Received: 20 Aug 2020 | Revised: 10 Sep 2020 | Accepted: 13 Sep 2020

Electronic Journal of Plant Breeding

Research Article

Understanding the role of different biochemical compounds responsible for inducing resistance in pigeon pea towards *Helicoverpa armigera* infestation

Mareyam Mukhtar[1], L. Arul[1], P. Jayamani[2] and E. Kokiladevi[1]*

[1]Department of Plant Biotechnology, CPMB & B, Tamil Nadu Agricultural University, Coimbatore 641003, India
[2]Department of Pulses, CPBG, Tamil Nadu Agricultural University, Coimbatore 641003, India
***E-Mail**: kokiladevi@tnau.ac.in

Abstract
Pigeon pea's productivity has been stagnant over the years because of the devastating pest *Helicoverpa armigera* which causes major yield losses. The present study explored different responses of various defense strategies in leaves and pods of four pigeon pea cultivars (ICP-12142, ICP-11543, LRG-41 and CO-8) after infestation. Two pigeon pea cultivars *viz;* ICP-11543 and CO-8 were ascertained to be susceptible to infestation with excessive leaf and pod damage compared to the other two cultivars. Decreased activities of defensive enzymes/compounds like peroxidase (POD), superoxide dismutase (SOD), polyphenol oxidase (PPO) and total phenols were reported following infestation in cultivars of ICP-11543 and CO-8 which resulted in intensification of infestation. The cultivars; LRG-41 and ICP-12142 were found to be moderately resistant to pest infestation because of the up-regulation of PPO, POD and SOD along with accumulation of total phenols which might be held accountable for shifting the oxidative status of the respective cultivars.

Key words
H. armigera, POD, PPO, SOD, pigeon pea

INTRODUCTION

Plants have been exposed to herbivorous insects for at least 100 million years (Stotz *et al.,* 1999). To deter the feeding of herbivorous insects, extensive ranges of physicochemical strategies have evolved in plants (Rasmann *et al.,* 2009). Such defense mechanisms may be constitutive, *i.e.,* it will be present regardless of any stress and establishing the front line of defense to herbivorous insects or inducible, *i.e.* activated when attacked (Franceschi *et al.,* 2005). The herbivorous-stressed plants develop active defensive reactions at site of damage in systemic manner in undamaged tissue (Kessler *et al.,* 2002). The *Helicoverpa armigera*, is a polyphagous pest far spread to Africa, Asia, Australia and southern parts of Europe. It attacks over 200 species of plants, including cotton, pigeon pea, chickpea, maize, sorghum and groundnut and has grown resistance to almost all the insecticides (Sharma *et al.,* 2005). It also drastically reduces the yield of various crops. Pigeon pea is considered as one of the key host for *H. armigera (*Rajapakse *et al.,* 2007; Srikanth *et al.,* 2017). While the neonate instars thrive on leaves and reproductive parts (flowers), the older instars nourish inside the pods, causing critical damage to the seeds. Therefore, alternative methods are required to control this plague (Karban *et al.,* 2011). One of the most significant and extensive herbivory defenses embraced by plants is induced resistance. Induced resistance in plants towards herbivorous insect attack is generated by up-regulating oxidative enzymes such as polyphenol oxidase (PPO), ascorbate oxidase (AOX) lipoxygenases (LOXs), polyamine oxidase (PO), *etc.* This metabolism shift is called oxidative shift which is due to the up-regulation of oxidative enzymes activated upon infestation. These oxidative enzymes play many possible roles in anti-herbivore defense plants: (1) direct

oxidative injury towards the herbivore (2) indirect injury to the herbivore mediated by oxidative damage to lipids, antioxidants, proteins, vitamins, *etc.,* (Felton *et al.,* 1994). Due to oxidative shifts, induced resistance is developed in pigeon pea after *Helicoverpa armigera* infestation. Therefore, the plants protect themselves by creating a nutritional and oxidative stress against herbivores through the exhibition of reactive oxidants and activated oxygen. Infestation of pest in plants is always accompanied with oxidative stress which produces tremendous production of free radicals like hydrogen peroxide (H_2O_2) and super-oxide anion ($O_2^{-\bullet}$) *etc.,* which initiate membrane damage, DNA modification and lipid peroxidation generating irreversible structural and metabolic impairment. The abrupt increase in free radicals advancing to oxidative bursts and resulting in decrease of antioxidants which could be correlated with drastic oxidative damage to important biomolecules like proteins, pigments, lipids and nucleic acids *etc., (*Scheler *et al.,* 2013; Wang *et al.,* 2014). These free radicals also exhibit binary role, both as signaling molecules vital for plant development and as injurious metabolism by-products that cause damage to lipids, DNA and RNA (Mitler *et al.,* 2017). A proper balance must be maintained between free radical production and free radical detoxification enzymes/compounds in order to reduce damage to plant tissues owing to oxidative stress. An anti-oxidative defense system has been developed encompassing a number of antioxidants that can inactivate or quench free radicals so as to minimize the oxidative stress caused by accumulation of free radicals (Nagar *et al.,* 2017). Enzymes responsible for defense like peroxidase (POD), superoxide dismutase (SOD), polyphenoloxidase (PPO) and secondary metabolites including phenols are key players in metabolizing the free radicals (Gill and Tuteja 2010). Nevertheless, tolerating oxidative stress is not a single step mechanism but unified counter attack regulated by the concentration of defensive enzymes and antioxidants. For this reason, it is crucial to comprehend the role of different enzymes or compounds affiliated with the defense mechanism for the development of cultivars with insect resistance. The present study was framed to distinguish the different biochemical responses involved in defense like antioxidant enzymes, defensive enzymes/ compounds in leaves and pods of different pigeon pea cultivars at various intervals of time after *H. armigera* infestation.

MATERIALS AND METHODS

Experimental material, pest infestation and statistical design:Seeds of *Cajanus cajan* were obtained from Department of Pulses, CPBG, Tamil Nadu Agricultural University and International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, India. Four pigeon pea genotypes namely ICP-12142, ICP-11543, LRG-41 and CO-8 were sown in pots with three replications at greenhouse of Department of Biotechnology, Tamil Nadu Agricultural University, Coimbatore. Leaves and pods of 4 months old plants were used for the infestation treatment. To prevent any natural infestation of different cultivars, individual plants were covered by muslin cloths of 42 cm × 25 cm size wire mesh before flowering of each genotype and were maintained till the end of experiment. Two numbers of third instar larvae of *H. armigera* were released on individual pigeon pea plants inside the muslin cloth cage with camel hair brush for each replication per cultivar. Experiments with no larvae released were used as control. Leaf as well as pod samples were collected from the control and infested plants of four pigeon pea genotypes after 24 and 36 hr of infestation.

Damage score of leaves was calculated from individual pigeon pea cultivars by measuring the area on which *H. armigera* fed on using scale ranging from 1-9, where 1 is for ≤ 10% leaf area eaten; 2 is for 10–20% leaf area eaten; 3 is for = 21–30% leaf area eaten and 9 is for ≥ 80% leaf area eaten. The pod damage was noted in per cent after harvesting by counting the total pods as well as damaged pods by the *H. armigera* using the mathematical equation:

$$\text{Percentage of pod damage} = \frac{\text{Number of damaged pods}}{\text{Total number of pods}} \times 100$$

The percent pod damage was changed to pest susceptibility/resistance (%) by using the formula taken from Abbott (1925):

$$\text{Susceptibility (\%)} = \frac{\text{P.D.of check} - \text{P.D.of test genotype}}{\text{P.D.check}} \times 100$$

(Table 1.)
Pest Susceptibility percentage was converted to pest susceptibility rating (PSR) scale (1–9) (Kooner and Cheema 2006).

Around 3 mg of leaf as well as pod sample was taken separately in pre-chilled pestle and mortar and then macerated with ice cold 0.1 M phosphate buffer (pH 7) comprising of 2% PVP, 1 mM EDTA, 10 mM β-mercaptoethanol. Homogenized mixture was centrifuged at 10,000×g at 4° C for 15 mins and supernatant was used for determination of enzyme assay. Each enzyme activity were checked in three replications. The activity of SOD was analyzed by using 0.1 mM Tris HCl buffer (pH 8) 5 mM EDTA, 0.1 ml of enzyme extract and 5 mM pyrogallol solution. The absorbance was recorded at 420 nm using spectrophotometer with an interval of 30 s up to 3 min (Marklund and Marklund 1974). PPO assay was done according to the method given by (Arnnok *et al.,* 2010). The supernatant was mixed with 0.05 M phosphate buffer and 1 ml of 0.1 M catechol. The absorbance was noted at 410 nm at 30 sec intervals for 3 min. POD assay was performed by using 2 ml of 0.05 M guaiacol, 30 µl of enzyme extract and 0.1 ml of 0.8 M H_2O_2. H_2O_2 initiated the reaction and absorbance was measured at 470 nm in accordance with the method given by (Shannon *et al.,* 1966).

Table 1. Damage in leaves and pods of different pigeon pea cultivars following *H. armigera* infestation.

Cultivars	Leaf damage (%)	Leaf damage score	Pod damage (%)	Pest susceptible rating (PSR)
ICP-12142	15	2	10.4	2
ICP-11543	20	3	14.67	2
LRG-41	14	2	10	2
CO-8	21	3	16.79	4

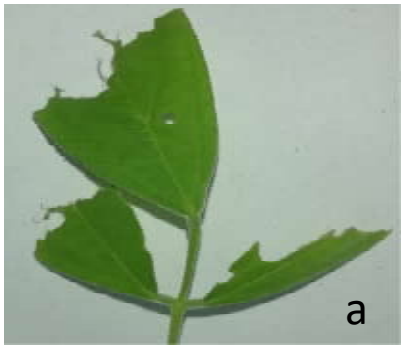
Assay of defensive compounds- Total phenols was analyzed using Folin-Denis reagent according to the protocol stated by (Swain and Hillis 1959).

Statistical analysis- Statistical parameters like mean and standard deviation (SD) were calculated from the data set. Data was examined by factorial CRD (Software SPSS) and the differences between the cultivars were analyzed by Duncan test (Software SPSS) with $P \leq 0.05$ whereas MS Excel 2010 was used regarding the correlation analysis.

RESULTS AND DISCUSSION

Total phenol was reported to be significantly higher in the *H. armigera* infested pigeon pea genotypes and there was a rapid induction of phenolics due to the infestation in all the pigeon pea cultivar used for the study (**Fig. 1 a and b**). Maximum phenol content was observed in the *H. armigera* infested ICP-12142 cultivar as compared to other cultivars. The concentration of total phenols were higher in the leaves of ICP-12142 (1.23 fold), ICP-11543 (1.22 fold), LRG-41 (1.16 fold) and CO-8 (1.01 fold) after 36 hrs of infestation. Total phenols drastically escalated in pods of all pigeon pea cultivars after infestation, but it was observed to be less in CO-8.

In all the pigeon pea cultivars, peroxidase activity were reported to be considerably high in cultivars after infestation of *H. armigera* compared to healthy plants (**Fig. 2 a and b**). The peroxidase activity were found to be elevated in leaves of all the pigeon pea cultivars after 36 hrs of infestation, except in CO-8. After 36 hrs of infestation, it was reported to be increased by 1.2 folds in pods of all the cultivars, except ICP-11543.



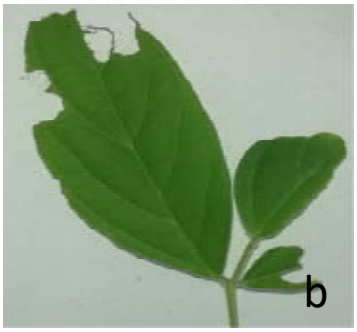
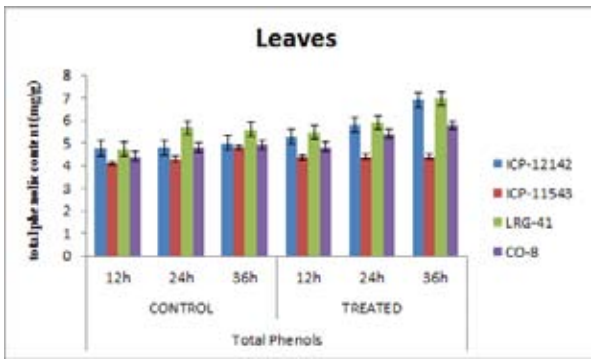


Fig. a and b – Infestation by H. armigera after 24 hours in cultivar CO-8 (a) and ICP-12142 (b).



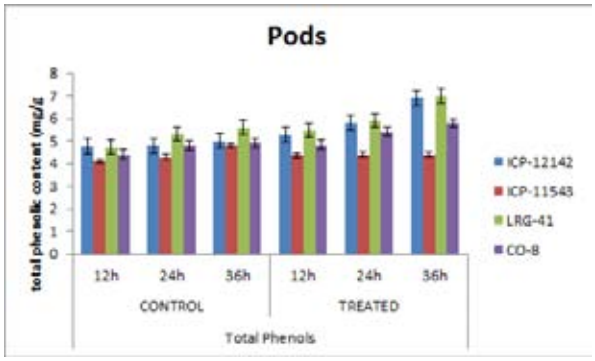


Fig. 1 (a) Total phenols in leaves and in pods (1b) of healthy/control and *H. armigera* infested pigeon pea cultivars. Mean of three replications are pooled and is presented in the data. SD of triplicates is represented by error bars

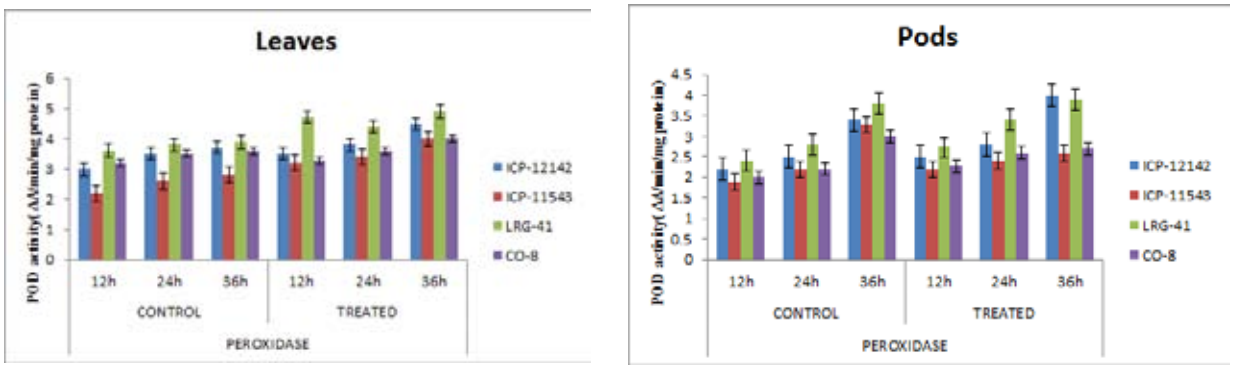


Fig. 2 (a) Peroxidase in leaves and in pods (b) of healthy/control and *H. armigera* infested pigeon pea cultivars. Mean of three replications are pooled and is presented in the data. SD of triplicates is represented by error bars.

All the cultivars which were infested with *H. armigera*, the activity of PPO was observed to be significantly higher compared to the control (**Fig. 3 a and b**). PPO activity was reported to be increased in leaves and pods of all the pigeon pea genotypes following infestation. In LRG-41 it was found to be significantly increased *i.e.,* 1.4 folds than other cultivars after infestation. PPO activity wasn't significantly affected after infestation in CO-8. In leaves, it were found that ICP-11543 showed 1.4 fold increases than other cultivars. In pods, the trend was not the same as LRG-41 was showing a significant increase in PPO than other genotypes.

High levels of constitutive SOD activity were observed in the healthier tissues of the cultivars tested (Fig. 4 a and b). It increased (1.1 fold to 1.2 fold) in leaves of all the pigeon pea cultivars following 24 hr of infestation with *H. armigera*. However, after 36 hr of infestation, the trend observed was not identical as it increased in some cultivars such as ICP-12142 and LRG-41 and decreased in ICP-11543 and CO-8 cultivars. After 24 hr of infestation, the SOD activity was detected to increase by 1.21–2.23 folds in all the cultivars except in ICP-11543. In pods, it were same in ICP-12142 and LRG-41. It was higher in ICP-12142 by 1.2 folds than the other cultivars.

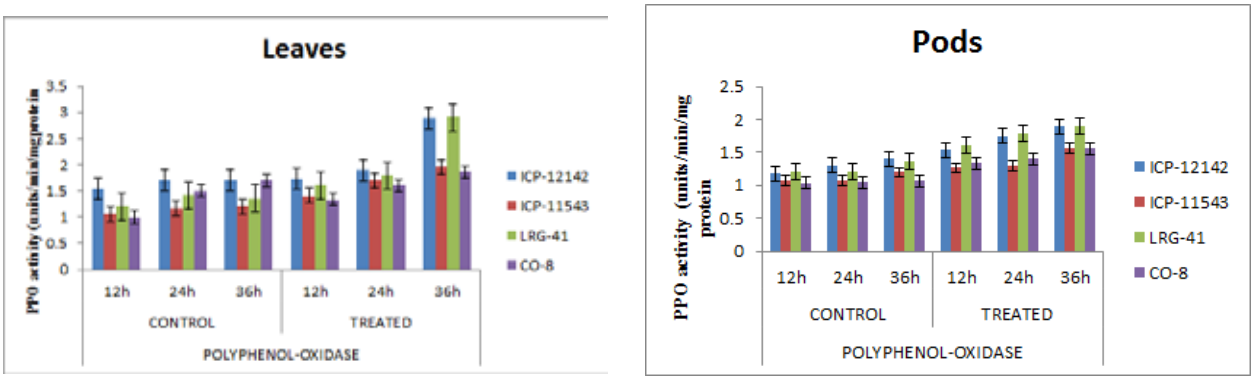


Fig. 3. (a) Polyphenol oxidase in leaves and in pods (b) of healthy/control and *H. armigera* infested pigeon pea cultivars. Mean of three replications are pooled and is presented in the data. SD of triplicates is represented by error bars.

Pest susceptible rating - Damage induced by *H. armigera* in leaves were reported to be 14-15% in LRG- 41 and ICP-12142 and in other cultivars *i.e.,* ICP-11543 and CO-8 it was found to be 20-21%. The pod damage was found to be 10.4 and 10% in LRG- 41 and ICP-12142 and 14.67 and 16.79 in ICP-11543 and CO-8. The pest susceptible rating was found to be highest in CO-8 than rest of cultivars due to high damage caused by *H. armigera* infestation. Plants try to avoid insect infestation through detailed set of resistance mechanism involving cascade of defense mechanism which consists of protective enzymes, ROS scavenging enzymes and signaling molecules. To mitigate the effects of infestation, the plant depends on its capability to detect the approaching stimuli that decodes it and communicates a quick physiological or morphological reaction to escape the damage. Due to the sudden elevation in ROS following the infestation of the pests which ultimately leads to oxidative bursts (Bhattacharjee *et al.*, 2005). In order to maintain the ROS levels in cells, well-defined machinery has been established in plants that generate the enzymatic and non-enzymatic antioxidants known as the defensive enzymes (Huang and

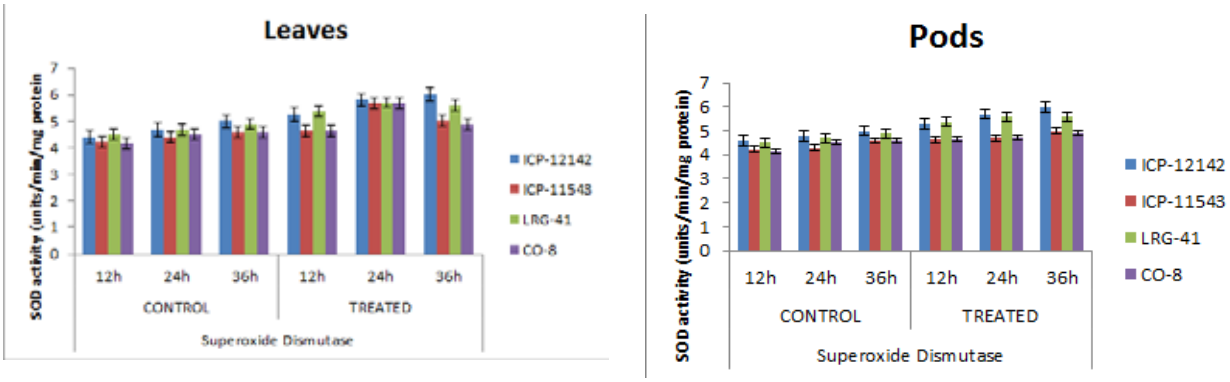


Fig. 4. (a) Superoxide dismustase in leaves and in pods (b) of healthy/control and *H. armigera* infested pigeon pea cultivars. Mean of three replications are pooled and is presented in the data. SD of triplicates is represented by error bars.

Song 2013). Herbivory leads to change in the oxidative status as a result of imbalance between ROS species, anti-oxidants like ascorbate and phenolic components which impart pest tolerance (Bi *et al.,* 1997). The first and foremost defense against insect infestation is established by superoxide dismutase as it converts superoxide radical into molecular O_2 and H_2O_2 (Usha Rani *et al.,* 2010; Devi *et al.,* 2017). The SOD activity is elevated in the leaves and pods of resistant pigeon pea cultivars as reported in ICP-12142 and LRG-41 following *H. armigera* infestation which can help minimize damage to the membranes as a result of scavenging the free radicals thus deterring oxidation of lipids (**Fig. 4 a and b**). War *et al.,* (2013) noted the fact of escalating SOD levels reduce the free radicals generated in plants following infestation, thus generating the accumulation of H_2O_2 which suggested that increasing concentration of H_2O_2 serves as a signal to produce the defensive compounds in cells. Elevated levels of POD and PPO in leaves and pods of ICP-12142 and LRG-41 cultivars following infestation might be protecting them from damage caused due to infestation compared to ICP-11543 and CO-8 as leaf injury caused after infestation was negatively correlated with SOD, POD, PPO and total phenols ($r = -0.68$, $r = -0.95$, $r = -0.57$ and $r = -0.95$ respectively) (**Table 2**). Peroxidase not only help in scavenging of H_2O_2 but also executes other functions like generating quinones and semi-quinones which inhibit the insect feeding (Zhu-Salzman *et al.,* 2008); conversion of hydroxylcinnamyl alcohols to free radical intermediates, phenol oxidation and production of anti-nutritional metabolites (He *et al.,* 2011).

Table 2. Correlation coefficient (r) between various defense related enzymes/molecules with leaf and pod damage following *H. armigera* infestation.

Defense related enzymes/ molecules	Time interval after infestation	Leaves after infestation	Pods after 36 hr after infestation
SOD	24	- 0.68*	- 0.67*
	36		
POD	24	- 0.95*	- 0.85*
	36		
PPO	24	- 0.57**	- 0.79*
	36		
Phenols	24	- 0.95*	- 0.97**
	36		

**Correlation is significant at 0.01 level

PPO was found to increase in the infested cultivars and was observed to be more in mildly resistant cultivars like ICP-12142 and CO-8. Polyphenoloxidase, a metallo-enzyme that oxidizes monophenols and diphenols to quinones, highly reactive intermediate compounds that easily polymerize and react with nucleophilic side chains of amino acids and crosslink proteins, in that way lowers the availability of proteins, developing into brown coloration in injured plant tissues and control feeding and growth of pests (He *et al.,* 2011; Zhang *et al.,* 2008).

The increased content of total phenols after infestation in tissues ICP-12142 and LRG-41 and CO-8 might be held accountable for enhancing the defensive enzymes as compared to ICP-11543 (**Table 2**). Also, it may be noted that there was a higher negative correlation between leaf and pod damage with peroxidase activity as well as with total phenols (**Table 2)**. A key role is played by phenols in reducing ROS *eg.,* singlet oxygen and H_2O_2 (Maffei *et al.,* 2007). Phenol oxidation are one of the probable defense strategies against herbivorous insects.

Phenols can act as an antioxidant which is attributed to their redox nature and thus acts as reducing agent and scavenges free radicals (Yildiz-Aktas *et al.,* 2009). Observation made by Dixit *et al.,* (2017) concluded that phenolics like p-coumaric and cinnamic acid may be effective to get rid of pests as they are reported destructive to *Helicoverpa armigera* infestation.

To conclude, ICP-11543 and CO-8 were found to be susceptible to *H. armigera* infestation because of greater leaf and pod damage as compared to other cultivars. The induced mechanism of defense in cultivars like ICP-12142 and LRG-41 in leaves and pods infested with *H. armigera* is contributed to the amalgamation of various defense related enzymes like SOD, POD and PPO and total phenols as compared to the susceptible cultivars like ICP-11543 and CO-8.

ACKNOWLEDGEMENT

The authors are thankful for the financial assistance received from DBT (DBT Grant No.: BT/PR25127/NER/95/1030/2017 dt 12-09-2018) Government of India, New Delhi. Authors are also thankful to Dr. D. Amirtham, Assistant Professor (Biochemistry) for providing with biochemical protocols to conduct the experiments.

REFERENCES

Arnnok. P., Ruangviriyachai C. Mahachai R. Techawongstien S. and Chanthai S. 2012. Determination of total phenolics and anthocyanin contents in the pericarp of hot chilli pepper (*Capsicum annuum L.). Int Food Res J*. **19**(1).

Bhattacharjee S., 2005. Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transduction in plants. *Current Science*. 1113-1121.

Bi J.L., Murphy J.B. and Felton G.W. 1997. Antinutritive and oxidative components as mechanisms of induced resistance in cotton to *Helicoverpa zea*. *Journal of Chemical Ecology*. **23**(1): 97-117. [Cross Ref]

Chance B., and Maehly A. C. 1955. Assay of catalase and peroxidases. *Methods Enzymol* **2:** 764–775. [Cross Ref]

Devi B N., Chitdeshwari T and Ravikesavan R. 2017. Antioxidative enzyme activities in maize genotypes grown under saline water irrigation. Electronic Journal of Plant Breeding. **8**(2): 636-642. [Cross Ref]

Dixit G., Praveen A., Tripathi T., Yadav V.K. and Verma, P.C. 2017. Herbivore-responsive cotton phenolics and their impact on insect performance and biochemistry. *Journal of Asia-Pacific Entomology*. **20**(2): 341-351. [Cross Ref]

Felton G.W., Bi J.L., Summers C.B., Mueller A.J. and Duffey, S.S. 1994. Potential role of lipoxygenases in defense against insect herbivory. *J. Chem. Ecol.* **20**(3): 651-666. [Cross Ref]

Franceschi V.R., Krokene P., Christiansen E. and Krekling T. 2005. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. *New Phytol.* **167**(2): 353-376. [Cross Ref]

Gill S.S. and Tuteja, N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **48(12):** 909-930. [Cross Ref]

He J., Chen F., Chen S., Lv G., Deng Y., Fang W., Liu Z., Guan Z. and He C. 2011. Chrysanthemum leaf epidermal surface morphology and antioxidant and defense enzyme activity in response to aphid infestation. *J. Plant Physiol.* **168(7):** 687-693. [Cross Ref]

Hillis W.E. and Swain, T. 1959. The phenolic constituents of Prunus domestica. II—The analysis of tissues of the Victoria plum tree. *J. Sci.* **10(2):** 135-144. [Cross Ref]

Karban R. 2011. The ecology and evolution of induced resistance against herbivores. *Funct. Ecol.* **25(2**): 339-347. [Cross Ref]

Kessler A. and Baldwin I.T. 2002. Plant responses to insect herbivory: the emerging molecular analysis. *Annu. Rev. Plant Biol.* **53(1):** 299-328. [Cross Ref]

Kooner B.S. and Cheema H.K. 2006. Evaluation of pigeon pea genotypes for resistance to pod borer complex. *Indian J. Agric. Sci.* **1(1-2):** 194-196.

Maffei M.E., Mithöfer A. and Boland W. 2007. Insects feeding on plants: rapid signals and responses preceding the induction of phytochemical release. *Phytochemistry*, **68(22-24)**: 2946-2959. [Cross Ref]

Marklund S. and Marklund G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem.* **47(3):** 469-474. [Cross Ref]

Mittler R., 2017. ROS are good. *Trends Plant Sci.* **22(1):** 11-19. [Cross Ref]

Nagar A., Sharma V. and Chhipa A.S., 2017. Role of antioxidants in biological system. *Mintage Journal of Pharmaceutical and Medical Sciences*. **1:** 7-12.

Rajapakse C.N.K. and Walter G.H. 2007. Polyphagy and primary host plants: oviposition preference versus larval performance in the lepidopteran pest *Helicoverpa armigera*. *Arthropod-Plant Interactions*. **1(1):** 17-26. [Cross Ref]

Rani P.U. and Jyothsna Y. 2010. Biochemical and enzymatic changes in rice plants as a mechanism of defense. *Acta Physiol. Plant.* **32(4):** 695-701. [Cross Ref]

Rasmann S. and Agrawal A.A. 2009. Plant defense against herbivory: progress in identifying synergism, redundancy, and antagonism between resistance traits. *Curr Opin Plant Biol*. **12(4)**: 473-478. [Cross Ref]

Scheler C., Durner J. and Astier J., 2013. Nitric oxide and reactive oxygen species in plant biotic interactions. *Curr Opin Plant Biol*. **16(4):** 534-539. [Cross Ref]

Shannon L.M., Kay E. and Lew, J.Y. 1966. Peroxidase isozymes from horseradish roots I. Isolation and physical properties. *J. Biol.* **241(9):** 2166-2172.

Sharma H.C., Pampapathy G., Dhillon M.K. and Ridsdill-Smith J.T. 2005. Detached leaf assay to screen for host plant resistance to *Helicoverpa armigera*. *J. Econ. Entomol.* **98(2**): 568-576. [Cross Ref]

Srikanth S., Swathi M., Kollipara P., Rao M. V. and Mallikarjuna N. 2017. Protease inhibitors of Cajanus conferring resistance to pod borer of pigeonpea (*Cajanus cajan* L. Millsp.). *Electronic Journal of Plant Breeding*. **8**(1)**:** 29-37. [Cross Ref]

Stotz H.U., Kroymann J. and Mitchell-Olds T. 1999. Plant-insect interactions. *Curr Opin Plant Biol*. **2(4)**: 268-272. [Cross Ref]

Torres M.A. 2010. ROS in biotic interactions. *Physiol. Plant*. **138(4):** 414-429.Wang J. and Higgins V.J., 2005. Nitric oxide modulates H_2O_2-mediated defenses in the *Colletotrichum* coccodes–tomato interaction. *Physiol. Mol. Plant Pathol.* **67(3-5):** 131-137. [Cross Ref]

War A.R., Paulraj M.G., Ignacimuthu S. and Sharma H.C. 2013. Defensive responses in groundnut against chewing and sap-sucking insects. *J. Plant Growth Regul.* **32(2):** 259-272. [Cross Ref]

Yildiz-Aktas, L.A.L.E., Dagnon S., Gurel A., Gesheva E. and Edreva A. 2009. Drought tolerance in cotton: involvement of non-enzymatic ROS-scavenging compounds. *J Agron Crop Sci***. 195(4):** 247-253. [Cross Ref]

Zhu-Salzman K., Luthe D.S. and Felton G.W. 2008. Arthropod-inducible proteins: broad spectrum defenses against multiple herbivores. *Plant Physiol.* **146(3):** 852-858. [Cross Ref]

.