virguliforme* are among the Fusarium species responsible to produce vascular wilt and root rot, resulting in crop yield loss. Similarly, phytopathogenic bacteria such as *Pseudomonas* and *Xanthomonas* cause blights and spotting in legume crops (Rubiales *et al*., 2015). Root-knot nematode (*Meloidogyne* spp.) and cyst nematode (*Heterodera* spp. and *Globodera* spp.) have the greatest impact on legume plants (Dhandaydham *et al*., 2008). *Callosobruchus chinensis* and *C. maculatus* are the most significant legume seed pests in storage because they directly damage the seeds, resulting in a loss of quality and quantity parameters and severely affecting seed viability and germination (Cruz *et al*., 2016; Mainali *et al*., 2015). These biotic factors have a negative impact on legume crops, potentially resulting in significant financial losses and a decrease in global food production (Jat *et al*., 2024).

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How to cite this article: Jat, B.L., Sharma, H.C., Pagaria, P., Meena, A.K., Mali, G.R. and Khan, T. (2024). Legumes: Source of Bioactive Compounds and Their Potential Use in Legume Crops Improvement: A Review. Legume Research. 47(11): 1827-1834. doi: 10.18805/LR-4945.

Submitted: 20-04-2022 **Accepted:** 21-12-2022 **Online:** 07-02-2023

Because plants lack an immune system, they have evolved several antifungal proteins and peptides (Praxedes *et al*., 2011). Studies have been carried out on confer resistance or retardation in the physiology of insect-pests fed on diets containing protease inhibitors (PIs) or on transgenic plants expressing PIs (DeLeo and Gallerani, 2002; Murdock and Shade, 2002; Telang *et al*., 2003).

Vicilins, seed storage proteins, thought to be defense mechanisms against phytopathogenic fungi because they bind spores to cell wall polymers, preventing them from germinating (Gomes *et al*., 1998). Proteins with biological activity have been discovered in the seeds of leguminous plants including *Pisum sativum* (Almeida *et al*., 2000; Ye *et al*., 2000), *Phaseolus vulgaris* (Wong and Ng, 2005), *P. mungo*

(Wang *et al*., 2004; Ye and Ng, 2005), *Glycine max* (Krishnaveni *et al*., 1999), *Vigna unguiculata* (Gomes *et al*., 1998; Carvalho *et al*., 2001), *Cicer arietinum* (Ye *et al*., 2002) and *Delandia umbellata* (Ye and Ng, 2002). Vicilins, which bind to chitin and chitinous structures, have been found to be poisonous to larval herbivores of Coleopteran and Lepidopteran as well as yeast and fungal cell walls (Gomes *et al*., 1998; Firmino *et al*., 1996; Uchoa *et al*., 2006) are a few examples. Souza *et al*. (2010) reported on the potential use of vicilins against *C. maculatus*, a seed storage bruchid beetles. They bind to the midgut, ovaries, eggs and excreta of these beetles.

Kunitz inhibitors, another class of proteins found in leguminous seeds, are the best described among other plant inhibitor families due to their abundance in legumes (Oliva *et al*., 2010; Hansen *et al*., 2007). These protein inhibitors, purified from leguminous seeds, have molecular weights of 20 kDa.

Plants have proteinase inhibitors as a reserve protein and as plant defense proteins (Richardson, 1991; Mosolov and Valueva, 2005). These proteins can be produced naturally or as a result of an herbivorous insect attack (Ryan and Pearce, 1998; Mosolov *et al*., 2001). The negative impact of these proteins on the growth and survival of insect larvae has been well documented, with parameters measured in pesticide experimental studies (Carlini and Grossi, 2002; Macedo *et al*., 2004). Chitinase (22000 Da) and a β-1, 3-glucanase (26000 Da) were extracted from cowpea, *V. unguiculata* seeds and shown to inhibit the development of phytopathogenic fungi in an *in vitro* test. The isolated chitinase harmed the physiology of the cowpea weevil, *C. maculatus* (Gomes *et al*., 1996).

The use of such bioactive chemicals (protease inhibitors) in reducing herbivory insect damage by targeting insect digestive enzymes has piqued the interest of many researchers. Plants that expressed these protease inhibitor proteins inhibited the growth and development of insects, eventually killing them. In several bioassay tests, insect mortality was demonstrated when insects fed transgenic plants expressing such proteins (Koiwa *et al*., 1998; Schuler *et al*., 1998). This review study summarized the potential bioactive chemicals (PIs) from cultivated legumes and their insecticidal potential as a transgenic pesticide against herbivores insects.

Efficacy of legume PIs against herbivores insects

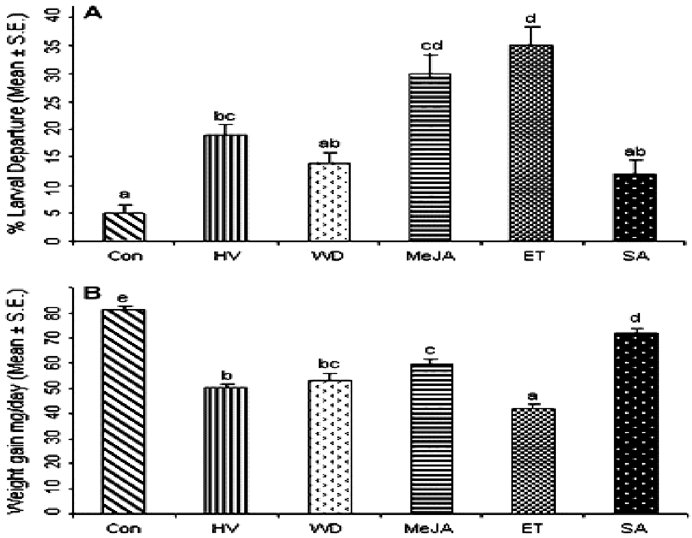
Protease inhibitors (PIs) found active against trypsin as phytochemical resistance factor against herbivorous insects (Broadway, 1996). PIs influence the growth and development of insect herbivory because they bind to the active site of digestive gut protease or by preventing proteolysis in the midgut of insect larvae leading to their starvation and death (Gatehouse *et al*., 1999). As a result, these enzymes lose their ability to produce amino acids from food, which leads to the organism's demise (Kessler and Baldwin, 2002) and (Zhu and Zeng, 2015). Kunitz and Bowman-Brik inhibitors are of two major families of PIs in the plants. Among them Kunitz inhibitors are of 8,000 to 22,000 Da proteins with a single reactive site of trypsin, whereas Bowman-Brik inhibitors are of 8000 to 10,000 Da proteins with two reactive sites of trypsin and chymotrypsin (Laskowski and Qasim, 2000).

Green and Ryan (1972) proposed in a seminal paper that demonstrated rapid accumulation of protease inhibitors in potato and tomato leaves attacked by Colorado potato beetles, both locally and systemically. A few years later, Hilder *et al*. (1987) produced PI-expressing transgenic tobacco lines by leveraging the potentials of plant genetic transformations. It contains a gene construct containing PI-encoding cowpea genes (Cowpea trypsin inhibitor, CpTI) that is linked to the CaMV 35S promoter. These transgenic tobacco lines with a high level of CpTI expression (about 1% of total soluble protein) showed increased resistance to the tobacco budworm, *Heliothis virescens* and other significant lepidopteran pests (Hilder *et al*., 1987). Ethylene (ET) and methyl jasmonate (MeJA) are important plant defense regulators that are produced in response to insect herbivory and injury in a variety of plant species (Truman *et al*., 2007).

Gram pod borer, *H. armigera* is a severe polyphagous insect that preys on legume crops. In response to insect attack, *H. armigera* infestation in chickpea plants activated a gene implicated in ethylene production (ACC oxidase) (Singh *et al*., 2008). To demonstrate induced plant defense in chickpea, stay/ dispersal assays were performed and the percentage dispersal from control plants (5±3.5) was significantly lower than ET (35±7.9) and MeJA (30±7.9) treated and pre-infested (19±4.18) plants (Fig 1A). The consequences of induced plant defense were investigated by feeding larvae on elicited plants under no-choice conditions (Singh *et al*., 2008). *H. armigera* larvae feeding on ET-treated plants had the lowest mean body mass change (41.73±1.97 mg), followed by pre-infested plants (50.73±1.31 mg). Larvae fed MeJA-treated plants (59.65±2.01 mg) and damaged plants (53.14±2.77 mg) gained significantly more body mass than control plants (Fig 1B).

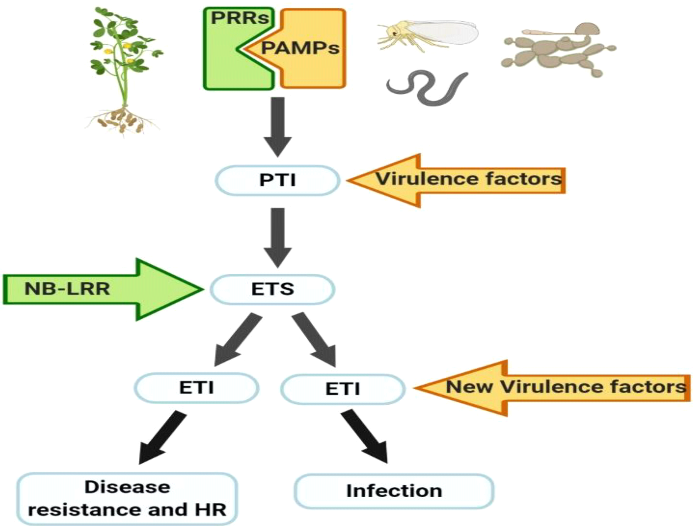
Swathi *et al*. (2015) discovered that a serine PI found in pigeonpea, *Cajanus cajan*, a wild type cousin of *C. platycarpus*, inhibited the gut trypsin-like protease activity of *H. armigera* (HGPs). This inhibitor was highly stable at 50°C in acidic or basic conditions and it contained numerous isoinhibitors that were also shown to be effective against HGPs and shared many similarities with Kunitz-type PIs. Golla *et al*. (2018) reported less pod damage by *H. armigera* in wild relatives of chickpea, *C. arietinum* compared to cultivated chickpea due to stronger insect gut trypsin and chymotrypsin inhibition induced by PIs present in wild relatives of chickpea. Similarly, trypsin inhibitor activity also observed in seed flour extracts in some chickpea varieties (Kansal *et al*., 2008). In all chickpea varieties trypsin inhibitor (molecular mass of 30,000 Da) showed inhibitory activity *in vitro* and *in vivo* against *H. armigera* gut protease. The activity of purified inhibitor was stable up to 80°C and showed its maximum activity between pH 6 to 10. Supplemented diet with increased concentrations of chickpea trypsin inhibitor reduced larval growth, weight and survival as well as adversely affect adult emergence.

About 60 per cent inhibition of *H. armigera* gut protease activity by PIs from chickpea seeds has been observed (Srinivasan *et al*., 2005). Gomes *et al*. (2005) reported 73 per cent inhibition of cotton boll weevil, *Anthonomus grandis* proteinase larvae by chickpea trypsin inhibitor. Trypsin inhibition activity have been reported in mungbean varieties seed flour extracts and have sown 37-59 per cent *H. armigera* gut protease inhibition by PIs (Kansal *et al*., 2008). The relative potential of SBTI and Bowman-Brik trypsin-chymotrypsin inhibitor (SBBI) against *H. armigera* have been studied *in vivo*. Reduction in *H. armigera* larvae biomass has been reported when larvae fed with soybean Kunitz trypsin inhibitor (SBTI) (Johnston *et al*., 1993), chickpea and mungbean protease inhibitors (Sudheendra



(Con: Control; HV: *Helicoverpa* infestation; WD: Wounding; MeJA: Methyl jasmonate; ET: Ethephon; SA: Salicylic acid). (A) Comparison of the mean (±SE) dispersal percentage of first-instar larvae from the control and treated plants. (B) Comparison of the mean (±SE) weight gain of the larvae fed on the control and treated plants. Values in the graph represent the fresh weight gain mg d^{-1} (mean ±SE) of 5th instar larvae of *Helicoverpa* fed on plants exposed to different treatments. Means of various treatments superscripted by different lowercase letters are significantly different (Tukey's Test, P<0.001). Source: Singh *et al.* (2008).

Fig 1: Elicitor-induced resistance in chickpea plants against *Helicoverpa armigera* infestation.



PRRs: Pathogen recognition receptor; PAMPs: Pathogen-associated molecular patterns; PTI: PAMP-triggered immunity; ETS: Effector-triggered susceptibility; NB-LRR: Nucleotide-binding and leucine-rich repeat domains; ETI: effector-triggered immunity; HR: hypersensitive cell death. Source: Lucio *et al*. (2020).

Fig 2: Plant defense mechanisms against the attack of pathogens.

and Mulimani, 2002). Reduced mean larval weight, larval growth and food intake have been observed when *H. armigera* fed with SBTI than SBBI.

Vicilins (7S storage proteins) isolated from the seeds of cowpea legumes, *Vigna unguiculata*, adzuki bean, *V. angularis*, jack bean, *Canavalia ensiformis*, soybean, *Glycine max*, common bean, *Phaseolus vulgaris* and lima bean, *P. lunatus* proved larval growth inhibitor of cowpea weevil, *C. maculatus* (Yunes *et al*., 1999). These vicilins bind to a chitin matrix of *C. maculatus*. Vicilins from all non-host seeds, including those from the *C. maculatus* resistance cowpea line IT81D-1045, suppressed larval development significantly (ED of 1.07±0.32% to 2.22±0.64%). This is because of low digestibility of vicilins by insect midgut proteinases.

Legume PIs *vs* phytopathogens

In response to biotic stresses, plants have evolved various defense systems. When a pathogen is present, the plant employs cellular proteins called pathogen recognition receptors (PRRs) to recognize innate pathogen compounds called pathogen-associated molecular patterns (PAMPs) as shown in Fig 2. Protease inhibitors are proteins produced by legumes that prevent phytopathogens from using their protease activity (Fig 3) (Lucio *et al*., 2020).

Vicilin storage proteins (7S globulins) isolated from cowpea seeds, *V. unguiculata* affect the growth and inhibit spore or conidia germination of *F. oxysporum*, *F. solani*, *Colletotrichum musae*, *Phytopthora capsici*, *Neurospora crassa* and *Ustilago maydis* sporidia and leads to abnormal development (sporulation) of *Saccharomyces cerevisiae* cells (Gomes *et al*., 1997 and 1998). The possible inhibitory effects of cowpea vicilins on fungi growth may be exerted through their ability to bind to the cell surface of the microorganisms, cell wall and/or plasma membrane. Vicilin proteins, peroxidase and 24 kDa protein in the soybean seed coat characterized and this protein fraction was able to inhibit the growth of *F. lateritium* and *F. oxysporum* phytopathogenic fungi (Santos *et al*., 2008). These seed coat antifungal proteins may help protect seeds from colonization by phytopathogenic fungi.

Resistance against PIs

So many classes of phytochemicals reported earlier in plants that are confer resistance against herbivorous insects. These classes include lectins (Chrispeels and Raikhel, 1991; Huesing *et al*., 1991a), phenolics (Summers and Felton, 1994), amino acids (Rosenthal and Dahlman, 1991), sugars (Juvik *et al*., 1994; Liedl *et al*., 1995), amylase inhibitors (Huesing *et al*., 1991b; Schroeder *et al*., 1995) and proteinase inhibitors (Oppert *et al*., 1993; Wolfson and Murdock, 1995). Wheat germ agglutinin (WGA) isolated from hexaploid wheat is made up of six isolectin dimeric forms and have detrimental effect on growth and development of cowpea weevil (Huesing *et al*., 1991a). These isolectins can be used against cowpea bruchid beetle. The toxic effects of α-amylase inhibitor (aAI) to cowpea weevil have been reported earlier (Huesing *et al*., 1991b).

When artificial seed containing aAI evaluated against cowpea weevil the 3.1 days delay in the developmental time of cowpea weevil with every 0.1 per cent increase in aAI dose. The cDNA encoding α-amylase inhibitor was transferred in pea (*Pisum sativum*) from common bean (*Phaseolus vulgaris*) through *Agrobacterium*-mediated transformation to know the toxic effects on pea weevil (Schroeder *et al*., 1995). The expression of α-amylase inhibitor in pea seeds was stable at T_5 seed generation and accumulated up to 3 per cent of soluble protein. The development of pea weevil, *Bruchus pisorum* larvae ceased at an early stage with the minimum seed damage and no adult beetles emerged from seeds in T_5 seed generation. With the help of such approach (α-AI) legume crops can be protect from bruchid beetles.

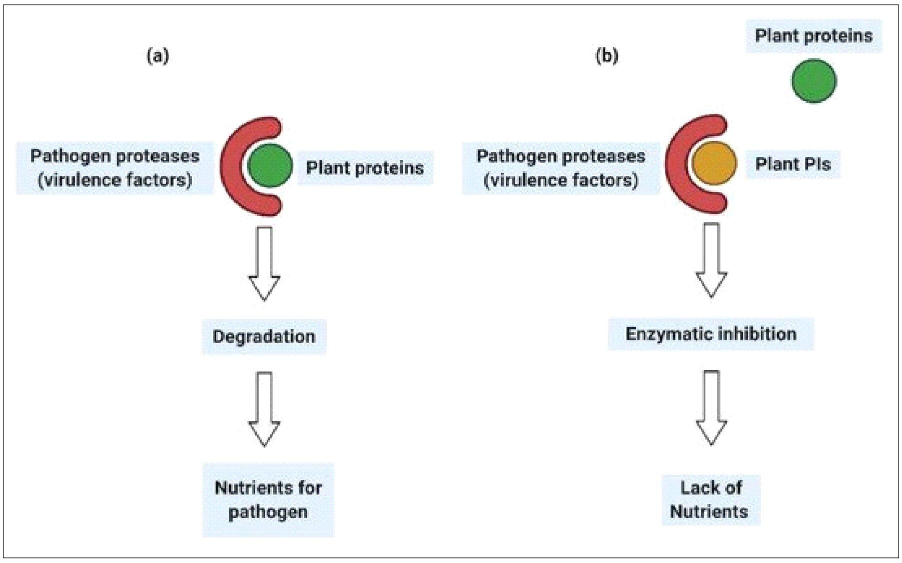


Fig 3: The action of pathogenic proteases (a), Action of protease inhibitors (PIs) over pathogen proteases (b). Source: Lucio *et al.* (2020).

Proteins of trypsin-like activity are present in insect midgut. Among these, some trypsin(s) are susceptible to inhibition by the PIs, while other trypsin(s) are not susceptible to inhibition (Broadway, 1996). The level of activity of "inhibitor-resistant" trypsin(s) is enhance in the midgut when "inhibitor-resistant" insect fed PI, thus insect easily digest dietary protein in the presence of PI. Insects could overcome the activity of proteinase inhibitors by secreting inhibitor resistant enzymes (Broadway, 1996). Resistance in *H. armigera* larvae against chickpea trypsin inhibitor have been also reported (Kansal *et al*., 2008). Entomotoxic proteins, such as lectins of leguminous seeds, have been identified as an effective alternative control plant lectin compounds of many herbivory insects. The effect of flour of leguminous seeds of common bean, *Phaseolus vulgaris* L. (Fabaceae), the broad bean, *Vicia faba* L. (Fabaceae) and soybean, *Glycine max* L. (Fabaceae) at different concentrations have been tested against 4th instar larvae of khapra beetle, *Trogoderma granarium* Everts (Mantzoukas *et al*., 2020). Among three leguminous seed flour, the flours of *P. vulgaris* and *V. faba* caused the highest mortality of *T. granarium* larvae. Similarly, the flour of *Phaseolus* spp. Disrupt the female fecundity, adult emergence and the developmental timespan of *Callosobruchus maculatus* (Karbache *et al*., 2011).

Sugars as sustainable crop pests and disease management

Sugars are included in various metabolic and other pathways, adding those that contribute to plant defense against insect herbivores and microbial pathogens. These sugars are characterized in monosaccharides and oligosaccharides. Among them, monosaccharides are rare sugars with limited biological functions. Most of the sugar-based molecules are oligosaccharides derived from the plant cell wall, or from beneficial or pathogenic microbes. They have been used for crop farming as bio pesticides, bio fertilizers, for seed coating formulation and agricultural film (Trouvelot *et al*., 2014). Rare sugars can inhibit plant pathogens with different lifestyles (Mochizuki *et al*., 2020; Ohara, 2011). The detrimental effects of several legume secondary compounds have been documented against the larvae of bruchid beetle, *Callosobruchus maculatus* (Janzen *et al*., 1977). The tropical legume *Derris elliptica* (Wall.) Benth. has been reported to be a good source of 2,5-dihydroxymethyl-3,4-hydroxypyrrolidine (DMDP), an analogue of β-D-fructofuranose (Welter *et al*., 1976) and the biocidal activity of (DMDP) against larvae of *C. maculatus* have been reported (Evans *et al*., 1985). The strong inhibitory effects of carbohydrates on beetles have documented for *Tenebrio molitor* (Leclercq, 1948), *Tribolium confusum* (Bernard and Lemonde, 1949), *Stegobium paniceum* and *Orycaephilus surinamensis* (Lemonde and Bernard, 1953). Similarly, various plant parts of legume *Lonchocarpus sericeus* (Poir) are reported to have insecticidal properties against insects (Irvine, 1961).

CONCLUSION

The process by which plants protect themselves from herbivory-insects and pathogen attack are of major interest because knowing them will aid in the development of novel ways for controlling infection in crop plants. Trypsin inhibitors, lectins, amylase inhibitors, phenolics and amino acids from different cultivated and wild pulses plants found that inhibit growth and development of different herbivorous insects in different ways. Because legumes use insect and pathogen-inhibiting proteins to fight these pests, these proteins can be use as bio-pesticide molecules in an environmentally benign alternative to hazardous agrochemicals. Protease inhibitors in legume crops are one of the promising bioactive weapons that confer resistance against herbivore insects by inhibiting proteases present in the gut of herbivore insects. Either naturally present or artificially incorporation of phytochemicals into legume plants completely blocks the survival of herbivorous insects. These bio compounds could be a new generation pesticide for sustainable agriculture.

Conflict of interest

Each and every author states that they have no conflicts of interest.

REFERENCES

Almeida, M.S., Cabral, K.M.S., Zingali, R.B. and Kurtenbach, E. (2000). Characterization of two novel defense peptides from pea (*Pisum sativum*) seeds. Archives of Biochemistry and Biophysics. 378: 278-286.

Atkinson, N.J. and Urwin, P.E. (2012). The interaction of plant biotic and abiotic stresses: From genes to the field. Journal of Experimental Botany. 63: 3523-3543.

Bernard, R. and Lemonde, A. (1949). Qualitative aspects of the carbohydrate requirements of *Tribolium confusum* Duval. Reviews of Canadian Biology. 8: 409-503.

Boye, J., Zare, F. and Pletch, A. (2010). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. Food Research International. 43: 414-431.

Broadway, M.M. (1996). Resistance of plants to herbivorous insects: Can this resistance fail? Canadian Journal of Plant Pathology. 18: 476-481.

Carlini, C.R. and Grossi-de-S, M.F. (2002). Plant toxic proteins with insecticidal properties. A review on their potentialities as bio-insecticides. Toxicon. 40: 1515-1539.

Carvalho, A.O., Machado, O.L.T., Cunha, M., Santos, I.S. and Gomes, V.M (2001). Antimicrobial peptides and immunolocalization of a LTP in *Vigna unguiculata* seeds. Plant Physiology Biochemistry. 39: 137-146.

Chrispeels, M.J. and Raikhel, N.V. (1991). Lectins, lectin genes and their role in plant defense. The Plant Cell. 3: 1-9.

Cruz, L.P., de Sá, L.F.R., Santos, L.A., Gravina, G.A., Carvalho, A.O., Fernandes, K.V.S., Freire Filho, F.R., Gomes, V.M. and Oliveira, A.E.A. (2016). Evaluation of resistance in different cowpea cultivars to *Callosobruchus maculatus* infestation. Journal of Pest Science. 89: 117-128.

DeLeo, F. and Gallerani, R. (2002). The mustard trypsin inhibitor 2 affects the fertility of *Spodoptera littorallis* larvae fed on transgenic plants. Insect Biochemistry and Molecular Biology. 32: 489-496.

Dhandaydham, M., Charles, L., Zhu, H., Starr, J.L., Huguet, T., Cook, D.R., Prosperi, J.M. and Opperman, C. (2008). Characterization of root-knot nematode resistance in *Medicago truncatula*. Journal of Nematology. 40: 46-54.

Evans, S.V., Gatehouse, A.M., Fellows, L.E. (1985). Detrimental effects of 2, 5-Dihydroxymethy l-3, 4-dihydroxypyrrolidine in some tropical legume seeds on larvae of the bruchid *Callosobruchus maculatus*. Entomologia Experimentalis et Applicata. 37: 257-261.

Firmino, F., Fernandes, K.V.S., Sales, M.P., Gomes, V.M., Miranda, M.R.A., Domingues, S.J.S. and Xavier-Filho, J. (1996). Cowpea (*Vigna unguiculata*) vicilins associate with putative chitinous structures in midgut and feces of the bruchid beetles, *Calloso bruchus maculatus* and *Zabrotes subfasciatus*. Brazilian Journal of Medical and Biological Research. 29: 749-756.

Franke, A.C., Brand, G.J.V.D., Vanlauwe, B. and Giller, K.E. (2018). Sustainable intensification through rotations with grain legumes in Sub-Saharan Africa: A review. Agriculture Ecosystems and Environment. 261: 172-185.

Gatehouse, AM.R., Norton, E., Davison, G.M., Babbe, S.M., Newll, C.A. and Gatehouse, J.A. (1999). Digestive proteolytic activity in larvae of tomato moth, *Lacanobia oleracea*; Effects of plant proteinase inhibitor *in vitro* and *in vivo*. Journal of Insect Physiology. 45: 545-558.

Golla, S.K., Rajasekhar, P., Akbar, S.M.D. and Sharma, H.C. (2018). Proteolytic activity in the midgut of *Helicoverpa armigera* (Noctuidae: Lepidoptera) larvae fed on wild relatives of chickpea, *Cicer arietinum*. Journal of Economic Entomology. 111: 2409-2415.

Gomes, A.P.G., Dias, S.C., Bloch, J.C., Melo, F.R., Furtado, J.J.R., Monnerat, R.G., Grossi-de-Sa M.F. and Franco, O.L. (2005). Toxicity to cotton boll weevil, *Anthonomus grandis* of a trypsin inhibitor from chickpea seeds. Comparative Biochemistry and Physiology-Part B. 140: 313-319.

Gomes, V.M., Cunha, M.D., Miguens, F.C., Fernandes, K.V.S., Rose, T.L. and Xavier-Filho, J. (1998). Ultrastructure and immonolabelling of fungi cells treated with *Vigna unguiculata* vicilins, 7S storage proteins. Plant Science. 138: 81-89.

Gomes, V.M., Mosqueda, M.I., Alejandro, B.L., Sales, M.P., Fernandes, K.V.S., Cordeiro, R.A. and Filho, J.X. (1997). Vicilin Storage proteins from *Vigna unguiculata* (Legume) seeds inhibit fungal growth. Journal of Agricultural and Food Chemistry. 45: 4110-4115.

Gomes, V.M., Oliveira, Antônia Elenir A. and Xavier-Filho, J. (1996). A Chitinase and a β-1, 3-Glucanase isolated from the seeds of cowpea [*Vigna unguiculata* (L.) Walp] inhibit the growth of fungi and insect pests of the seed. Journal of the Science of Food and Agriculture. 72: 86-90.

Green, T.R. and Ryan, C.A. (1972). Wound induced proteinase inhibitor in plant leaves: A possible defense mechanism against insects. Science. 175: 776-777. doi: 10.1126/Science.175.4023.776.

Hansen, D., Macedo-Ribeiro, S., Veríssimo, P., Yoo, I.S., Sampaio, U.M. and Oliva, M.L.V. (2007). Crystal structure of a novel cysteinless plant Kunitz-type proteinase inhibitor. Biochemical Biophysics Research Communication. 360: 735-740.

Hilder, V.A., Gatehouse, A.M., Sheerman, S.E., Barker, R.F. and Boulter, D. (1987). A novel mechanism of insect resistance engineered into tobacco. Nature. 330: 160-163.

Huesing, J.E., Shade, R.E., Chrispeels, M.J. and Murdock, L.L. (1991b). Alpha amylase inhibitor, not phytohemagglutinin, explains resistance of common bean seeds to cowpea weevil. Plant Physiology. 96: 993-996.

Huesing, J.E., Murdock, L.L. and Shade, R.E. (1991a). Effect of wheat germ isolectins on development of cowpea weevil. Phytochemistry. 30: 785-788.

Irvine, F.R. (1961). 'Woody Plants of Ghana,' Cambridge University Press, London.

Janzen, D.H., Juster, H.B. and Bell, E.A. (1977). Toxicity of secondary compounds to the seed-eating larvae of the bruchid beetle (*Callosobruchus maculatus*). Phytochemistry. 16: 223-227.

Jat, B.L., Dahiya, K.K., Yadav, S.S. and Mandhania, S. (2021a). Morphophysico-chemical components of resistance to pod borer, *Helicoverpa armigera* (Hubner) in pigeonpea [*Cajanus cajan* (L.) Millspaugh]. Legume Research. 44(8): 967-976. doi: 10.18805/LR-4182.

Jat, B.L., Dahiya, K.K., Lal, R. and Ram, N. (2017). Effect of weather parameters on seasonal incidence of pod borer complex in pigeonpea. Journal of Agrometeorology. 19(3): 255-258.

Jat, B.L., Dahiya, K.K. and Sharma, H.C. (2024). Screening of pigeonpea varieties through nylon bag no-choice bioassay for host plant resistance to *Helicoverpa armigera*. Legume Research. 47(2): 256-267. doi: 10.18805/LR-4659.

Jat, B.L., Jat, A.S. and Singh, I. (2021b). Impact of front-line demonstrations on the production and productivity of pulses in arid regions of nagaur district of Rajasthan. Agricultural Science Digest. doi: 10.18805/ag.D-5399.

Johnston, K.A., Gatehouse, J.A. and Anstee, J.H. (1993). Effect of soybean protease inhibitors on the growth and development of larval *Helicoverpa armigera*. Insect Physiology. 39: 657-664.

Juvik, J.A., Shapiro, J.A., Young, T.E. and Mutschler, M.A. (1994). Acylglucoses from wild tomatoes alter behavior and reduce growth and survival of *Helicoverpa zea* and *Spodoptera exigua*. Journal of Economic Entomology. 87: 482-492.

Kansal, R., Gupta, R.N., Koundal, K.R., Kuhar, K. and Gupta, V.K. (2008). Purification, characterization and evaluation of insecticidal potential of trypsin inhibitor from mungbean [*Vigna radiata* (L.) Wilczek] seeds. Acta Physiologiae Plantarum. 30: 761-768.

Kansal, R., Kumar, M., Kuhar, K., Gupta, R.N., Subrahmanyam, B., Koundal, K.R. and Gupta, V.K. (2008). Purification and characterization of trypsin inhibitor from *Cicer arietinum* L. and its efficacy against *Helicoverpa armigera*. Brazilian Journal of Plant Physiology. 20: 313-322.

Karbache, F., Mouhouche, F. and Fleurat-Lessard, F. (2011). Deterrant and insecticidal properties of bean seed (*Phaseolus vulgaris* L.) whole meal or protein extract incorporated into the diet of *Callosobrushus maculatus* (F.) (Coleoptera: Bruchidae). Journal of Stored Product Research. 47: 197-203.

Kessler, A. and Baldwin, I.T. (2002). Plant responses to insect herbivory: The emerging molecular analysis. Annual Review of Plant Biology. 53: 299-328.

Koiwa, H., Shade, R.E., Zhu-Salzman, K., Subramanian, L., Murdock, L.L., Nielsen, S.S., Bressan, R.A. and Hasegawa, P.M. (1998). Phage display selection can differentiate insecticidal activity of soybean cystatins. The Plant Journal. 14: 371-379.

Krishnaveni, S., Liang, G.H., Muthukrishnan, S. and Manickam, A. (1999). Purification and partial characterization of chitinases from sorghum seeds. Plant Science. 144: 1-7.

Laskowski, J.M. and Qasim, M.A. (2000). What can the structures of enzyme-inhibitor complexes tell us about the structures of enzyme substrate complexes? Biochemica et Biophysica Acta. 1477: 324-337.

Leclercq, J. (1948). Qualitative aspects of the carbohydrate requirements of *Tenebrio molitor* L. International Archives of Physiology. 56: 130-133.

Lemonde, A. and Bernard, R. (1953). Qualitative aspects of the carbohydrate requirements of larvae of *Stegobium paniceum* L. (Anobiidae) and *Oryzaephilus surinamensis* L. (Cucujidae). Le Naturaliste Canadien. 80: 125-142.

Liedl, B.E., Lawson, D.M., White, K.K., Shapiro, J.A., Cohen, D.E., Carson, W.G., Trumble, J.T. and Mutschler, M.A. (1995). Acylsugars of wild tomato *Lycopersicon pennellii* alters settling and reduces oviposition of *Bemisia argentifolii*. Journal of Economic Entomology. 88: 742-748.

Lucio, R.S., Jolanta, E.M., Cristina, C.H. and Sergio, O.S.S. (2020). Legumes protease inhibitors as biopesticides and their defense mechanisms against biotic factors. International Journal of Molecular Sciences. 21: 3322. doi: 10.3390/ijms21093322.

Macedo, M.L.R., Ribeiro, S.F.F., Taveira, G.B., Gomes, V.M., De Barros, K.M.C.A. and Maria-Neto, S. (2016). Antimicrobial activity of ILTI, a Kunitz-type trypsin inhibitor from *Inga laurina* (SW.) willd. Current Microbiology. 72: 538-544.

Macedo, M.L.R., Sa, C.M., Freire, M.G.M. and Parra, J.R.P.A. (2004). Kunitz-Type inhibitor of coleopteran proteinases, isolated from *Adenanthera pavonina* L. seeds and its effect on *Callosobruchus maculatus*. Journal of Agricultural and Food Chemistry. 52: 2533-2540.

Mainali, B.P., Kim, H.J., Park, C.G., Heon Kim, J., Yoon, Y.N., Oh, I.S. and Bae, S. (2015). Do oviposition preference and development of azuki bean weevil, *Callosobruchus chinensis* on five different leguminous seeds. Journal of Stored Products Research. 61: 97-101.

Mantzoukas, S., Korbou, G., Magita, A., Eliopoulos, P.A. and Poulas, K. (2020). Leguminous seeds powder diet reduces the survival and development of the khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae). Biology. 9: 204. doi: 10.3390/biology9080204.

Mauricio, P.S., Isabel, R.G., Maria, F.G. and Jose, X. (2000). Do legume storage proteins play a role in defending seeds against Bruchids? Plant Physiology. 124: 515-522.

Mochizuki, S., Fukumoto, T., Ohara, T., *et al*. (2020). The rare sugar d-tagatose protects plants from downy mildews and is a safe fungicidal agrochemical. Communications Biology. 3: 1-15. doi: 10.1038/s42003-020-01133-7.

Mosolov, V.V. and Valueva, T.A. (2005). Proteinase inhibitors and their function in plants: A review. Prikl Biokhim Mikrobiol. 41: 261-282.

Mosolov, V.V., Grigor'eva, L.I. and Valueva, T.A. (2001). Involvement of proteolytic enzymes and their inhibitors in plant protection (review). Applied Biochemistry and Microbiology. 37: 115-123.

Murdock, L.L. and Shade, R.E. (2002). Lectins and protease inhibitors as plant defense against insects. Journal of Agricultural and Food Chemistry. 50: 6605-6611.

Oddepally, R., Gopi, S. and Guruprasad, L. (2013). Purification and characterization of a stable Kunitz trypsin inhibitor from *Trigonella foenum graecum* seeds. Phytochemistry. 96: 26-36.

Ohara, T. (2011). Effect of D-tagatose on Plant Disease; Downy Mildew and Powdery Mildew; Proceedings of the 5th Symposium of International Society of Rare Sugars, Takamatsu and Miki; Kagawa, Japan. 9-12 November 2011.

Oliva, M.L., Silva, M.C., Sallai, R.C., Brito, M.V. and Sampaio, M.U. (2010). A novel sub classification for Kunitz proteinase inhibitors from leguminous seeds. Biochimie. 92: 1667-1673.

Oppert, B., Morgan, T.D., Culbertson, C. and Kramer, K.J. (1993). Dietary mixtures of cysteine and serine proteinase inhibitors exhibit synergistic toxicity toward the red flour beetle, *Tribolium castaneum*. Comparative Biochemistry and Physiology. 105: 379-385.

Praxedes, P.G., Zerlin, J.K., Dias, L.O. and Passoni, R.A.B. (2011). A novel antifungal protein from seeds of *Sesbania virgata* (Cav.) Pers. (Leguminosae-Faboideae). Brazilian Journal of Botany. 71: 687-692.

Richardson, M. (1991). Seed Storage Proteins: The Enzyme Inhibitors, Methods in Plant Biochemistry. 5: 259-305.

Rosenthal, G.A. and Dahlman, D.L. (1991). Incorporation of L canavanine into proteins and the expression of its antimetabolic effects. Journal of Agriculture and Food Chemistry. 39: 987-990.

Rubiales, D., Fondevilla, S., Chen, W., Gentzbittel, L., Higgins, T.J.V., Castillejo, M.A., Singh, K.B. and Rispail, N. (2015). Achievements and challenges in legume breeding for pest and disease resistance. Critical Reviews in Plant Science. 34: 195-236.

Ryan, C.A. and Pearce, G. (1998). Systemin: A polypeptide signal for plant defensive genes. Annual Review of Cell and Developmental Biology. 14: 1-17.

Santos, P.O., Santos, I.S., Gomes, V.M., Machado, O.L.T., Fernandes, K.V.S., Filho, J.X., Oliveira, A.E.A. (2008). *In vitro* evaluation of antifungal activity of soybean (*Glycine max*) seed coat proteins. Journal of Stored Products Research. 44: 310-315.

Schroeder, H.E., Gollasch, S., Moore, A., Tabe, L. M., Craig, S., Hardie, D.C., Chrispeels, M.J., Spencer, D. and Higgins, T.J.V. (1995). Bean alpha amylase inhibitor confers resistance to the pea weevil (*Bruchus pisorum*) in transgenic peas (*Pisum sativum* L.). Plant Physiology. 107: 1233-1239.

Schuler, T.N., Poppy, G.M., Kerry, B.R. and Denholm, I. (1998). Insect-resistant transgenic plants. Trends in Biotechnology. 16: 168-175.

Shewry, P.R., Napier, J.A. and Tatham, A.S. (1995). Seed storage proteins: Structures and biosynthesis. The Plant Cell. 7: 945-956.

Singh, A., Singh, I.K. and Verma, P.K. (2008). Differential transcript accumulation in *Cicer arietinum* L. in response to a chewing insect *Helicoverpa armigera* and defence regulators correlate with reduced insect performance. Journal of Experimental Botany. 59: 2379-2392.

Souza, S.M., Uchôa, A.F., Silva, J.R., Samuels, R.I., Oliveira, A.E.A., Oliveira, E.M., Linharesf, R.T., Alexandre, D. and Silva, C.P. (2010). The fate of vicilins, 7S storage globulins, in larvae and adult *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae). Journal of Insect Physiology. 56: 1130-1138.

Sparvoli, F., Laureati, M., Pilu, R., Pagliarini, E., Toschi, I., Giuberti, G., Fortunati, P., Daminati, M.G., Cominelli, E. and Bollini, R. (2016). Exploitation of common bean flours with low anti-nutrient content for making nutritionally enhanced biscuits. Frontiers in Plant Science. 7: 928. https://doi.org/10.3389/fpls.2016.00928.

Srinivasan, A., Giri, A.P., Harsulkar, A.M., Gatehouse, J.A. and Gopta, V.S. (2005). A Kunitz trypsin inhibitor from chickpea (*Cicer arietinum* L.) that exerts anti-metabolic effect on pod borer (*Helicoverpa armigera*) larvae. Plant Molecular Biology. 57: 359-374.

Sudheendra, K. and Mulimani, V.H. (2002). Effect of feeding legume proteinase inhibitors on *Helicoverpa armigera* gut proteinase activity. International Chickpea and Pigeonpea Newsletter. 9: 51-53.

Summers, C.B. and Felton, G.W. (1994). Prooxidant effects of phenolic acids on the generalist herbivore *Helicoverpa zea*: Potential mode of action for phenolic compounds in plant anti herbivore chemistry. Insect Biochemistry and Molecular Biology. 24: 943-953.

Swathi, M., Mohanraj, S.S., Swaroop, V., Gujjarlapudi, M., Mallikarjuna, N., Dutta Gupta, A. and Padmasree, K. (2015). Proteinase inhibitors from *Cajanus platycarpus* accessions active against pod borer *Helicoverpa armigera*. Acta Physiologiae Plantarum. 37: 242. DOI: 10.1007/s11738-015-1991-8.

Telang, M., Srinivasan, A., Patankar, A., Harsulkar, A., Joshi, V., Damle, A., Deshapande, V. *et al.* (2003). Bitter guard proteinase inhibitors: Potential growth inhibitors of *Helicoverpa armigera* and *Spodoptera litura*. Phytochemistry. 63: 643-652.

Trouvelot, S., Héloir, M.C., Poinssot, B., Gauthier, A., Paris, F., Guillier, C., Combier, M., Trdá, L., Daire, X., Adrian, M. (2014). Carbohydrates in plant immunity and plant protection: Roles and potential application as foliar sprays. Frontiers in Plant Science. 5: 1-14. doi: 10.3389/fpls.2014.00592.

Truman, W., Bennett, M.H., Kubigsteltig, I., Turnbull, C. and Grant, M. (2007). Arabidopsis systemic immunity uses conserved defense signaling pathways and is mediated by jasmonates. Proceedings of National Academy of Science. USA. 104: 1075-1080. doi: 10.1073/pnas.0605423104.

Uchoa, A.F., DaMatta, R.A., Retamal, C.A., Albuquerque-Cunha, J.M., Souza, S.M., Samuels, R.I., Silva, C.P. and Xavier-Filho, J. (2006). Presence of the storage seed protein vicilin in internal organs of larval *Callosobruchus maculatus* (Coleoptera: Bruchidae). Journal of Insect Physiology. 52: 169-178.

Wang, S.Y., Wu, J.H., Ng, T.B., Ye, X.Y. and Rao, P.F. (2004). A non-specific lipid transfer protein with antifungal and antibacterial activities from the mung bean. Peptides. 25: 1235-1242.

Welter, A., Jadot, J., Dardenne, G., Marlier, M. and Casmir, J. (1976). 2, 5-Dihydroxymethy l-3, 4-dihydroxypyrrolidine in leaves of *Derris elliptica*. Phytochemistry. 15: 747-749.

Wolfson, J.L. and Murdock, L.L. (1995). Potential use of protease inhibitors for host plant resistance: A test case. Environmental Entomology. 24: 52-57.

Wong, J.H. and Ng, T.B. (2005). Sesquin, a potent defensing like antimicrobial peptide from ground beans with inhibitory activities toward tumor cells and HIV-1 reverse transcriptase. Peptides. 26: 1120-1126.

Ye, X.Y. and Ng, T.B. (2002). Delandin, a chitinase-like protein with antifungal, HIV-1 reverse transcriptase inhibitory and mitogenic activities from the rice bean, *Delandia umbellate*. Protein Expression and Purification. 24: 524-529.

Ye, X.Y. and Ng, T.B. (2005). A chitinase with antifungal activity from the mung bean. Protein Expression and Purification. 40: 230-236.

Ye, X.Y., Ng, T.B. and Rao, P.F. (2002). Cicerin and arietin, novel chickpea peptides, with different antifungal potencies. Peptides. 23: 817-822.

Ye, X.Y., Wang, H.X. and Ng, T.B. (2000). Sativin, a novel antifungal miraculin-like protein isolated from legumes of the sugar snap *Pisum sativum var. macrocarpon*. Life Sciences. 67: 775-781.

Yunes, A.N.A. andrade, M.T.D., Sales, M.P., Morais, R.A., Fernandes, K.V.S., Gomes, V.M. and Filho, J.X. (1999). Legume seed vicilins (7S storage proteins) interfere with the development of the cowpea weevil (*Callosobruchus maculatus* F). Journal of the Science of Food and Agriculture. https://doi.org/10.1002/(SICI)1097-0010(199801)76:13.0.CO;2-4.

Zhu-Salzman, K. and Zeng, R. (2015). Insect response to plant defensive protease inhibitors. Annual Reviews of Entomology. 60: 233-252.