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Functional Constraints of Enzymatic activity of Tobacco Cutworm in Response to endophytic *Aspergillus terreus*

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ABSTRACT: Endophytic fungi which live within the plant tissues asymptomatically are important mediators of plant-herbivore interactions. Secondary metabolites produced by the fungi or by plants as a result of interactions with fungi have been related to the anti-herbivore properties of fungal endophytes. To cope up from the harmful effects of these mycotoxins, insects have evolved a number of defense mechanisms, such as the production of digestive and detoxifying enzymes. These enzymes help in the detoxification of harmful plant metabolites and have positive effects on insects by breaking down complex compounds into simpler forms. Keeping in view the insecticidal potential of endophytic fungi, the present investigation has been undertaken to analyze the effect of ethyl acetate extract of endophytic *Aspergillus terreus* on the digestive and detoxifying enzymes of *Spodoptera litura* (Fabricius). After feeding the larvae on a diet containing fungal extract (LC50 = 2.31 mg/ml), the enzyme activity was assessed after 48 and 96 hours. The findings showed that when the larvae were fed supplemented diet, the activity of digestive enzymes such as α-amylase, α and β-glucosidases, and α and β-galactosidases reduced. Similarly detoxifying enzymes i.e. phosphatases, esterases, and GSTs have also been shown to be inhibited. It was also determined how endophyte infected cauliflower plants influence the digestive and detoxifying enzymes of *S. litura.* The results revealed that the larvae consuming endophyte-infected plants had reduced levels of activity in their detoxifying and digestive enzymes. In conclusion, the endophytic *A. terreus* exhibits enzyme inhibitory activity against *S. litura*.

Keywords: *Aspergillus terreus, Spodoptera litura,* secondary metabolites, digestive and detoxifying enzymes.

INTRODUCTION

Alternative methods of insect-pest management have greatly benefited from the growing demands for less chemical input in agriculture and an increase in pesticide resistance. The use of myco-biocontrol, a sustainable strategy based on organic methods of pest control, offers an attractive substitute for the use of synthetic pesticides. Endophytic fungi are widely distributed throughout plants, frequently have no negative impact on them, and may even increase plant's ability to withstand biotic and abiotic challenges (Ownley *et al.,* 2008; Moloinyane and Nchu 2019). Through the stimulation of plant defenses or directly through the synthesis of fungal compounds with insecticidal capabilities, endophytic entomopathogenic fungi may assist in protecting plants against herbivores (Moloinyane and Nchu 2019). Additionally, it was shown that plants that had been artificially infected with endophytic fungus showed resistance to insect pests (Shiba *et al.,* 2010; Menjivar *et al.,* 2012). The principal metabolites typically produced by endophytic fungi within host plants include alkaloids, flavonoids and phenolic compounds (Espinoza *et al.,* 2019).

Microorganisms may deploy a variety of defense methods, including the suppression of vital enzymes of pests. According to reports (Kaur *et al.,* 2016; Thakur *et al.*, 2012), the mycotoxins produced by the endophytic fungus function as larvicides, growth retardants, as well as deterrents to feeding and oviposition. By altering the levels of digesting enzymes, antioxidant enzymes, and detoxifying enzymes (Carletto *et al.*, 2010; Homayoonzadeh *et al.,* 2020), these secondary metabolites can also have an impact on herbivore physiology.

In order to produce metabolites and energy, digestive enzymes break down complex food molecules into smaller units (Klowden, 2007; Zibaee and Stovtcheva 2011). The digestive enzymes amylase, lipase, glycosidases, and proteases are all significant ones that are found in insects. According to Terra and Ferriera (2012), α-amylase catalyzes the endo-hydrolysis of lengthy chains like starch and glycogen. While lipases hydrolyze the fat molecules, glycosidases break down carbohydrate oligomers into monosaccharides and catalyze the hydrolysis of terminal, non-reducing 1,4-linked alpha-D glucose residues (Terra and Ferriera 2012; Zibaee *et al.,* 2008, 2009). The activity of digestive enzymes has been found to vary or be altered by a variety of fungal endophytes (Kaur *et al.,* 2019; Singh *et al.*, 2015).

In order to deal with the toxic effects of plant allelochemicals, insect herbivores have also coevolved a variety of defensive strategies, such as the sequestration of plant toxins, increased rates of excretion, feeding on less-defended plant parts, or the

use of detoxification enzymes for biochemical metabolism (Pang *et al.,* 2010). Detoxification is a well-known biochemical adaptation used by insect herbivores to use plants that would otherwise be poisonous. It also serves as a key mechanism for pesticide resistance. Among the important detoxifying enzymes found in insects are glutathione-S-transferases (GSTs), phosphatases, and esterases (Zibaee and Stoytcheva 2011). The detoxification of organophosphorous pesticides and the development of pesticide resistance in insect species are both facilitated by the widely dispersed family of enzymes known as esterases, which hydrolyze amide, carboxylester, and thioester bonds (Georghiou and Lagunes-Tejeda 1991; Conyers *et al.*, 1998). According to Armstrong (1991), GSTs are multifunctional enzymes that catalyze the conjugation of reduced glutathione to electrophilic substances. GSTs detoxify the pesticides and toxic plant allelochemicals they utilize to manage insects. In acidic and alkaline conditions, respectively, acid phosphatases and alkaline phosphatases catalyze the elimination of organic and inorganic phosphate ester (Zibaee and Stoytcheva 2011; Nathan 2006). Although the insecticidal efficacy of digestive and detoxifying enzyme inhibitors from plants has received widespread reporting (Franco *et al.,* 2002; Esmaeily and Bandani 2015, 2016), there are few reports of investigations on the effectiveness of endophyte-derived enzyme inhibitors for biological control.

The majority of earlier research has focused on the hyphomycete genera *Verticillium Beauveria, Metarhizium* and *Paecilomyces*, and the insects that make up their primary targets are primarily from the orders Hemiptera, Coleoptera, Lepidoptera, Orthoptera, and Thysanoptera. *Cladosporium* sp., *Alternaria* sp., *Paecilomyces* sp., and other ascomycetes have recently been discovered to exhibit insecticidal activity (Namasivayam *et al.,* 2014; Abraham *et al.,* 2015; Mirhaghparast *et al.,* 2013). *Helicoverpazea* (Boddie) has been shown to be susceptible to insecticidal activity from endophytic *Aspergillus* species, including *A. nomius*, *A. leporis, A. sulphureus, A. tubingensis*, and *A. ochraceus* (Strobel and Daisy 2003; Azevado *et al.,* 2000). Similar reports of the insecticidal potential of *Aspergillus* species against *S. litura* and *S. frugiperda* include *A. sojae*, *A. awamori*, *A. fumigatus*, *A. niger*, and *A. flavus* (Kaur *et al.,* 2016; Guo *et al.,* 2017; Elango *et al.,* 2020; Singh *et al.,* 2021). However insecticidal potential of *Aspergillus terreus* scarcely explored but recently Ragavendran and Natarajan (2015) observed that three species of mosquitoes were resistant to the larvicidal and pupicidal effects of *A. terreus* metabolites. Our preliminary studies indicated adverse effects of *A. terreus* on survival, development and nutritional physiology of *Spodoptera litura* (Fabricius) (data submitted elsewhere). The polyphagous defoliator *S. litura*, which has 300 host plant species worldwide, is mostly managed by chemical pesticides (Tuan *et al.,* 2014; Tong *et al.,* 2013). *S. litura* has developed resistant populations as a result of the overuse of chemical pesticides in attempts to control it (Ahmad *et al.,* 2007). Considering the efficiency of endophytic *A. terreus* as an efficient biopesticide, the current study was conducted to evaluate the enzyme inhibitory effect of ethyl acetate extract of endophytic *A. terreus* on *S. litura.* Further studies were conducted on evaluation of enzyme inhibitory effect of artificially inoculated plants on *S. litura.*

MATERIAL AND METHODS

Insect rearing: The *S. litura* larvae have been collected from infested cauliflower and cabbage crops in the fields of Amritsar (Punjab), India. For two generations, the culture was maintained in the lab on castor leaves under controlled conditions of 25 ± 2°C temperature, 65 ± 5% RH, and 16:12 L:D photoperiod (Thakur *et al.,* 2013b). The larvae from this lab culture were reared on an artificial diet for experimental work. The artificial diet was comprised wheat bran, kidney bean flour, yeast, vitamin combination, agar-agar, distilled water, etc. with slight modifications (Thakur *et al.,* 2013a; Koul *et al.,* 1997).

Fungal culture and preparation of fungal extract: *Catharanthus roseus* leaf was used to isolate endophytic fungus, which was subsequently maintained on PDA (potato dextrose agar) plates. Using morphological and molecular techniques, the isolated fungus was identified as *Aspergillus terreus* (data submitted elsewhere). The production of fungal extract was carried out in 50 ml malt extract broth (malt extract = 20 g/l, dextrose = 20 g/l, peptone = 1 g/l, pH = 5.5) in 250 ml Erlenmeyer flask by inoculating one plug (approximately 1 cm^2) taken from the periphery of an actively growing culture. The flasks were incubated at 30°C and 250 rpm for 10 days. After 10 days, the fungal mycelia were harvested and extraction was carried out twice using ethyl acetate at 120 rpm and 40°C. Rotavapor was used to concentrate the extracts, which were then dissolved in 1 ml of HPLC-grade water and kept at 4°C for later use.

Enzymatic Assay: Estimation of various digestive and detoxifying enzymes was carried out for third instar larvae of *S. litura* (12 days old). The larvae were fed on artificial diet amended with ethyl acetate extract of endophytic *A. terreus* at concentration 2.31 mg/ml (LC_{50} determined after bioassay studies) as well as with control diet for 48 and 96 hours. In case of artificially inoculation, cauliflower plants were individually infected with 150 ml of *A. terreus* spore solution containing 4.28×10^6 spores/ml, whereas control plants were given 150 ml of water containing 0.01% Tween 80. Leaf samples from six randomly chosen endophyte-infected (FE+) and uninfected (FE-) plants were collected after three weeks of fungal infection in order to confirm the endophyte infection. As more than 20% of the endophytes grown in the FE+ plants were identical to the infected ones, the cultivation of endophytes proved successful (Jallow *et al.,* 2008); however these cultures were entirely missing in the FE- plants. All studies on infected and uninfected cauliflower plants were carried out at a temperature of 25 ± 2°C and a relative humidity of 65 ± 5%. The larvae were fed on FE+ and FE- plants for 48 and 96

hours after 3 weeks of inoculation. Ten larvae from each treatment were chosen at random from each time interval in order to examine a particular digestive or detoxifying enzyme. The temperature and humidity levels during the experiment were maintained at 25°C and 65°F, respectively. All experiments were replicated thrice.

Estimation of digestive enzymes: The Zibaee (2012) protocol was used to measure the activity of the digestive enzymes. Larval midgut was homogenized to yield the gut homogenate (1% w/v). The homogenate was centrifuged for 20 minutes at 4°C at 13,000 rpm, and the supernatant was collected and kept at 20°C for further use. All digestive enzymes were extracted using the same method, and the enzyme activity was represented as µM/mg of fresh larval weight (Mehrabadi *et al.,* 2011).

(a) α-Amylase: For the purpose of estimating α-amylase, a mixture of 20 µl of enzyme extract, 100 µl of phosphate buffer (0.02 M, pH 7.1), and 40 µl of soluble starch (1%) was incubated at 35ºC for 30 min. The reaction was stopped by adding 100 µl of Dinitrosalicyclic acid (DNS) reagent and heated in boiling water for 10 minutes. The absorbance was measured using microplate reader at 540 nm (Eon BioTek).

(b) Glucosidases: By mixing 20 ml of enzyme extract with 40 ml of p-nitrophenyl-α-D-glucopyranoside (pNG) (5 mM) and 100 ml of 0.02 M phosphate buffer (pH 7.1) at 37ºC for 10 minutes, the enzyme activity of α-glucosidases was examined. By adding 150 µl of sodium carbonate (1 M), the process stopped (Terra and Ferreira 1983). Similar steps were taken to analyze the activity of β-Glucosidases, with the exception that p-nitrophenyl-β-D-glucopyranoside (pNG) (5mM) was the substrate. At 450 nm, the absorbance was measured.

(c) Galactosidases: By mixing 20 µl of enzyme extract with 40 µl of p-nitrophenyl-α-D-galactopyranoside (5 mM) and 100 µl of phosphate buffer (0.02 M, pH 7.1) at 37ºC for 10 minutes, the activity of α-galactosidases was calculated. The addition of 150 µl of sodium carbonate (1 M) stopped the reaction. Similar procedures were used to estimate the activity of β-galactosidases, with the exception that p-nitrophenyl-β-D-galactopyranoside (5 mM) was the substrate. At 450 nm, the absorbance was measured.

Estimation of detoxifying Enzymes:

(a) Esterases: According to the Katezenellenbogen and Kafatos (1971) methodology, esterase activity was assessed (EST). In cold 0.1M sodium phosphate buffer (pH 6.5), the larvae (1% w/v) were homogenized. The experimental combination included 1ml of postcoupling (Sodium lauryl sulphate (4%) (w/v) and quick red TR salt (1%) (w/v) solutions made in phosphate buffer (0.1M), pH-6.5) solution, 0.05ml of enzyme extract, and 2ml of 1mM -naphthyl acetate prepared in buffer. At 540 nm, the absorbance was measured.

(b) Phosphatases: The MacIntyre (1971) protocol was used to estimate the phosphatases. By homogenizing the larvae (1% w/v) in 0.05M Tris buffer (pH - 8.6), alkaline phosphatase (Akp) was isolated. The test combination contained 0.2 ml of enzyme extract, 2 ml of post coupling solution, and 0.005 M sodium naphthyl phosphate produced in 0.05 M Tris buffer. With the exception of the enzyme extraction, which was done in 0.05 M acetate buffer (pH 8.6) and absorbance was measured at 540 nm on a microplate reader (Eon BioTek), the method for estimating acid phosphatase (Acp) was the same as for Akp.

(c) Glutathione-S-transferase: Chien and Dauterman (1991) protocol was used to calculated glutathione-S-transferase activity. In 0.1M sodium phosphate buffer (pH 7.6) with 0.1mM phenyl thiourea (PTU), the larvae (2% w/v) were homogenized. The test combination contained 40 µl of 0.1M sodium phosphate buffer (pH 7.6), 200 µl of reduced glutathione (GSH), 100 µl of enzyme extract, and 60 µl of ethanolic CDNB solution. At 25°C, the rise in absorbance was measured at 340 nm at 1and 5 minutes intervals.

Statistical analysis: Each value was represented as its mean ± standard error. The Student's t-test was used to assess the data from biochemical experiments. The statistical analysis was carried out using Microsoft Office Excel 2007 and SPSS software for Windows version 19.0 (SPSS Inc, Chicago).

RESULTS AND DISCUSSION

For the successful management of agricultural pests in the agroecosystem, entomopathogenic fungi are a good substitute for synthetic pesticides (Inglis *et al.,* 2001; Shah and Pell 2003). Fungal endophytes distinguish themselves from many other biocontrol agents by having the capacity to invade the interior tissues of plants (Zimmermann 2007; Bamisile *et al.,* 2021). Endophytic fungi's secondary metabolites don't interfere with normal plant growth and development, but they do make plant tissues less palatable to herbivores (Chen *et al.,* 2018; Laib *et al.,* 2020). Insects counteract these effects by secreting a variety of enzymes viz. digestive and detoxifying enzymes.

Effects of ethyl acetate extract on digestive enzymes activities: Addition of ethyl acetate extract of *A. terreus* (LC_{50}) to the larval diet suppressed the activity of α–amylase when fed for 48 hours. Relative to control, there was a significantly decrease of 20.51% due to addition of fungal extract ($t = 32.34$, $p \leq 0.05$). The enzyme-inhibitory action of fungal metabolites increased along with the increase of feeding durations. After 96 hours of feeding, α–amylase activity significantly decreased from 67.29 µM/mg in control larvae to 36.50 µM/mg in larvae fed on amended diet ($t = 36.31$, $p \leq 0.05$) (Table 1). Fungal metabolites significantly suppressed the activity of α-glucosidases. Larvae fed for 48 hours on amended diet showed29.22% reduction in α-glucosidase activity ($t = 42.68$, $p \leq 0.05$). However, after 96 hours the reduction rate further increased by 58.80% over control ($t = 38.81$, $p \leq 0.05$) (Table 1). Addition of fungal extract in larval diet increased the level of β-glucosidases from 14.62 µM/mg in control larvae to 19.62 µM/mg in *S. litura* after 48 hours of feeding ($t = 22.37$, $p \leq 0.05$), however, a significant drop was recorded after 96 hours of larval exposure to amended diet ($t = 37.73$, $p \leq 0.05$) (Table 1). A similar trend was detected in the level of α

–galactosidases of *S. litura* that tended to increase initially (t = 41.00, p≤0.05) followed by a significant decline after 96 hrs of exposure (t = 36.63, p≤0.05) (Table 1). Consumption of diet amended with fungal extract significantly decreased the level of β-galactosidases by 20.41% after 48 hours (t = 56.83, p ≤ 0.05) in *S. litura* larvae followed by further decline after 96 hours of treatment (t = 170.03, p ≤ 0.05) (Table 1).

Effect of endophyte infected cauliflower plant on digestive enzymes: The larvae feeding on FE+ plants exhibited a significantly decrease of 4.94% in the activity of α–amylase when fed for 48 hours relative to larvae fed on FE- plants (t = 5.93, p≤0.05). However, after 96 hours of feeding, α–amylase activity significantly increased from 95.48 μM/mg in control larvae to 110.94 μM/mg in larvae fed on treated plants (t = 28.30, p≤0.05) (Table 2). Consumption of FE+ plants significantly suppressed the activity of α-glucosidase after 48 hours (t = 6.33, p≤0.05). Similarly, after 96 hours the enzyme activity rate decreased by 28.54% over FE- plants (t = 22.28, p≤0.05) (Table 2). Feeding on FE+ diet increased the level of β-glucosidases from 25.70 μM/mg in control to 30.23 μM/mg in *S. litura* larvae fed on treated plants for 48 hours (t = 18.40, p≤0.05), however, a significant drop was detected after 96 hours (t = 17.30, p≤0.05) (Table 2). Feeding on FE+ plants significantly decreased the activity of α –galactosidases of *S. litura.* For both the time intervals (48 hours, t = 9.28, p≤0.05, 96 hours t = 30.99, p≤0.05). Similar trend was observed for β-galactosidases (t = 78.75, p ≤ 0.05) (Table 2).

A reduction in the activity of α-amylase, α-and β-glucosidases, α-and β-galactosidases was found in the present study in *S. litura* larvae feeding on diet containing fungal metabolites. The decrease in the level of digestive enzymes may be due to the metabolites produced by endophytic *A. terreus* which is in line with other reports (Ramdanis *et al.,* 2012; Singh *et al.,* 2016; Centko *et al.,* 2017; Kaur *et al.,* 2018). Similarly, *S. litura* larvae feeding endophyte inoculated plants showed significant decrease in activity of α-amylase, α-and β-glucosidases, α-and β-galactosidases. This is the first study reporting the enzyme inhibitory potential of an endophytic *A. terreus*against *S. litura*. Recently Singh *et al.,* (2016) reported a significant reduction in α-amylase and α-glucosidases activity of *S. litura* when treated with chorogenic acid isolated from a partially purified extract of the endophytic *Cladosporium velox*. Although enzyme inhibitory effects of endophytic fungi with insecticidal potential has not been studied much, but the metabolites of entomopathogenic fungi like *Metarhizium anisopliae* and *Beauveria bassiana* have previously been reported to act as enzyme inhibitors. The metabolites of *Beauveria bassiana* fraction 2 (BBF2) significantly interfered with the digestive enzymes of the red bug, leading to starvation and weight loss (Sahayaraj and Tomson 2010). The activity of digestive enzymes determines the extent to which food is converted after being consumed and digested. It has been shown that secondary metabolites, including as phenols, alkaloids, and terpenoids, alter the insect's midgut epithelium (Zibaee, 2011; Senthil-Nathan, 2013). The peritrophic membrane and midgut epithelial cells, which are the primary locations for the production and release of numerous enzymes in insects, have been shown to be damaged in literature on the cytotoxicity of endophytic fungi (Ferreira *et al.,* 1990; Seetharaman *et al.,* 2017; Contreras-Cornejo *et al.,* 2018). In our work, the dropped enzyme activity may have resulted from the diverse extracts' direct impacts on the digestive enzymes produced by midgut epithelial cells or on their indirect effects on enzymes in insect guts (Jbilou *et al.,* 2008; Franco *et al.*, 2002). According to Bede *et al.* (2007), the midgut epithelial cells' cytological characteristics also control the activity of digestive enzymes and express the nutritional value of the food that has been consumed.

While consuming plants, herbivorous insects are exposed to a variety of plant defense substances. Thus, insects have evolved a number of strategies to get around plant challenges or even occasionally exploit them to their advantage by secreting enzymes that act as detoxifiers. Detoxifying enzymes are important for removing foreign substances and support the maintenance of regular physiological processes (Fan *et al.,* 2013). Insects may occasionally store plant-derived xenobiotics as a kind of defense against natural enemies or quickly excrete them or convert them into non-toxic molecules (Ibanez *et al.,* 2012). The primary detoxification enzymes in insects are esterases, glutathione-S-transferases, alkaline and acid phosphatases, which are also involved in the metabolism of biologically active substances, the detoxification of pathogenic products, and the repair of physiological processes (Zibaee, 2009; Serebrov *et al.,* 2001; 2006). The results of present studies indicated that the fungal endophyte suppressed the defense mechanism of *S. litura*.

Effect of ethyl acetate extract on activities of detoxifying enzymes: After 48 hours, addition of ethyl acetate extract to the larval diet considerably reduced the levels of Acp and Akp.Acp and Akp activity decreased by 22.77% and 34.53%, respectively, compared to controls. (Acp: t = 56.68, p ≤ 0.05; Akp: t = 234.47, p ≤ 0.05) (Table 3). Similar declining trend was observed even after 96 hours for both the enzymes of *S. litura* (Acp: t = 217.80, p ≤ 0.05; Akp: t = 442.90, p ≤ 0.05). As is evident from table 3, consumption of fungal metabolite supplemented diet led to drop in the activity of esterases. With respect to control, the treated larvae showed 23.12% and 36.38% reduction in esterases respectively after 48 and 96hours (t = 56.49, p ≤ 0.05; t = 96.84, p ≤ 0.05). Similar declining trend was recorded for GSTs with 26.23% and 42.81% suppression over control after 48 and 96 hrs of treatment respectively (t = 117.44, p ≤ 0.05; t = 147.06, p ≤ 0.05) (Table 3).

Effect of endophyte infected cauliflower plant on detoxifying enzymes: Ingestion of FE+ plants significantly increased the activity of Acp by 5.48% over control after 48hours of exposure (t = 3.85, p≤0.05), however, a significant drop was observed after 96 hours of feeding (t = 14.69, p≤0.05) (Table 4). As is

evident from table 4, consumption of FE+ plants led to drop in the activity of Akp. With respect to control, the treated larvae showed 4.79% and 10.48% reduction in Akp activity respectively after 48 and 96 hours ($t = 7.40, p \leq 0.05; t = 26.63, p \leq 0.05$) (Table 4). A similar trend was detected in the level of esterases of *S. litura* that tended to decrease by 8.68% and 15.99% respectively after 48 and 96 hours of exposure ($t = 8.77, p \leq 0.05; t = 17.36, p \leq 0.05$) (Table 4). The results presented in table 4 indicates similar declining trend for GST activity.

Consumption of metabolites of endophytic *A. terreus* significantly suppressed the activity of detoxifying enzymes viz. acid and alkaline phosphatases, esterases and glutathione-S-transferases. Similarly, larvae feeding on endophyte infected plants also showed suppressed levels of acid and alkaline phosphatases, and glutathione-S-transferases whereas an induction in the level of esterases was observed at 96 hours interval. This suggests that esterases could be involved in the detoxification of fungus-produced toxins. Dubovskiy *et al.* (2012) also revealed that esterases play an important role in *Locusta migratoria* in detoxification of metabolites produced by *Metarhizium anisopliae.* Reduced activity of Akp, GST and esterases was also observed previously in *S. littoralis* fed with ethyl acetate extract of the endophytic fungus *Sarocladium strictum* (El-Sayed *et al.*, 2020). Earlier reports also indicated induced esterase activity in lepidopteran in response to phenolic glycosides (Hemming and Lindroth 2000; Lindroth *et al.,* 1991). Insect detoxification enzymes serve a variety of purposes, including the restoration of physiological processes, the detoxification of harmful substances, and the metabolization of physiologically useful substances. Therefore, a change in their activity may affect how effectively the insect adapts to its environment (Serebrov *et al.,* 2001, 2006). Reduced enzyme activity shows that these substances have an impact on ion transport and other physiological processes in the gut. Therefore, modifications to the midgut's physiological balance may have an impact on the enzyme activity (Nathan *et al.,* 2004).

Table 1: Influence of ethyl acetate extract of *A. terreus* on digestive enzymes of *S. litura* larvae.

Digestive enzymes	Status	Enzyme activity (µmol/mg/min)	
		48 hours (Mean ± S.E.)	96 hours (Means ± S.E.)
α – amylase	Control	57.63 ± 0.24	67.29 ± 0.43
	Treatment	45.81 ± 0.28	36.50 ± 0.73
	t – value	32.34**	36.31**
α – glucosidase	Control	23.95 ± 0.12	25.73 ± 0.16
	Treatment	16.95 ± 0.11	10.60 ± 0.36
	t – value	42.68**	38.81**
β – glucosidase	Control	14.62 ± 0.14	17.54 ± 0.09
	Treatment	19.62 ± 0.18	12.20 ± 0.10
	t – value	22.37**	37.73**
α - galactosidase	Control	12.37 ± 0.04	20.36 ± 0.26
	Treatment	14.44 ± 0.04	12.84 ± 0.44
	t – value	41.00**	36.63**
β - galactosidase	Control	20.92 ± 0.02	24.07 ± 0.03
	Treatment	16.65 ± 0.07	14.22 ± 0.05
	t – value	56.83**	170.03**

Means and standard error are given Student's t-test, ($p \leq 0.05$). **Significant at 1%; Treatment was given by LC_{50} (2.31 mg/ml)

Table 2: Effect of fungal endophyte infected (FE+) and uninfected (FE-) cauliflower plants on digestive enzymes of *S. litura* larvae.

Digestive enzymes	Status	Enzyme activity(µmol/mg/min)	
		48 hours (Mean ± S.E.)	96 hours (Mean ± S.E.)
α - amylase	FE-	73.62 ± 0.55	95.48 ± 0.41
	FE+	69.98 ± 0.27	110.94 ± 0.36
	t – value	**5.93****	**28.30****
α – glucosidase	FE-	24.75 ± 0.25	27.54 ± 0.12
	FE+	22.98 ± 0.13	19.68 ± 0.33
	t – value	**6.33****	**22.28****
β – glucosidase	FE-	25.70 ± 0.15	31.46 ± 0.17
	FE+	30.23 ± 0.19	26.85 ± 0.20
	t – value	**18.40****	**17.30****
α - galactosidase	FE-	18.64 ± 0.11	20.41 ± 0.17
	FE+	17.39 ± 0.08	14.77 ± 0.07
	t – value	**9.28****	**30.99****
β - galactosidase	FE-	22.33 ± 0.08	24.49 ± 0.08
	FE+	16.64 ± 0.18	13.23 ± 0.12
	t – value	**29.10****	**78.75****

Means and standard error are given Student's t-test, ($p \leq 0.05$). **Significant at 1%

Table 3: Influence of ethyl acetate extract of *A. terreus* on detoxifying enzymes of *S. litura* larvae.

Detoxifying enzymes	Status	Enzyme activity (µmol/mg/min)	
		48 hours (Mean ± S.E.)	96 hours (Mean ± S.E.)
Acid Phosphatases	Control	38.82 ± 0.11	47.36 ± 0.09
	Treatment	29.98 ± 0.10	26.96 ± 0.03
	t – value	56.68**	217.80**
Alkaline Phosphatases	Control	48.30 ± 0.05	62.54 ± 0.07
	Treatment	31.62 ± 0.04	28.42 ± 0.03
	t – value	234.47**	442.90**
Esterases	Control	16.65 ± 0.06	20.75 ± 0.07
	Treatment	12.80 ± 0.02	13.20 ± 0.02
	t – value	56.49**	96.84**
Glutathione-S-transferases	Control	26.34 ± 0.03	29.08 ± 0.02
	Treatment	19.43 ± 0.05	16.63 ± 0.08
	t – value	117.44**	147.06**

Means and standard error are given Student's t-test, ($p \leq 0.05$). **Significant at 1%; Treatment was given by LC_{50} (2.31 mg/ml)

Table 4: Effect of fungal endophyte infected (FE+) and uninfected (FE-) cauliflower plants on detoxifying enzymes of *S. litura* larvae.

Detoxifying enzymes	Status	Enzyme activity (µmol/mg/min)	
		48 hours (Mean ± S.E.)	96 hours (Mean ± S.E.)
Acid Phosphatases	FE-	10.94 ± 0.11	12.57± 0.12
	FE+	11.54 ± 0.15	9.67 ± 0.16
	t – value	**3.85***	**14.69****
Alkaline Phosphatases	FE-	42.98± 0.17	52.47 ± 0.16
	FE+	40.92 ± 0.22	46.97 ± 0.12
	t – value	**7.40****	**26.63****
Esterases	FE-	20.62 ± 0.08	24.95 ± 0.13
	FE+	18.83 ± 0.19	28.94 ± 0.19
	t – value	**8.77****	**17.36****
Glutathione-S-transferases	FE-	28.66 ± 0.21	32.64 ± 0.23
	FE+	29.77 ± 0.16	27.01 ± 0.09
	t – value	**4.27***	**22.45****

Means and standard error are given Student's t-test, ($p \leq 0.05$). **Significant at 1%, *Significant at 5%

CONCLUSIONS

It is evident from this work that *S. litura's* digestive and detoxifying enzyme activity was significantly affected by the diet supplemented with ethyl acetate extract and endophyte-infected plants. The intestinal epithelium's ability to absorb nutrients can also be hindered by suppressed levels of digestive enzymes. The induction of digestive enzymes depends on feeding. As a result, a recent study by Singh *et al.,* (2021) suggests that reduction of the activity of several digestive enzymes may be correlated with decreased consumption rate. Similarly, reduced detoxification enzyme activity is associated with the toxic effects of secondary metabolites produced by endophytic fungi on *S. litura* midgut epithelial cells, as secondary metabolites are known to induce cytotoxic effect (Edriss *et al.,* 2012; Wink 2018; Mousavi and Karami 2022). Secondary metabolites of entomopathogenic fungi cause death by interfering with the insect host's defense mechanisms (Gillespie and Claydon 1989, Zibaee *et al.,* 2011). Thus this study shows secondary metabolites produced by endophytic *A. terreus* act as enzyme inhibitors.

FUTURE SCOPE

This work can be used to develop eco-friendly formulations for insect pest control.

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Conflict of Interest. None.

REFERENCES

Abraham, S., Basukriadi, A., Pawiroharsono, S. and Sjamsuridzal, W. (2015). Insecticidal activity of ethyl acetate extracts from culture filtrates of mangrove fungal endophytes. *Mycobiology*, *43*(2), 137-149.

Ahmad, M., Arif, M. I. and Ahmad, M. (2007). Occurrence of insecticide resistance in field populations of *Spodoptera litura* (Lepidoptera: Noctuidae) in Pakistan. *Crop Protection*, *26*(6), 809-817.

Armstrong, R. N. (1991). Glutathione S-transferases: reaction mechanism, structure, and function. *Chemical Research and Toxicology, 4*(2), 131–140.

Azevedo, J. L., Maccheroni Jr, W., Pereira, J. O. and De Araújo, W. L. (2000). Endophytic microorganisms: a review on insect control and recent advances on tropical plants. *Electronic Journal of Biotechnology*, *3*(1), 15-16.

Bamisile, B. S., Akutse, K. S., Siddiqui, J. A. and Xu, Y. (2021). Model application of entomopathogenic fungi as alternatives to chemical pesticides: Prospects,

challenges, and insights for next-generation sustainable agriculture. *Frontiers in Plant Science*, *12*, 741804.

Bede, J. C., McNeil, J. N. and Tobe, S. S. (2007). The role of neuropeptides in caterpillar nutritional ecology. *Peptides*, *28*(1), 185-196.

Carletto, J., Martin, T., Vanlerberghe Masutti, F. and Brévault, T. (2010). Insecticide resistance traits differ among and within host races in *Aphis gossypii*. *Pest Management Science: formerly Pesticide Science*, *66*(3), 301-307.

Centko, R. M., Ratnaweera, P. B., Tysoe, C., Withers, S. G., de Silva, E. D. and Andersen, R. J. (2017). Alpha-glucosidase and alpha-amylase inhibiting thiodiketopiperazines from the endophytic fungus *Setosphaeria rostrata* isolated from the medicinal plant *Costus speciosus* in Sri Lanka. *Phytochemistry Letters*, *22*, 76-80.

Chen, D., Zhang, P., Liu, T., Wang, X. F., Li, Z. X., Li, W. and Wang, F. L. (2018). Insecticidal activities of chloramphenicol derivatives isolated from a marine alga-derived endophytic fungus, *Acremonium vitellinum*, against the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Molecules*, *23*(11), 2995.

Chien, C. and Dauterman, W. C. (1991). Studies on glutathione S-transferase in *Helicoverpa* (= Heliothis) *zea*. *Insect Biochemistry*, *21*(8), 857-864.

Contreras-Cornejo, H. A., Macías-Rodríguez, L., del-Val, E. and Larsen, J. (2018). The root endophytic fungus *Trichoderma atroviride* induces foliar herbivory resistance in maize plants. *Applied Soil Ecology*, *124*, 45-53.

Conyers, C. M., MacNicoll, A. D. and Price, N. R. (1998). Purification and characterisation of an esterase involved in resistance to organophosphorus insecticides in the saw-toothed grain beetle, *Oryza ephilussurinamensis* (Coleoptera: Silvanidae). *Insect Biochemistry and Molecular Biology*, *28*(7), 435-448.

Dubovskiy, I. M., Slyamova, N. D., Kryukov, V. Y., Yaroslavtseva, O. N., Levchenko, M. V., Belgibaeva, A. B. and Glupov, V.V. (2012). The activity of nonspecific esterases and glutathione-S-transferase in *Locusta migratoria* larvae infected with the fungus *Metarhizium anisopliae* (Ascomycota, Hypocreales). *Entomological Review*, *92*, 27-31.

Edriss, A. E., Satti, A. A. and Alabjar, Z. A. (2012). Preliminary studies on phytochemicals and larvicidal effects of *Acacia nilotica* L. extracts against *Anopheles arabiensis* Patton. *Scientific Research and Essays*, *7*(50), 4253-4258.

Elango, D., Manikandan, V., Jayanthi, P., Velmurugan, P., Balamuralikrishnan, B., Ravi, A. V. and Shivakumar, M. S. (2020). Selection and characterization of extracellular enzyme production by an endophytic fungi *Aspergillus sojae* and its bio-efficacy analysis against cotton leaf worm, *Spodoptera litura*. *Current Plant Biology*, *23*, 100153.

El-Sayed, A. S., Moustafa, A. H., Hussein, H. A., El-Sheikh, A. A., El-Shafey, S. N., Fathy, N. A. and Enan, G. A. (2020). Potential insecticidal activity of *Sarocladium strictum*, an endophyte of *Cynanchum acutum*, against *Spodoptera littoralis*, a polyphagous insect pest. *Biocatalysis and Agricultural Biotechnology*, *24*, 101524.

Esmaeily, M. and Bandani, A. R. (2015). Interaction between larval α-amylase of the tomato leaf miner, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) and proteinaceous extracts from plant seeds. *Journal of plant protection research*.

Esmaeily, M. and Bandani, A. R. (2016). The effect of proteinaceous extract of triticale on α-amylase activity of tomato leaf miner, *Tuta absoluta* Meyrick (Lep.: Gelechiidae). *Plant Pest Research*, *6*(1).

Espinoza, F., Vidal, S., Rautenbach, F., Lewu, F. and Nchu, F. (2019). Effects of *Beauveria bassiana* (Hypocreales) on plant growth and secondary metabolites of extracts of hydroponically cultivated chive (*Allium schoenoprasum* L. [Amaryllidaceae]). *Heliyon*, *5*(12).

Fan, J., Xie, Y., Xue, J. and Liu, R. (2013). The effect of *Beauveria brongniartii* and its secondary metabolites on the detoxification enzymes of the pine caterpillar, *Dendrolimus tabulaeformis*. *Journal of Insect Science*, *13*(1), 44.

Ferreira, C., Bellinello, G. L., Ribeiro, A. F. and Terra, W. R. (1990). Digestive enzymes associated with the glycocalyx, microvillar membranes and secretory vesicles from midgut cells of *Tenebrio molitor* larvae. *Insect Biochemistry*, *20*(8), 839-847.

Franco, O. L., Rigden, D. J., Melo, F. R. and Grossi-de-Sá, M. F. (2002). Plant α-amylase inhibitors and their interaction with insect α-amylases: Structure, function and potential for crop protection. *European journal of biochemistry*, *269*(2), 397-412.

Georghiou, G. P. and Lagunes-Tejeda, A. (1991). *The occurrence of resistance to pesticides in arthropods* (No. 04; QH545. P4, G4.). Rome: Fao.

Gillespie, A. T. and Claydon, N. (1989). The use of entomogenous fungi for pest control and the role of toxins in pathogenesis. *Pesticide Science*, *27*(2), 203-215.

Guo, Z., Gai, C., Cai, C., Chen, L., Liu, S., Zeng, Y. and Dai, H. (2017). Metabolites with insecticidal activity from *Aspergillus fumigatus* JRJ111048 isolated from mangrove plant *Acrostichum specioum* endemic to Hainan Island. *Marine drugs*, *15*(12), 381.

Hemming, J. D. and Lindroth, R. L. (2000). Effects of phenolic glycosides and protein on gypsy moth (Lepidoptera: Lymantriidae) and forest tent caterpillar (Lepidoptera: Lasiocampidae) performance and detoxication activities. *Environmental Entomology*, *29*(6), 1108-1115.

Homayoonzadeh, M., Moeini, P., Talebi, K., Allahyari, H., Torabi, E. and Michaud, J. P. (2020). Physiological responses of plants and mites to salicylic acid improve the efficacy of spirodiclofen for controlling *Tetranychus urticae* (Acari: Tetranychidae) on greenhouse tomatoes. *Experimental and Applied Acarology*, *82*, 319-333.

Ibanez, S., Gallet, C. and Després, L. (2012). Plant insecticidal toxins in ecological networks. *Toxins*, *4*(4), 228-243.

Inglis, G. D., Goettel, M. S., Butt, T. M. and Strasser, H. E. R. M. A. N. N. (2001). Use of hyphomycetous fungi for managing insect pests. In *Fungi as biocontrol agents: progress, problems and potential* (pp. 23-69). Wallingford UK: CABI publishing.

Jallow, M. F., Dugassa-Gobena, D. and Vidal, S. (2008). Influence of an endophytic fungus on host plant selection by a polyphagous moth via volatile spectrum changes. *Arthropod-Plant Interactions*, *2*, 53-62.

Jbilou, R., Amri, H., Bouayad, N., Ghailani, N., Ennabili, A. and Sayah, F. (2008). Insecticidal effects of extracts of seven plant species on larval development, α-amylase activity and offspring production of *Tribolium castaneum* (Herbst) (Insecta: Coleoptera:

Tenebrionidae). *Bioresource Technology*, *99*(5), 959-964.
Katzenellenbogen, B. S. and Kafatos, F. C. (1971). General esterases of silk worm moth moulting fluid: Preliminary characterization. *Journal of Insect Physiol*ogy, *17*(6), 1139–1151.
Kaur, J., Kaur, R., Dutta, R., Kaur, S. and Kaur, A. (2018). Exploration of insecticidal potential of an alpha glucosidase enzyme inhibitor from an endophytic *Exophiala spinifera*. *Journal of Applied Microbiology, 125*(5), 1455–1465.
Kaur, J., Sharma, A., Sharma, M., Kumari Manhas, R., Kaur, S. and Kaur, A. (2019). Effect of α-glycosidase inhibitors from endophytic fungus *Alternaria destruens* on survival and development of insect pest *Spodoptera litura* Fab. and fungal phytopathogens. *Scientific Reports*, *9*(1), 1-13.
Kaur, T., Kaur, J., Kaur, A. and Kaur, S. (2016). Larvicidal and growth inhibitory effects of endophytic *Aspergillus niger* on a polyphagous pest, *Spodoptera litura*. *Phytoparasitica*, *44*, 465-476.
Klowden, M. J. (2007). *Physiological systems in insects*. 688.
Koul, O., Shankar, J. S., Mehta, N., Taneja, S. C., Tripathi, A. K. and Dhar, K. L. (1997). Bioefficacy of crude extracts of *Aglaia* species (Meliaceae) and some active fractions against lepidopteran larvae. *Journal of Applied Entomology*, *121*(1-5), 245-248.
Laib, D. E., Benzara, A. and Akkal, S. (2020). The insecticidal activity of the endophytic fungus *Isaria fumosorosea* Wize isolated from the leaves of the *Ricinus communis* L. against *Locusta migratoria* L. and *Acanthoscelides obtectus* Say. *Acta Scientifica Naturalis*, *7*(1), 126-135.
Lindroth, R. L., Barman, M. A. and Weisbrod, A. V. (1991). Nutrient deficiencies and the gypsy moth, *Lymantria dispar*: effects on larval performance and detoxication enzyme activities. *Journal of Insect Physiology*, *37*(1), 45-52.
MacIntyre, R. J. (1971). A method for measuring activities of acid phosphatases separated by acrylamide gel electrophoresis. *Biochemical Genetics*, *5*, 45-56.
Mehrabadi, M. B. A. R., Bandani, A. R, Saadati, F. and Mahmudvand, M. (2011). α-Amylase activity of stored products insects and its inhibition by medicinal plant extracts. *Journal of Agricultural Science and Tech*nology, *13*, 1173–1182.
Menjivar, R. D., Cabrera, J. A., Kranz, J. and Sikora, R. A. (2012). Induction of metabolite organic compounds by mutualistic endophytic fungi to reduce the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) infection on tomato. *Plant and soil*, *352*, 233-241.
Mirhaghparast, S. K., Zibaee, A. and Hajizadeh, J. (2013). Effects of *Beauveria bassiana* and *Metarhizium anisopliae* on cellular immunity and intermediary metabolism of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae). *Invertebrate Survival Journal*, *10*(1), 110-119.
Moloinyane, S. and Nchu, F. (2019). The effects of endophytic *Beauveria bassiana* inoculation on infestation level of *Planococcus ficus*, growth and volatile constituents of potted greenhouse grapevine (*Vitis vinifera* L.). *Toxins*, *11*(2), 72.
Mousavi, S. S. and Karami, A. (2022). Application of Endophyte microbes for production of secondary metabolites. *Application of Microbes in Environmental and Microbial Biotechnology*, 1-37.
Namasivayam, S. K. R., Sekar, S. and Bharani, R. A. (2014). Pesticidal activity of endophytic fungal metabolites against major groundnut defoliator *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *Journal of Biopesticides*, *7*, 116.
Nathan, S. S. (2006). Effects of *Melia azedarach* on nutritional physiology and enzyme activities of the rice leaf folder *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae). *Pesticide Biochemistry and Physiology*, *84*(2), 98-108.
Nathan, S. S., Chung, P. G. and Murugan, K. (2004). Effect of botanical insecticides and bacterial toxins on the gut enzyme of the rice leaf folder *Cnaphalocrocis medinalis*. *Phytoparasitica*, *32*, 433-443.
Ownley, B. H., Griffin, M. R., Klingeman, W. E., Gwinn, K. D., Moulton, J. K. and Pereira, R. M. (2008). *Beauveria bassiana*: endophytic colonization and plant disease control. *Journal of invertebrate pathology*, *98*(3), 267-270.
Pang, Q., Chen, S., Dai, S., Chen, Y., Wang, Y. and Yan, X. (2010). Comparative proteomics of salt tolerance in *Arabidopsis thaliana* and *Thellungiella halophila*. *Journal of proteome research*, *9*(5), 2584-2599.
Ragavendran, C. and Natarajan, D. (2015). Insecticidal potency of *Aspergillus terreus* against larvae and pupae of three mosquito species *Anopheles stephensi, Culex quinquefasciatus, and Aedes aegypti*. *Environmental Science and Pollution Research*, *22*, 17224-17237.
Ramdanis, R., Soemiati, A. and Mun'im, A. (2012). Isolation and α-glucosidase inhibitory activity of endophytic fungi from mahogany (*Swietenia macrophylla* King) seeds. *International Journal of Medicinal and Aromatic Plants*, *2*(3), 447-452.
Sahayaraj, K. and Tomson, M. (2010). Impact of two pathogenic fungal crude metabolites on mortality, biology and enzymes of *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae). *Journal of Biopesticides*, *3*(Special Issue), 163.
Seetharaman, P., Gnanasekar, S., Chandrasekaran, R., Chandrakasan, G., Syed, A., Hodhod, M. S. and Sivaperumal, S. (2017). Isolation of limonoid compound (Hamisonine) from endophytic fungi *Penicillium oxalicum* LA-1 (KX622790) of *Limonia acidissima* L. for its larvicidal efficacy against LF vector, *Culex quinquefasciatus* (Diptera: Culicidae). *Environmental Science and Pollution Research*, *24*, 21272-21282.
Senthil-Nathan, S. (2013). Physiological and biochemical effect of neem and other Meliaceae plants secondary metabolites against Lepidopteran insects. *Frontiers in physiology*, *4*, 359.
Serebrov, V. V., Alekseev, A. A. and Glupov, V. V. (2001). Changes in the Activity and Pattern of Hemolymph Esterases in the Larvae of Greater Wax Moth *Galleria mellonella* L. (Lepidoptera, Pyralidae) during Mycosis. *Biology Bulletin of the Russian Academy of Sciences*, *28*, 499-503.
Serebrov, V. V., Gerber, O. N., Malyarchuk, A. A., Martemyanov, V. V., Alekseev, A. A. and Glupov, V. V. (2006). Effect of entomopathogenic fungi on detoxification enzyme activity in greater wax moth *Galleria mellonella* L. (Lepidoptera, Pyralidae) and role of detoxification enzymes in development of insect resistance to entomopathogenic fungi. *Biology Bulletin*, *33*, 581-586.
Shah, P. A. and Pell, J. K. (2003). Entomopathogenic fungi as biological control agents. *Applied Microbiology and Biotechnology*, *61*(5), 413-423.
Shiba, T. and Sugawara, K. (2010). Inhibitory effect of an endophytic fungus, *Neotyphodiumlolii*, on the feeding

and survival of *Ostrinia furnacalis* (Guenee) (Lepidoptera: Pyralidae) and *Sesamia inferens* (Walker)(Lepidoptera: Noctuidae) on infected *Lolium perenne*. *Applied Entomology and Zoology*, *45*(1), 225-231.

Singh, B., Dhaliwal, R. S., Kumar, P. and Singh, A. (2021). Insecticidal activity of a proteinaceous α-glycosidase inhibitor isolated from an endophytic *Aspergillus awamori* and its biosafety evaluation. *Physiological and Molecular Plant Pathology*, *116*, 101707.

Singh, B., Kaur, T., Kaur, S., Manhas, R. K. and Kaur, A. (2015). An alpha-glucosidase inhibitor from an endophytic *Cladosporium* sp. with potential as a biocontrol agent. *Applied biochemistry and biotechnology*, *175*, 2020-2034.

Singh, B., Kaur, T., Kaur, S., Manhas, R. K. and Kaur, A. (2016). Insecticidal potential of an endophytic *Cladosporium velox* against *Spodoptera litura* mediated through inhibition of alpha glycosidases. *Pesticide biochemistry and physiology*, *131*, 46-52.

Strobel, G. and Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews*, *67*(4), 491-502.

Terra, W. R. and Ferreira, C. (1983). Further evidence that enzymes involved in the final stages of digestion by *Rhynchosciara* do not enter the endoperitrophic space. *Insect Biochemistry*, *13*(2), 143-150.

Terra, W. R. and Ferreira, C. (2012). Biochemistry and molecular biology of digestion. In *Insect molecular biology and biochemistry* (pp. 365-418). Academic press.

Thakur, A., Kaur, S., Kaur, A. and Singh, V. (2012). Detrimental effects of endophytic fungus *Nigrospora* sp. on survival and development of *Spodoptera litura*. *Biocontrol Science and Technology*, *22*(2), 151-161.

Thakur, A., Kaur, S., Kaur, A. and Singh, V. (2013b). Enhanced resistance to *Spodoptera litura* in endophyte infected cauliflower plants. *Environmental Entomology*, *42*(2), 240-246.

Thakur, A., Singh, V., Kaur, A. and Kaur, S. (2013a). Insecticidal potential of an endophytic fungus, *Cladosporium uredinicola*, against *Spodoptera litura*. *Phytoparasitica*, *41*, 373-382.

Tong, H., Su, Q., Zhou, X. and Bai, L. (2013). Field resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates and four newer chemistry insecticides in Hunan, China. *Journal of Pest Science*, *86*, 599-609.

Tuan, S. J., Lee, C. C. and Chi, H. (2014). Population and damage projection of *Spodoptera litura* (F.) on peanuts (*Arachis hypogaea* L.) under different conditions using the age stage, two sex life table. *Pest Management Science*, *70*(5), 805-813.

Wink, M. (2018). Plant secondary metabolites modulate insect behavior-steps toward addiction?. *Frontiers in Physiology*, *9*, 364.

Zibaee, A. (2012). Digestive enzymes of large cabbage white butterfly, *Pieris brassicae* L. (Lepidoptera: Pieridae) from developmental and site of activity perspectives. *Italian Journal of Zoology*, *79*(1), 13-26.

Zibaee, A. and Stoytcheva, M. (2011). Botanical insecticides and their effects on insect biochemistry and immunity. *Pesticides in the modern world-pests control and pesticides exposure and toxicity assessment*, 55-68.

Zibaee, A., Bandani, A. R., Kafil, M. and Ramzi, S. (2008). Characterization of α-amylase in midgut and salivary glands of *Chilo suppressalis* Walker (Lepidoptera: Pyralidae), rice striped stem borer. *Journal of Asia-Pacific Entomology*, *11*(4), 201-205.

Zibaee, A., JalaliSendi, J., Ghadamyari, M., Alinia, F. and Etebari, K. (2009). Diazinon resistance in different selected strains of *Chilo suppressalis* (Lepidoptera: Crambidae) in northern Iran. *Journal of Economic Entomology*, *102*(3), 1189-1196.

Zimmermann, G. (2007). Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. *Biocontrol Science and technology*, *17*(9), 879-920.

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