EFFECTS OF SOME PLANTS SEED EXTRACTS ON *HELICOVERPA ARMIGERA* HÜBNER (LEPIDOPTERA: NOCTUIDAE) MIDGUT PROTEASE ACTIVITY

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ABSTRACT: *Helicoverpa armigera* is one of the most important pests of crops, such as cotton, cereals and vegetables. Using plant derived enzyme inhibitors in transgenic plants is one of the safe methods in IPM programs. In this study protease inhibitory activity of some plants seed extracts from poaceae and fabaceae family were studied. Insects reared in controlled condition and the last larval instars alimentary canal were used in enzymatic assays. The crude seed extracts were subjected for ammonium sulfate precipitation. The seed extracts of six plants were fractionated into four fractions (0-30 %, 30-50, 50-70 and 70-80%). The percentage of inhibition to cotton bollworm midgut protease activity obtained in crude and each protein fraction of ammonium sulfate. Proteolytic activity of midgut enzyme extracts was evaluated using the azocasein as substrate. The results revealed that seed extracts of *Phasaeolus vulgaris and Cicer arietinum* are potentially effective in inhibiting the proteolytic activity of cotton bollworm (54.5 and 53.2% respectively). Also total extracts of *Triticum aestivum, Hordeum vuIgare, Zea mays,* and *sophora alopecuroides* inhibited HGP activity by 17.7, 18.74, 20.62 and 29.31% respectively. Results revealed that the F1 fraction protein of all studied plants showed less than 20% inhibitory activity against HGP, and the F2 and F3 fraction exhibited the same inhibitory activity in the range of 10-20% in poaceae species. The legume plants especially in F1, F2 and F3 fractions exhibited near 15-50% inhibitory activity on HGP. In over all, among studied plants, *Z. mays, P. vulgaris* and *C. arietinum* have strong inhibitory activity in compare with others.

KEY WORDS: Inhibitory activity, poacea, fabacea, azocasein, ammonium sulfate, cotton bollworm

Cotton bollworm, *Helicoverpa armigera* (Hübner), is one of the most important pests worldwide. It is polyphagous insect with a wide range of host plants including cultivated and wild plants such as cotton, mays, chickpea, tomato, vegetables, and other crops (Harsulkar et al., 1999; Nair et al., 2013). The wide application of chemical insecticides has been the main strategy for the control of *H. armigera* in different parts of the world. High levels of resistance to conventional insecticides also harmful effects on the environment and human health were developed due to improper use of insecticides (Giri et al., 1998; Parde et al., 2010).

Pests such as cotton bollworm rely on a proteinases enzymes present in their guts to digest protease present in seeds, leaves and flowers of host plants (Shukla et al., 2005; Mohammadi et al., 2010). Insect pests depend on peptides obtained from proteolytic digestion for growth and development. Disruption in an insect's ability to digest protein by transgenic plants expressing proteinase inhibitors, seems to be an alternative approach to conventional insecticides (Fan & Wu, 2005).

Proteinase inhibitors are generally small proteins (less than 20 kDa) that mainly have been identified in storage tissues, such as tubers, seeds and aerial parts of plants such as flowers and leaves (Grover et al., 2014; Ryan, 2006). They

are also inducible in plants in response to attack by herbivores (Ryan, 2006). Protease inhibitors have been isolated and characterized from a large number of organisms, including plants, animals, and microorganisms (Christeller, 2005; Nair et al., 2013). A useful strategy for enhancing plant defense systems is to identify PIs with high activity against the target pests.

Protease inhibitors (PIs) are compounds that form complexes with proteases and inhibit their proteolytic activity and suppression of the normal assimilation of food proteins (Ryan, 1990; Fan & Wu, 2005). Plants utilize proteinase inhibitors in order to moderate the adverse effects from attacking herbivores.

So many studies have been carried on protease inhibitors, which active against different insect species, both in *in vitro* and in *in vivo* (Dorrah, 2004; Shukla, 2005).

Reduction in fecundity and fertility, reducing larval growth and development and delay in pupation period after feeding of *C. annum* leaf extracts to *H. armigera* larvae through artificial diet has reported by Tamhane et al. (2005). Also they observed that about 91-98% of protease activity of *H. armigera* gut protease was inhibited by extracts of *C. annum*.

Protein proteinase inhibitors extracted from the seeds of *Momordica charantia* L. were identified as effective inhibitor of cotton bollworm gut proteinases (Telong et al., 2003).

Five plants including *Arachis hypogaea*, *Vigna sinensis*, *Dolichos lablab*, *Phaseolus aureus* and *Cassia siamea* reported inhibitory active plants with 22.91 to 58.33 % inhibition against *H. armigera* protease activity (Padul, 2012).

Serine protease activities of *S. littoralis* midgut were inhibited by soybean trypsin inhibitor in vitro. Consumption of SBI by the larvae, causes variable effects such as reduction in weight gain and survival of the larvae (Dorrah, 2004).

Partially purified inhibitor from soybean seeds extracts inhibiting cotton bollworm total protease activity by 91%. While inhibition of trypsin and chymotrypsin like proteases were found near 65 and 40% respectively (Ghodke, 2013).

In a study aimed to test the efficacy of pigeonpea genotypes against *H. armigera* development were observed that, insects fed with diet containing seed powder exhibited larval and pupal weight reduction. also certain abnormalities such as larval-pupal intermediates were reported (Grover, 2014). Grover et al. (2014) reported that cotton bollworm fed with diet containing pigeon pea seed powder exhibited larval and pupal weight reduction and certain abnormalities.

Some studies about screening of host and non-host plant-derived inhibitors has resulted in effective proteins that demonstrated high levels of inhibitory and biological effects against various insects. Some researchers reported that non-host plant PIs showed more inhibitory activity than host plants (Gruden et al., 1998; Harsulkar et al., 1999; Jamal et al., 2013). Parde et al. (2010) in their studies reported that, *in vivo* studies indicated that non-host plant PIs were good candidates as inhibitors of the HaGPs. The PIs from the non-host plants can be expressed in transgenic plants to confer resistance to insect pests.

Most of plants proteinase inhibitors that have been characterized are from the poaceae, fabaceae, and solanaceae families.

Usually seed extracts of plants showed more inhibitory activity than leaf and flower tissues and this may be due to higher accumulation of proteins in seeds than leaves and flowers (Qutchkourov et al., 2003; Harrison et al., 2012; Chougule et al., 2005). Currently, the main emphasis of plant proteinase inhibitor studies is on identifying potential inhibitors of digestive proteinases of the target insects and present study was conducted to evaluate the in vitro assays of the some poaceae and fabaceae family crude seed extracts and partial purified fractions proteins against the cotton bollworm gut proteases activity.

MATERIALS AND METHODS

Insect rearing

Cotton bollworm larvae were provided by a colony in plant protection department of Tabriz University. Larvae were reared on artificial diets based on cowpea (Shorey & Hall, 1965) in controlled condition of 26±2°C, 50±5% relative humidity and a photoperiod of 16:8 (L: D) h.

Enzyme preparation

One day old last-larval instars of cotton bollworm were selected for gut extraction. The individuals were chilled and dissected in cold petri dishes. Each gut with lumen contents was moved to 1.5 ml micro tubes containing 1 ml cold Glycine-NaOH, pH 10, buffer. The collected guts were then homogenized using Ultra turrax T8 homogenizer then centrifuged for 10 minutes at 10000 rpm and 4°C. The supernatant was used as enzyme solution (Mohammadi et al., 2015).

Crude extracts and protein fractions preparing of Inhibitors

Plant seeds were prewashed with distilled water and dried in room temperature then ground using mortar and pestle. The prepared flour was soaked in Glycine-NaOH buffer, pH 10.0 for 90 minutes in 6°C. The homogenates were centrifuged at 10000 rpm for 30 minutes at 4°C.The proteins collected from the supernatant were used for protease inhibition assay (Baker, 1987; Melo et al., 1999).

For partial purification of proteins, crude extracts obtained from the seeds of studied plants were precipitated at 0-30, 30-50, 50-70 and 70-80% saturation with ammonium sulfate and four protein fractions (F1 – F4) were obtained (Mohammadi et al., 2010).

Determination of protein concentration

Protein concentration was estimated by the method of Bradford (1976) using bovine serum albumin (BSA) as the standard.

Enzyme activity and inhibitory assays

Total protease activity determined using azocaseinolytic assay. Azocasein at final concentration of 1% (w/v) was incubated with the enzyme fraction in Glycine-NaOH 200 mM buffer, pH 10, containing 5 mM $CaCl_2$, at 37°C for 60 min. The reaction was terminated by the addition of 300 µl of TCA (10% v/v) and the sample was centrifuged for 10 min at 10000 rpm. The supernatant was added to 1M NaOH in equal volumes. And the absorbance of the supernatant was read at 450 nm. Protease and inhibitory activity was defined as the amount of enzyme that increased the absorbance by 1.0 OD under the given assay condition.

For the inhibitory assays, a suitable volume of seed extract was added to the gut proteinase extract and incubated at room temperature (27°C) for 15 min. The residual proteinase activity was then estimated for every assay.

Statistical analysis

Statistical analyses were done using SPSS 15 software. The effects of PIs from plant materials on *H. armigera* last larval instars. enzyme activity analysed using one way-ANOVA. When a significant effect was found the Duncan's multiple range test was performed to compare the means (P=0.05). All experiments carried out in three replications.

RESULTS AND DISCUSSION

Efficiency of crude seed extracts against HGP inhibitory activity

The crude extracts possess activities in the range between 18.11 to 55.33 % which are considered to have strong inhibitory activity (Fig. 1). The fabaceae species showed strong inhibitory activity against HGP (mean 45%) in while poaceae family species showed a moderate inhibitory activity (mean 19%).

All the species showed inhibitory activity *in vitro* against HGP activity while *P. vulgaris* crude seed extract showed a strong inhibitory activity of 55% against HGP among studied plant species.

HGP inhibitory activity of different protein fractions of Poaceae species

In different fractions of Poaceae species, inhibitory activity without significant differences was observed. Only *Z. mays* F4 fraction protein significantly affected HGP activity more than other fractions by 30% (Fig. 2).

HGP inhibitory activity of different protein fractions of Fabaceae species

Analysis of variances showed significant differences among F1 to F4 protein fractions in Fabaceae species. HGP activity strongly affected by *F. vulgaris* fractions and specially 70-80% fraction inhibited HGP activity more than 45%. About C. *arietinum* 70-80% protein fraction has more efficiency than other ones. The first fraction in all plants showed moderately to weak inhibitory activity in the range of 10-16%. The most inhibitory activity in *S. alopecuroides* was measured in 50-70% fraction by 26% (Fig. 3).

The use of conventional insecticides to control insect pests poses hazards to human health, non-target species, beneficial insects and environment. Indiscriminate use of chemical insecticides can also select insecticide resistance populations of pests (Harrison & Bonning, 2010). The digestive enzymes such as proteolytic and amylolytic enzymes are a target for insect pest management programs that are safe and environmentally friend method. Digestive enzymes play important roles in insect growth, development and reproduction; other functions including enzyme activation and detoxification are in relation with protease enzymes in insects digestive system (Christeller et al., 1992; Terra et al., 1996). Digestive systems of the lepidopteran larva contain proteases such as trypsin, chymotrypsin and elastase. Özgur et al. (2009) studies on *H. armigera* digestive protease showed that, serine proteases are dominant protease in the *H. armigera* midgut.

Several families of proteinase inhibitors has recognized among the animal and plant kingdom. Majority of proteinase inhibitors studied in plant kingdom originates from three main families namely Fabaceae, Solanaceae and Poaceae (Wee, 2000). Many investigators have isolated and characterized enzyme inhibitors from poaceae species such as barley, wheat and maize. Divya et al. (2014), reported that *Z. mays* contains PIs with trypsin and chymotrypsin inhibitory activity and these enzymes are abundant in cotton bollworm gut (Ozgur et al., 2009). Also Gourinath et al. (2000) reported that the members of Poaceae family have serine protease inhibitors. Odani et al. (1983) has reported that a large number of inhibitors in poaceae family have only α-amylase-inhibitory activity; however inhibitors from barley, rye and tall fescue are active against trypsin.

Maize and ragi inhibitors showed dual activities and can inhibit serine proteinases as well as α-amylase (Mahoney et al., 1984; Shivraj & Pattabiraman, 1981; Habib & Fazili, 2007). Boisen (1983), reported that, Inhibitors of trypsin, chymotrypsin and microbial proteases are the most common PIs in barley and are present mostly in seeds also trypsin and chymotrypsin inhibitory activity was detected in barley by Casaretto et al. (2004). Constantin et al. (2008) and Poerio et al. (2003) demonstrated that protease inhibitors are present in leaf and seeds of wheat. In present study protease inhibitory activity of different poaceae species has observed and all fractions with minor differences contains HGP inhibitory activity which is in agreement above mentioned reports. The present study concluded that all the species possess the potential to inhibitory *H. armigera* gut protease activity. Results revealed that the F1 fraction protein of all studied plants

showed less than 20% inhibitory activity against HGP, and the F2 and F3 fraction exhibited the same inhibitory activity in the range of 10-20% in Poaceae species.

Fabaceae species are rich of proteins and protease inhibitors and plenty of studies isolated and characterized different PIs from leaves, seeds and foliage of different Fabaceae species (Giri et al., 1998; Franco, 2003; Fan & Wu, 2005; Kansal, 2008; Abd El-latif, 2015). The PIs from the wild relatives of pigeonpea showed considerable potential against the HaGPs (Parde et al., 2012).

Our results were concurrent with those by Nair et al. (2013), *C. arietinum* (chickpea) seeds are known to contain, inhibitors of proteases. This study has shown that *P. vulgaris* and *C. arietinum* protease inhibitor caused a significant decrease in proteolytic activity in the gut of *H. armigera* compared to the other inhibitors. The legume plants especially in F1, F2 and F3 fractions exhibited near 15-50% inhibitory activity on HGP. In over all, among studied plants, *Z. mays, P. vulgaris* and *C. arietinum* have strong inhibitory activity in compare with other plants that are in agreement with above mentioned reports.

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LITERATURE CITED

Abd El-latif, A. O. 2015. Protease purification and characterization of a serine protease inhibitor from Egyptian varieties of soybean seeds and its efficacy against *Spodoptera littoralis*. J. Plant Prot. Res., 55: 16-25.

Baker, J. E. 1987. Electrophretic analysis of amylase isozymes in geographical strains of *Sitophilus oryzae*, *S. zeamays* and *S. granarius* (Coleoptera: Curculionidae). J. Stored Prod. Res., 23: 125-131.

Boisen, S. 1983. Effects of gut flora on intestinal activities of trypsin, chymotrypsin, elastase and amylase in growing rats fed purified diets with cellulose, pectin or sand. Denmark J. Anim. Physiol a Anim Nutr., 53: 245-254.

Bradford, M. M. 1976. A Rapid and sensitive method for the quantitation of microgeram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Casaretto, J. A., Zúñiga, G. E. & Corcuera, L. J. 2004. Abscisic acid and jasmonic acid affect proteinase inhibitor activities in barley leaves. J.plant physiol., 161: 389-396.

Constantin, C., Quirce, S., Grote, M., Touraev, A., Stoecklinger, I. S., Adriano Mari, A., Thalhamer, J., Heberle-Bors, E. & Valenta, R. 2008. Molecular and Immunological Characterization of a Wheat Serine Proteinase Inhibitor as a Novel Allergen in Baker's Asthma1. J. Immunolog., 180: 7451-7460.

Christeller, J. T., Laing, W. A., Markwick, N. P. & Burgess, E. P. J. 1992. Midgut protease activities in 12 phytophagous lepidopteran larvae: dietary and protease inhibitor interactions. Insect Biochem. Mol. Biol., 22: 735-746.

Dorrah, M. A. 2004. Effects of soybean trypsin inhibitor on digestive proteases and growth of larval *Spodoptera litturalis* (BOISD). Efflatounia, 4: 23-30.

Fan, S. G. & Wu, G. J. 2005. Characteristics of plant proteinase inhibitors and their applications in combating phytophagous insects. Bot. Bull. Acad. Sin., 46: 273-292.

Franco, O. L., DosSantos, R. C., Batista, J. A. N., Mendes, A. C. M., Dearaujo, M. A. M., Monnerat, R. G., Grossa-se, M. F. & De Freitas, S. M. 2003. Effect of black-eyed trypsin/chymotrypsin inhibitor on the proteolytic activity and on the Development on *Anthonomus grandis*. Phytochem., 63: 343-349.

Giri, A. P., Harsulkar, A. M., Deshpande, V. V., Sainani, M. N., Gupta, V. S. & Ranjekar, P. K. 1998. Chickpea Defensive Proteinase Inhibitors Can Be Inactivated by Podborer Gut Proteinases. Plant Physiol., 116: 393-401.

Ghodke, A. B., Chavan, S. G., Sonawane, B. V. & Bharose, A. A. 2013. Isolation and in vitro identification of proteinase inhibitors from soybean seeds inhibiting *Helicoverpa armigera* gut proteases. J. Plant Interact., 8: 170-178.

Grover, S., Grewal, S. K., Gupta, A. K. & Taggar, G. K. 2014. Exploring the potential of seed flour from pigeon peage notypes to retard the growth and development of *Helicoverpa armigera*. J. Appl. Nat. Sci., 6: 633-637.

Gruden, K., Strukelj, B., Popovic, T., Lenarcic, B., Bevec, T., Brzin, J., Kregar, I., Herzog, V. J. & Stiekema, W. J. 1998. The cysteine protease activity of Colorado potato beetle (*Leptinotarsa decemlineata* Say) guts, which is insensitive to potato protease inhibitors, is inhibited by thyroglobulin type-1 domain inhibitors. Insect Biochem Mol. Biol., 28: 549-560.

Gourinath, S., Alam, N., Srinivasan, A., Betzel, C. & Singh, T. P. 2000. Structure of the bifunctional inhibitor of trypsin and alpha-amylasefrom ragi seeds at 2.2 A resolution. Acta Crystallographica Section D, 56 (3): 287-293.

Harrison, R. L. & Bonning, B. C. 2012. Proteases as Insecticidal Agents. Toxins, 2: 935-953.

Harsulkar, A. M., Giri, A. P., Patankar, A. G., Gupta, V. S., Sainani, M. N., Ranjekar, P. K. & Deshpande, V. V. 1999. Successive Use of Non-Host Plant Proteinase Inhibitors Required for Effective Inhibition of *Helicoverpa armigera* Gut Proteinases and Larval Growth. Plant Physiol., 121: 497-506.

Habib, H. & Fazili, K. M. 2007. Plant protease inhibitors: a defense strategy in plants. Biotechnol. Mol. Biol. R., 2: 68-85.

Jamal, F., Pandey, P. K., Singh, D. & Khan, M. Y. 2013. Serine protease inhibitors in plants: nature's arsenal crafted for insect predators. Phytochem. Rev., 12: 1-34.

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

Melo, F. R., Sales, M. P., Pereira, L. S., Bloch, C., Franco, O. L. & Ary, M. B. 1999. Alpha-amylase inhibitors from cowpea seeds. Protein and peptide Lett., 6: 385-390.

Mahoney, W. C., Hermodson, M. A., Jones, B. & Powers, D. D. 1984. Aminoacid sequence and secondary structural analysis of the corn inhibitorof trypsin and activated Hageman factor. J. Biol. Chem., 259: 8412-8416.
Mohammadi, D., Farshbaf Pour Abad, R., Rashidi, M. R. & Mohammadi, S. A. 2010. Activity and some properties of *Helicoverpa armigera* Hübner and *Spodoptera exigua* Hübner (Lep.: Noctuidae) midgut protease. Munis Ent. Zool., 5: 697-706.
Mohammadi, D., Farshbaf Pour Abad, R., Rashidi, M. R. & Mohammadi, S. A. 2015. Circadian variation in midgut digestive enzyme activity in cotton bollworm *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). J. Asia-Pac. Entomol., 18: 117-121.
Nair, M., Sandhu, S. S. & Babbar, A. 2013. Purification of trypsin inhibitor from seeds of *Cicer arietinum*(L.) and its insecticidal potential against *Helicoverpa armigera* (Hübner). Theor. Exp. Plant Physiol., 25: 137-148.
Odani, S., Koide, T. & Ono, T. 1983. The complete amino acid sequence ofbarley trypsin inhibitor. J. Biol. Chem., 258: 7998-8003.
Özgur, E., Yücel, M. & Öktem, H. A. 2009. Identification and characterization of hydrolytic enzymes from the midgut of the cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). Turk. J. Agric. For., 33: 285-294.
Parde, V. D., Sharma, H. C. & Kachole, M. S. 2010. In vivo inhibition of *Helicoverpa armigera* gut pro-proteinase activation by non-host plant protease inhibitors. J. Insect Physiol., 56: 1315-1324.
Padul, M. V., Patil, M. T., Chougale, A. D., Zambare, V. P., Patil, R. M., Ghule, R. B., Naikwade, S. V., Garad, A. S., Shaikh, F. K., Gadge, P. P., Shinde, K. D., Dama, L. B. & Salve, A. N. 2012. In vitro screening of proteinase inhibitors (trypsin, chymitrypsin and *Helicoverpa* gut proteinase inhibitors) in different plant tissue extracts. TIBSR, 1: 7-14.
Poeriol, E., Di Gennaro, S., Di Maro, A., Farisei, F., Ferranti, P. & Parente, A. 2003. Primary Structure and Reactive Site of a Novel Wheat Proteinase Inhibitor of Subtilisin and Chymotrypsin. Biol. Chem., 384: 295-304.
Qutchkourov, N. S., Rogelj, B., Strukelj, B. & Jongsma, M. A. 2003. Expression of Sea Anemone Equistatin in Potato. Effects of Plant Proteases on Heterologous Protein Production. J. Plant Physiol., 133: 379-390.
Ryan, C. A. 2006. The systemin signaling pathway: different activation of plat defensive genes. Biochem. Biophys. Acta, 1477: 112-121.
Shukla, S., Arora, R. & Sharma, H. C. 2005. Biological activity of soybean trypsin inhibitor and plant lectins against cotton bollworm/legume pod borer, *Helicoverpa armigera*. Plant Biotechnol., 22: 1-6.
Shorey, H. H. & Hale, R. L. 1965. Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. J. Econ. Entomol., 58: 522-524.
Shivraj, B. & Pattabiraman, T. N. 1981. Natural plant enzyme inhibitors.Characterization of an unusual alpha amylase/trypsin inhibitor fromragi (*Eleusine coracana* Geartn.). Biochem. J., 193: 29-36.
Tamhane, V. A., Chougule, N. P., Giri, A. P., Dixit, A. R., Sainani, M. N, & Gupta, V. S. 2005. In vivo and in vitro effect of *Capsicum annum* proteinase inhibitors on *Helicoverpa armigera* gut proteinases. Biophys. Acta, 1722: 156-167.
Telang, M., Srinivasan, A., Patanker, A., Harsulkar, A., Joshi, V., Damle, A., Deshpande, V., Sainani, M., Ranjekar, P., Gupta, G., Birah, A., Rani, S., Kachole, M., Giri, A. & Gupta, V. 2003. Bitter ground proteinase inhibitors: potential growth inhibitors of *Helicoverpa armigera* and *Spodoptera litura*. Phytochem., 63: 643-652.
Terra, W. R., Ferreira, C., Jordao, B. P. & Dillon, R. J. 1996. Digestive enzymes. In: Lehane, M. J. & Billingsley, P. F. editors. Biol. Insect Midgut, Chapman & Hall, pp. 153-194.
Vinod, D., Parde, V. D., Sharma, H. C. & Kachole, M. S. 2012. Protease Inhibitors in Wild Relatives of Pigeonpea against the Cotton Bollworm/Legume Pod Borer, *Helicoverpa armigera*. American. J. Plant Sci., 3: 627-635.
Wee, K. E., Yonan, C. R. & Chang, F. N. 2000. A new broad-spectrum protease inhibitor from the entomopathogenic bacterium, *Photorhabdus luminescens*. Microbiology, 146: 3141-3147.

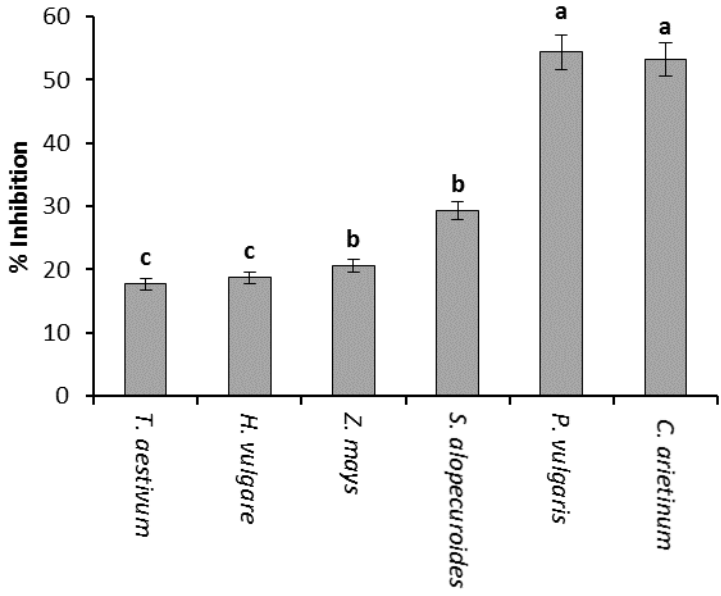

Figure 1. PI activity of different plant species crude seed extracts on *H. armigera* midgut protease activity (The means followed by different letters are significantly different, p=0.01).

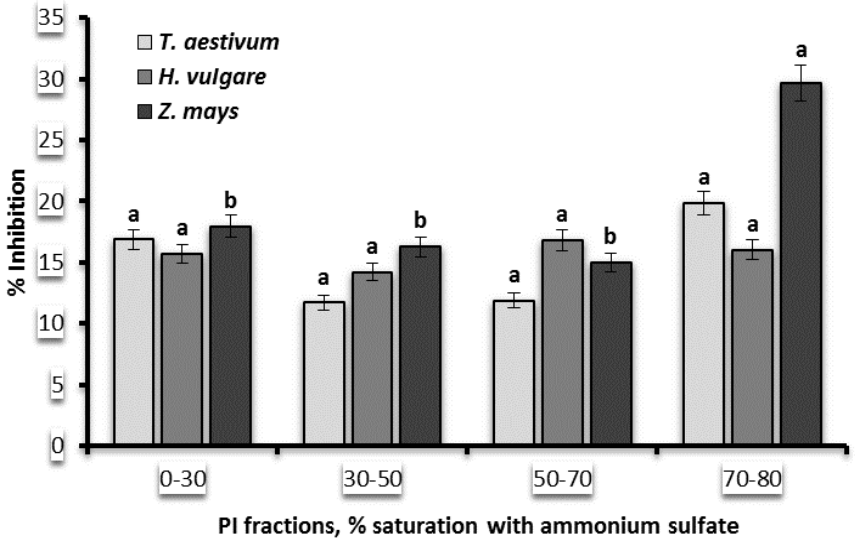

Figure 2. HGPI activity of different protein fractions of Poaceae family plants prepared with saturation in Ammonium sulfate (The means followed by different letters in each plant are significantly different, p=0.01).

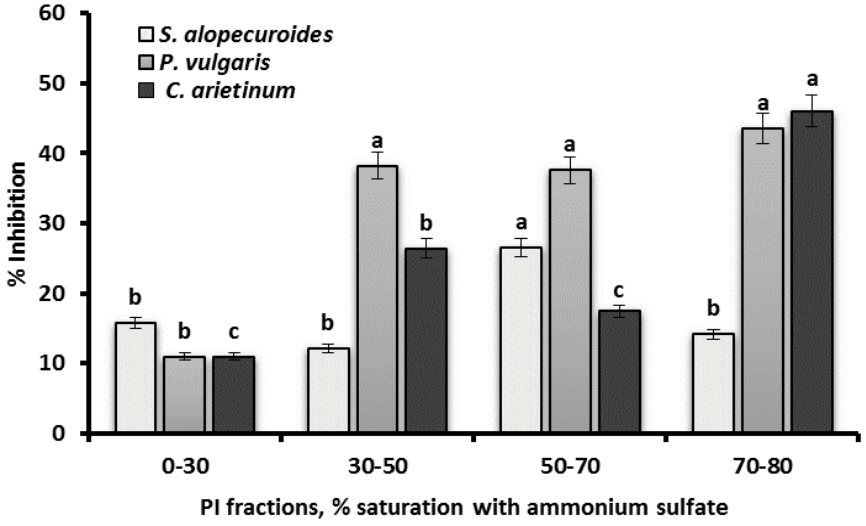

Figure 3. PI activity of different protein fractions of Fabaceae family plants prepared with saturation in Ammonium sulfate (The means followed by different letters in each plant are significantly different, p=0.01).