



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

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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,

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potentially resulting in significant financial losses and a decrease in global food production (Jat *et al.*, 2022).

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 1 A). 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 1 B).

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 iso-inhibitors 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-Birk 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 and Mulimani, 2002). Reduced mean

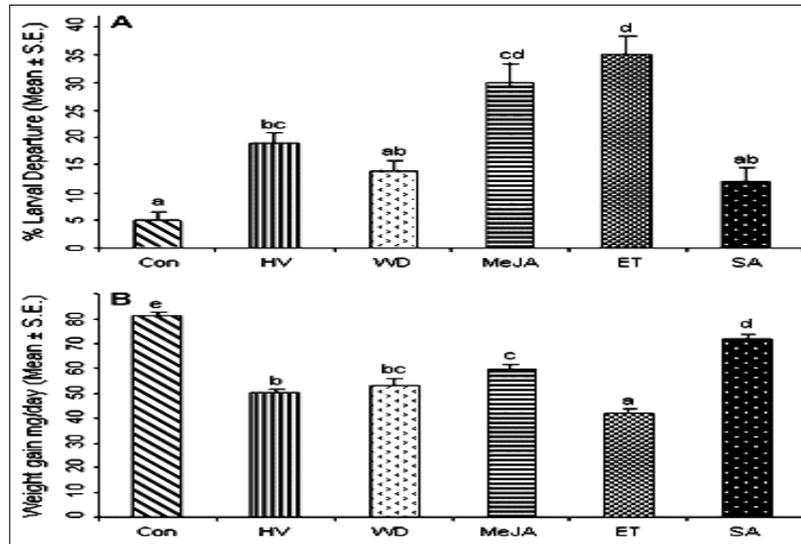


Fig 1: Elicitor-induced resistance in chickpea plants against *Helicoverpa armigera* infestation (Con, control; HV, *Helicoverpa* infestation; WD, wounding; MeJA, methyl jasmonate; ET, ethephon; SA, salicylic acid). (A) Comparison of the mean (\pm SE) dispersal percentage of first-instar larvae from the control and treated plants. (B) Comparison of the mean (\pm 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 \pm 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).

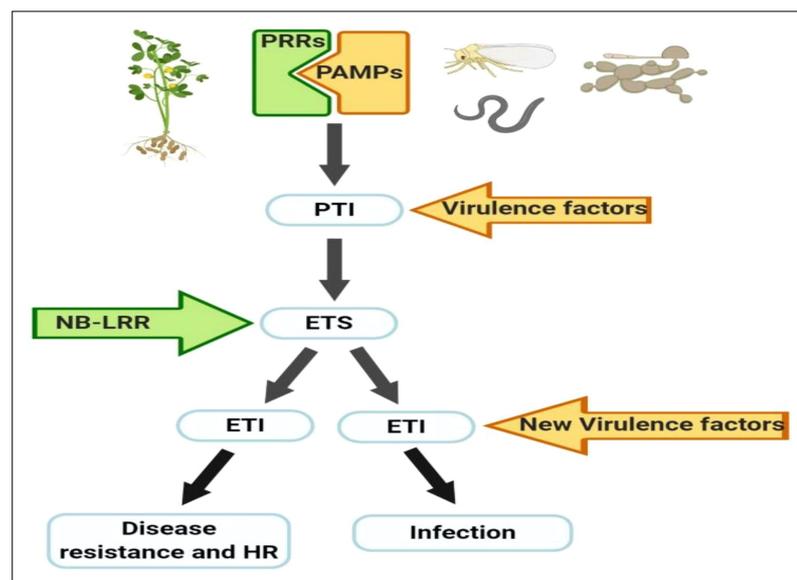


Fig 2: Plant defense mechanisms against the attack of pathogens. Pathogen recognition receptor (PRRs); pathogen-associated molecular patterns (PAMPs); PAMP-triggered immunity (PTI); effector-triggered susceptibility (ETS); nucleotide-binding and leucine-rich repeat domains (NB-LRR); effector-triggered immunity (ETI); hypersensitive cell death (HR) Source: Lucio *et al.* (2020).

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 $1.07 \pm 0.32\%$ to $2.22 \pm 0.64\%$). 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*, *Phytophthora 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.

Proteins of trypsin-like activity are present in insect midgut. Among these, some trypsin(s) are susceptible to

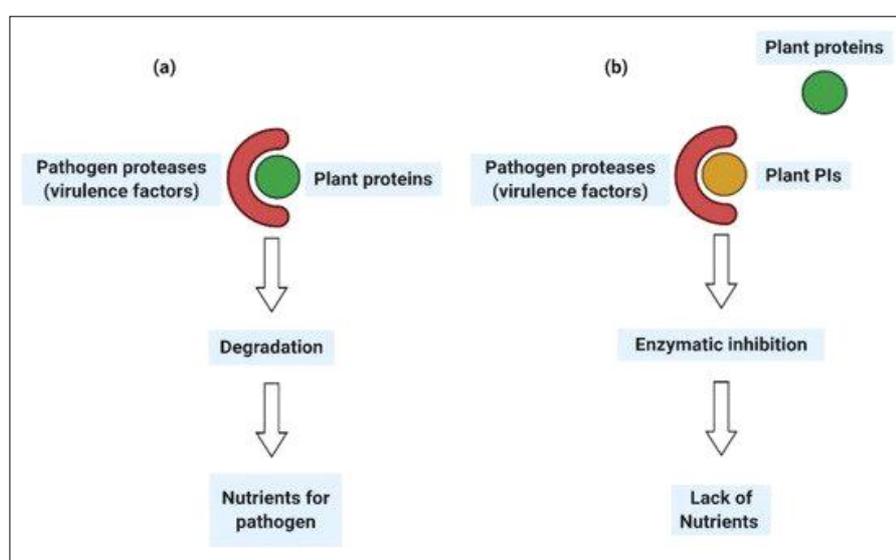


Fig 3: The action of pathogen proteases (a), Action of protease inhibitors (PIs) over pathogen proteases (b) Source: Lucio *et al.* (2020).

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 enhanced 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 *Oryzaephilus 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 used 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 artificial 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: None.

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