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ORIGINAL ARTICLE

Effectiveness of Selected Entomopathogenic Fungi against the Tobacco Caterpillar, Spodoptera Litura (Fabricius) (Lepidoptera: Noctuidae)

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ABSTRACT

The Tobacco caterpillar, *Spodoptera litura,* is a destructive pest that causes severe injury losses to various crops worldwide. The potential of different entomopathogenic fungi (EPFs) against *S. litura* was assesed at Institute of Plant Protection, MNS-University of Agriculture, Multan, using leaf dip method. The eggs and larvae were the most susceptible while pupae were less susceptible to the tested entomopathogenic fungal isolates (EPFs). The early instar larvae were highly susceptible to EPFs as compared to the later instar larvae. The median lethal concentration (LC50) values for third instar larvae were 1.13×107 conidia ml-1 and 2.16×107 conidia ml-1 for *Beauveria bassiana* 25 and *Isaria fumosorosea* 32, respectively. Entomopathogenic fungi pathogenicity was increased with increase in conidial concentration. Median lethal time (LT50) of *S. litura* was increased with decreased in fungus conidial concentrations. *Metarhizium anisopliae* L6, *Beauveria bassiana* and *Isaria fumosorosea* 32 showed LC50 of 1.22×106, 2.33×107 and 4.91×106, respectively, to eggs. No significant effect was recorded among all EPFs on adult emergence, while the *I. fumosorosea* had significant virulence on pupal formation.

Key words: Armyworm, Entomopathogens, Pathogenicity, Biological Control

INTRODUCTION

Insecticides are widely practiced by farmers to control the agricultural and horticultural crops pests like chewing and sucking insect pests (Ramzan et al., 2019a, b). Synthetic pesticides are used repeatedly to suppress pest populations, but they negatively impact humans and the ecosystem due to their residual effect (Gu et al., 2018; Cai et al., 2017). Due to very modest toxicity hazards and insignificant residual impact on ecosystems, researchers focused on the application of natural chemicals as biopesticides at the turn of the century (Idrees et al., 2016; Idrees et al., 2017; Luo et al., 2018; Qadir et al., 2021; Idrees et al., 2021). Entomopathogenic fungi (EPFs) are an alternative management strategy to control insect pests (Idrees et al., 2021). There is a need to adopt microbial agents such as entomopathogenic fungi to control insect pests like *Spodoptera litura*. Entomopathogenic fungi offer several advantages: ecofriendly (Lacey et al., 2001), low cost, natural availability, high virulence, safety to humans, birds, animals, and biological agents (predators, parasitoids, parasite etc.). EPFs are found naturally in the soil and plants, while Lepidoptera and Coleoptera are mainly infested, hosts. EPFs have been used against insect pests such as *Leptinotarsa decemlineata*, *Anastrepha ludens* (Daglish, 1998), *Ostrinia nubilalis*, *Popillia japonica*, *Laspeyresia pomonella*, *Pieris brassicae*, *Blissus leucopterus* (Tanadaand Kaya, 1993), *Odontotermis brunneus* (Khader Khan et al., 1993), *Melanoplus sanguinipes* (Askary et al., 1998) and *Plutella xylostella* (Vandenberg et al., 1998). Entomopathogenic fungi have unique features to invade their hosts. The Fungus infests the hosts via conidial attachment to the host's cuticle. EPFs attacked all stages of insects such as eggs (Ujian & Shahzad, 2007) larvae, pupae (Nguyen et al., 2007) and adults. Several EPFs like *M. anisopliae*, *B. bassiana*, *Nomuraea rileyi* and *Verticillium lecanii* have effectively managed insect pests (Lingappa et al., 2005). Different EPFs have different efficacy against insect pests like *S. litura* (Amer et al., 2008; Lin et al., 2007). Thackar (2002) has reported 800 species from 90 genera used against target insect pests (Hajek & Leger, 1994). EPFs are different in conidia production, enzyme activity, radial growth and germination rate (Petlamul & Prasertsan, 2012). The efficacy and identification of most virulent and toxic fungi can be needed to control chewing and sucking insect pests. The current study was carried out to assess the effectiveness of EPFs pathogenicity against different eggs and larvae of *S. litura*.

MATERIALS AND METHODS

Fungus culture: M. anisopliae, B. bassiana 25, L. lecanii and I. fumosorosea isolates of EPFs were cultured on potato dextrose agar (starch 20g, glucose 20g, agar 20g solved in1000 ml of distilled). Glass test tubes to contain PDA media were autoclaved at 121°C (15 Psi) for 15-20 minutes. Test tubes containing PDA medium were incubated at 27±1°C and 80±5% r.h. under 12L:12D photoperiod for 15 days.

Source of Entomopathogenic Fungus: EPFs were obtained from Department of Entomology, University of Agriculture, Faisalabad, Punjab Pakistan.

Conidia harvesting: Conidia were harvested from the 14-15 days old culture with the help of inoculation needle and placed in distilled water. The conidia containing solution were stirred with the help of glass stirrer for fifteen minutes to for homogenous suspension. The fine mesh cloth/sieve was used to remove the debris from prepared solution. The conidial concentration was calculated through Haemocytometer. The serial dilutions and final suspensions were prepared in distilled water. The serial dilution was well-maintained at 6°C.

Insect rearing: S. litura larvae were collected from cotton fields from Multan during 2019 and reared in plastic cages (Size should be mentioned). Before shifting, Cages were washed with 70% ethanol and sterilized with ultraviolet light. The cages were incubated at 26±1°C. Cotton leaves were collected from MNS-University of Agriculture Research Farm, washed with running water, sterilized with 0.5% v/v solution of sodium hypochlorite and air dried for an hour. After drying, leaves were provided to L_1 and L_2instars. For 3rdand later instars, sterilized mature cotton leaves were used. On daily basis, rearing cages were cleaned. Larvae were kept in separate plastic cages to avoid overcrowding and cannibalism. The pupae were kept into small petri dishes with

tissue paper lining at for adult emergence and ovi-position. Cotton swab was soaked in honey solution (10%) and placed in each cage as adult diet. Eggs were collected on daily basis and kept into small size petri dishes for hatching purpose. The culture was maintained up to seven generations and 6th generation was used in bioassay.

Efficacy of EPFs against S. litura eggs: During the study, four different concentrations of fungi (1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia ml^{-1}) were prepared. Four treatments were arranged in four replicates. Newly laid 50 eggs were randomly selected and dipped into each concentration for three minutes. Eggs dipped in distilled water were used as control. The treated eggs were shifted into petri plates, bottom of plates covered with tissue paper. The petri plates were kept at 26±1°C temperature and 55-65% r.h. On daily basis, petri plates were observed, and fresh cotton leaves were provided to hatched larvae. After 12 hours, emerging larvae were removed from treated eggs. Anand and Tiwary, (2009) have recorded eggs hatching data for up to 18 days. Eggs failed to hatch after 18 days of application, eggs were considered dead.

Efficacy of EPFs on S. litura larvae: Second, third and fourth instar larvae (L_2-L_4) were randomly selected and dipped singly into 1×10^7 conidia ml^{-1} solution for 30 seconds while on control treatment only distilled water was applied. The treated larvae were shifted individually into a container having (3.5×1.3 cm) and bottom covered with tissue paper. Cages were maintained at 26±1°C temperature and 55-65% relative humidity. On daily basis, cages were observed and newly fresh cotton leaves placed into cages for larval feeding. Each treatment contains 20 larvae with four replicates and mortality was noted up to 15 days.

Efficacy of EPFs against Spodoptera litura pupae: Bioefficacy of entomopathogenic fungi against 1-2 days old pupae was determined. Pupae (20) were collected randomly from culture, washed with distilled water and dipped in $1x10^8$ conidia ml^{-1} concentration. Pupae were well shaked in spore concentration with the help of stirrer for 2 minutes, placed into vials and kept in incubator at 26±1°C temperature and 50-60% relative humidity. Anand et al. (2008) have recorded that in control treatment, pupae were dipped in distilled water, both treated and untreated pupae kept in dark to examine the adult emergence for 18 days of application. Data for adult emergence was recorded and after 18 days of post treatment, un-emerged pupae were regarded as dead.

Statistical analysis: Data was subjected to analysis of variance (SAS System 2004) mortalities calculated by using Abbot formula and means compared by the Tukey HSD test at P = 0.05. LC_{50} and LT_{50} values were determined through Probit analysis.

RESULTS

Pathogenicity of entomopathogenic fungi against eggs: Bioefficacy of entomopathogenic fungi viz. I. fumosorosea 32, M.anisopliae L6 and B. bassiana 25 used during this experiment were very effective to newly laid eggs. Significantly different ($P<0.001$) mortalities were caused by EPFs at each tested conidial concentration. B. bassiana 25, M. anisopliae L6 and I. fumosorosea32 resulted in 32.64-69.66% egg mortality above 1×10^5 conidia ml^{-1} while L. lecanii 17 didn't showed any good egg mortality and caused 31.96%egg mortality at 1×10^8 conidia ml^{-1} conidial concentration. The egg mortality was low at lowest conidial concentration while increase by increasing the conidial concentration. LC50 values are also varies at different conidial concentration as shown in **Table 1**.

Table 1- Efficacy of EPFs against eggs of S. litura

Fungal isolate	1×10^5	1×10^6	1×10^7	1×10^8
M. anisopliae L6	31.42 ± 3.99a	46.50 ± 3.59a	63.88 ± 4.09a	69.66± 3.16a
B. bassiana 25	17.52 ±2.48bc	32.64 ± 2.94bc	45.27 ± 4.29b	53.36 ± 4.70a
I. fumosorosea 32	22.99 ±2.31ab	39.80 ± 3.24ab	51.99 ± 3.25ab	60.99 ± 3.87a
L. lecanii 17	6.89± 1.33c	11.99 ± 2.54c	20.54 ± 3.25c	31.96 ± 4.99b

Table 3. Median lethal concentration for S. litura eggs treated with three fungal isolates

Isolates	LC50 (fudicial limits)	Regression Equation	X	P
B. bassiana 25	2.33×10^7 (9.41×10^6-1.15×10^8)	0.1319x–2.5191	1.46	0.37
M. anisopliae L6	1.22×10^6 (3.37×10^5-2.59×10^6)	0.1424x–1.8974	1.5	0.46
I. fumosorosus 32	4.91×10^6 (2.30×10^5-1.23×10^7)	0.1541x-2.439	0.86	0.53

Table 2. Mortality of different larval instars of S. litura 10 days after exposed to fungal isolates (1×10^7 conidia ml^{-1}).

Larval instars	Mean	Larval	mortality	(%±SE)
	B. bassiana25	I. fumosorosea 32	M. anisopliaeL6	L. lecanii17
L2	53.08 ± 4.11a	49.4± 6.47ab	40.89 ± 2.79 bc	21.26 ± 3.33c
L3	47.32 ± 6.22a	43.87 ± 5.34a	32.89 ± 2.78a	11.25± 2.44b
L4	21.29 ± 2.78a	29.44 ± 3.51a	12.88 ± 3.68b	6.90 ± 2.46b

Table 4. LC50 values of fungal isolates against third instar larvae

Fungal Isolates	LC50	LFL	UFL	Regression Equation	X	P
I. fumosorosea32	2.16×10^7	1.18×10^7	5.61×10^7	0.1702x-2.9571	5.94	0.05
B. bassiana 25	1.13×10^7	5.49×10^6	2.77×10^7	0.1856x-3.0116	3.36	0.17

Table 5. Dose-mortality effect of isolates against third instar larvae ten-day after application/treatment

Conidia (ml^{-1})	Mean Larval mortality		(%± Standard Error of Means)	
Isolates	1×10^5	1×10^6	1×10^7	1×10^8
M. anisopliae L6	10.21 ± 2.32b	12.94 ± 2.17b	37.21 ± 2.94b	45.05 ± 2.75b
I. fumosorosus	32	15.90 ± 1.09ab 22.32 ± 3.31ab	55.62 ± 2.49a	60.83 ± 3.70a
B. bassiana25	22.26 ± 2.16a	29.27 ± 2.89a	61.31 ± 2.9a	65.29 ± 3.62a

Table 6. LT50 values of fungal isolates at conidial concentration of $1x10^8$ conidial ml^{-1} against third instar larvae

Isolate	LT50 (Hours) LFL	UF	Regression Equation	X	P
I. fumosorosea	32	190	183.90 199.88	0.0166x- 3.2141 37.80	0.00
B. bassiana	25	184	179.19 197.89	0.1672x -3.1333 43.71	0.00

Table 7. Effect of fungal isolates against pupal stage at conidial concentration of 1×10^8 conidia ml^{-1}.

Sr. no.	Fungal isolates	Adult Emergence 14 days post treatment	(%± Standard Error of Means) 18 days post treatment
	L. lecanii17	91.51±2.51a	92.66± 1.25ab
	I. fumosorosea32	77.257 ± 3.76b	86.01± 2.04c
	B. bassiana 25	82.76 ± 4.28ab	91.45± 2.04abc
	M. anisopiae L6	81.51± 4.34ab	89.77± 2.39bc
	Control	98.51± 1.45a	98.51± 1.43a

M. anisopliae L6, I. fumosorosea 32 and B. bassiana 25 with LC_{50} values of 1.22×10^6, 4.91×10^6 and 2.33×10^7 conidia ml^{-1}, respectively were effective against egg of S. litura. Among all tested entomopathogenic fungi, M. anisopliae L6 was found most effective fungi followed by I. fumosorosea 32 and B. bassiana 25. Based on LC_{50}, B. bassiana 25 was found less effective as compared to other fungi against egg of S. litura. The median lethal concentration (LC50) value of B. bassiana 25 was 2.33×10^7 conidia ml^{-1} which was less virulence than others fungi (Table 3).

Virulence of entomopathogenic fungi against different larval instars: Significantly different mortalities were caused by entomopathogenic fungi on different larval instars of S.litura ($F = 16.3$, $P < 0.001$ for second instar; $F = 17.3$, $P < 0.001$ for third instar while $F = 21.3$, $P < 0.001$ for fourth instar) at conidial concentration of $1x10^7$ conidia ml^{-1} ten days after application. B. bassiana 25 showed highest pathogenicity against second larvae while minimum against fourth instars (**Table 2**).

I. fumosorosea 32, M. anisopliaeL6and L. lecanii17 were found more effective against second instars while less against fourth instar. All entomopathogenic isolates were virulence against all larval instar but pathogenicity of fungi decreases with increase in larval instar. The second instars were more susceptible to fungus than third and fourth. B. bassiana 25 and I. fumosorosea32 mortalities were significantly higher but statistically equal as compared to L. lecanii (Table 4).

Dose-mortality effect of entomopathogenic fungi against third instar larvae: All fungal isolates were proved virulence against third instar larvae of S. litura under controlled conditions. The mortality rate was low at low conidial concentration while increase with increase in concentrations. The mortalities rate was varied at different ($P < 0.01$) conidial concentrations against third instar larvae. B. bassiana25 was given highest mortalities against third instar larvae followed by I. fumosorosea 32 and M. anisopliae L6 (**Table 5**).

LC50 values for 3rd instar larvae were 2.17×10^7 and 1.11×10^7 conidia ml-1, respectively in I. fumosorosea32 and B. bassiana 25 (Table 4). The median lethal time (LT50) for 3rd instar larvae were190 hours and 184 hours in I. fumosorosea 32 and B. bassiana 25at $1x10^8$ conidia ml-1, respectively (Table 6).

Impact of fungal isolates on S. litura pupae: It was observed that pupae were shown to be less vulnerable to fungal isolates than all other biological parameters such as eggs and treated pupae take time in adult conversion or emergence. There was statistically significant difference ($df = 4$, $F = 6.03$, $P < 0.01$) of fungal isolates on pupae fourteen (14) days after application. There was statistically significant difference ($df = 4$, $F = 6.38$, $P < 0.01$) of fungal isolates on pupae eighteen (18) days after application.

In B. bassiana25, M. anisopliae L6 and L. lecanii17 no difference was found in adult emergence as compared with control treatment while different in I. fumosorosea (Table 7).

Eighteen days of fungus application, adult emergence in I. fumosorosea (86.01%) was significantly different from control treatment. It was observed that pupae were found highly resistant to EPFs (Table 7).

DISCUSSION

I. fumosorosea was found highly pathogenic against *S. littoralis* and *Mamestra brassicae* eggs (Rodriguez-Rueda & Fargues, 1980). *B. bassiana*, *I. fumosorosea*, *I. javanicus* and *M. anisopliae* were highly effective against eggs of *S. frugiperda* (Idrees et al., 2021; Lezama- Gutierrez et al., 1996). The mortality rate of eggs varies according to the conidial concentration. The present study results revealed that the mortality rate increases with conidial concentration (Idrees et al., 2021). Eggs are found less vulnerable to entomopathogenic fungi than all other biological parameters of insects like larvae and pupae (Abdel-Baky et al., 1998; Mochi et al., 2009).

The infection rate of fungal isolates varies depending upon strain and insect species like *S. litura* (Dayakar & Kanaujia, 2003; Lin et al., 2007). The present study results further revealed that the mortality rate was high on earlier instars than on older instars (Purwar and Sachan, 2005). The low mortality on older instars is due to the presence of cuticle that reduced the efficacy of fungal isolates by increasing humoral immune (Asi et al., 2013).

The highest mortality was recorded at high concentration against second instar *S. litura* larvae. The present study results are in line with (Anand and Tiwary, 2009; Amer et al., 2008). The lower dose of *M. anisopliae* increase the median lethal time (LT50) of insect such as *S. littoralis* (Abou-Bakar, 1997).

In the present study, pupae were found less susceptible against entomopathogenic fungal isolates while few earlier researchers have suggested that pupa found resistant against fungal isolates due to hard and sclerotized cuticle that covered the pupal body (De et al., 2002). The current study findings are similar to some earlier study findings (Asi et al., 2013), reported that adult emergence was delayed and abnormal emergence occur. The body size and wings of adults reduced due to fungal infection. The life period of adults reduced and fertility as well as fecundity affected due to infection of fungi.

Due to fungal infection, most of the adults can't continue the mating and died during and after mating. Infected pupae, which are converted into adults causing size reduction and wing deformation of adults which resulting death. Similar findings have been reported by many researchers (Ekesi et al., 2002; Anand et al., 2008). The present study findings are in line with the results of many earlier studies that suggested that all life stages were not evenly vulnerable to fungal isolates (Angel-Sahagun et al., 2005).

The current study reported that among all biological parameters, larvae and eggs were highly susceptible while pupae less against fungal isolates. Mortality rate of eggs to fungus infection was high due to the absence of defensive mechanism (Bulet et al., 2003) while pupa has this defensive system that makes it less susceptible (Gorman et al., 2004). The entomopathogenic fungi such as *I. fumosorosea* 32, *B. bassiana* 25 and *M. anisopliae* L6, are highly against tested instar and egg of *S. litura*. Proper concentration of EPFs should be applied in the presence of larvae and eggs.

CONCLUSION

The current study concluded *B. bassiana* 25, *M. anisopliae* L6 and *I. fumosorosea* were proved to be an effective biocontrol agent against all tested stages (egg, larva, pupa and adult) of *S. litura*. The growth, and developmental parameters of insect pest reduced during high conidial concentration. Such strategy can prove an effective tool in insect pest control under laboratory as well as filed conditions.

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REFERENCES

1. Abdel-Baky NF, Arafat NS, Abdel-Salam AH, 1998. Three Clasporium spp. as promising biological control candidates for controlling whiteflies (Bemisia spp.) in Egypt. Pakistan Journal of Biological Sciences, 1(3), 188-195.
2. Abou-Bakar H, 1997. Metahrizium anisopliae (Metsch.) Sorokin, a potential entomogenous fungus for controlling cotton leaf worm, Spodoptera littoralis (Boised.) in clover fields. Egyptian Journal of Biological Control, 7(1-2), 111-114.
3. Al-Deghairi MA, 2008. Bioassay evaluation of the entomopathogenic fungi, Beauveria bassianaagainst eggs and nymphs of Bemisia tabaci Gennadius (Homoptera:

Aleyrodidae). Pakistan Journal of Biological Sciences, 11(12), 1551-1560
4. Amer MM, El-Sayed TI, Bakheit HK, Moustafa SA, El-Sayed YA, 2008. Pathogenicity and genetic variability of five entomopathogenic fungi against Spodoptera littoralis. Research Journal of Agricultural Sciences, 4(5), 354-367.
5. Anand R, Prasad B, Tiwary BN, 2008. Relative susceptibility of Spodoptera litura pupae to selected entomopathogenic fungi. Biocontrol, 54(1), 85-92.
6. Anand R, Tiwary BN, 2009. Pathogenicity of entomopathogenic fungi to eggs and larvae of Spodoptera litura, the common cutworm. Biocontrol Science and Technology, 19(9), 919-929.
7. Angel-Sahagún CA, Lezama-Gutiérrez R, Molina-Ochoa J, Galindo-Velasco E, López-Edwards M, Rebolledo-Domínguez O, Cruz-Vázquez C, Reyes-Velázquez WP, Skoda SR, Foster JE, 2005. Susceptibility of biological stages of the horn fly, Haematobia irritans, to entomopathogenic fungi (Hyphomycetes) Journal of Insect Science, 5, 50-58
8. Asi M, Bashir R, Afzal MH, Zia MK, Akram M, 2013. Potential of entomopathogenic fungi for biocontrol of Spodoptera litura Fabricius (Lepidoptera: Noctuidae). Journal of Animal and Plant Science, 23(3), 913-918.
9. Askary HY, Carriere R, Belanger R, Bordeur J, 1998. Pathogenicity of Verticillium lecanii to aphids and powdery mildew. Biocontrol Science and Technology, 8, 23-32.
10. Bulet P, Charlet M, Hetru C, 2003. Insect antimicrobial peptides. In: Ezekowitz, R.A.B. and J.A. Hoffmann (eds.), Innate Immunity. NJ: Humana Press, Totowa 89-107.
11. Daglish GJ, 1998. Efficacy of six grain protectants applied alone or in combination against three species of Coleoptera. Journal of Stored Products Research, 34, 263-268.
12. Dayakar S, Kanaujia KR, 2003. Evaluation of the pathogenicity of Beauveria bassiana, Metarhizium anisopliae and Nomuraea rileyi on different larval stages of tobacco caterpillar, Spodopteralitura (F.). Indian Journal of Plant Protection, 8(2), 9-12.
13. De La Rosa W, Lopez FL, Liedo P, 2002. Beauveria bassiana as a pathogen of the Mexican fruit fly (Diptera: Tephritidae) under laboratory conditions. Journal of Economic Entomology, 95(1), 36-43.
14. Ekesi S, Maniania NK, Lux SA, 2002. Mortality in three African tephritid fly puparia and pupa caused by entomopathogenic fungi, Metarhizium anisopliae and Beauveria bassiana. Biocontrol Science and Technology, 12(1), 7-17.
15. Gandhi K, Patil RH, Srujana, Y, 2016. Field resistance of Spodoptera litura (Fab.) to conventional insecticides in India. Crop Protection, 88, 103–108.
16. Gorman MJ, Kankanala P, Kanost MR, 2004. Bacterial challenge stimulates innate immune responses in extra-embryonic tissues of tobacco hornworm eggs. Insect Molecular Biology, 13(1), 19-24.
17. Hajek AE, Leger RJS, 1994. Interactions between fungal pathogens and insect hosts. Annual Review of Entomology, 39, 293-322.
18. Jian AA, Shahzad S, 2007. Pathogenicity of Metarhizium anisopliae Var. Acridum strains on Pink Hibiscus Mealy bug (Maconellicoccus hirsutus) affecting cotton crop. Pakistan Journal of Botany, 39 (3), 967-973.
19. Khaderkhan H, Jayaraj S, Gopalan M, 1993. Muscardine fungi for the biological control of agro forestry termite Odontotermes obesus (Rambur). Insect Science and Applied, 14, 529.
20. Lacey LA, Frutos R, Kaya HK, Vails P, 2001. Insect pathogens as biological control agents: Do they have future? Biological Control, 21(3), 230- 248.
21. Lezama GR, Alatorre RR, Bojalil JLF, Molina OJ, Arenas VM, Gonzalez RM, Rebolledo DO, 1996. Virulence of five entomopathogenic fungi (Hyphomycetes) against Spodoptera frugiperda (Lepidoptera: Noctuidae) eggs and neonate larvae. Vedalia, 3, 35-39.
22. Lin HP, Yang XJ, Gao YB, Li SG, 2007. Pathogenicity of several fungal species on Spodopteralitura. Chinse Journal of Applied and Ecology, 18(4), 937-940.
23. Lingappa S, Saxena H, Devi VPS, 2005. Role of biocontrol agents in management of Helicoverpa armigera (Hubner). In: Recent advances in Helicoverpa armigera Management (Hem Saxena, A. B. Rai, R. Ahmad and Sanjeev Gupta eds.). Indian Society of Pulses Research and development, IIPR, Kanpur. 159-184.
24. Liu XZ, Li SD, 2004. Fungi secondary metabolites in biological control of crop pests. In: Z.Q (ed) Handbook of Industrial Mycology, An. Marcel Dekker, New York, 723–744.
25. Mochi DA, Monteiro AC, Machadoa ACR, Yoshida L, 2009. Efficiency of entomopathogenic fungi in the control of eggs and larvae of the horn fly Haematobia irritans (Diptera: Muscidae). Veterinary Parasitology, 167(1), 62- 66.
26. Nguyen NC, Borgemeister H, Poehling Zimmermann G, 2007. Laboratory investigations on the potential of entomopathogenic fungi for biocontrol of Helicoverpa armigera (Lepidoptera: Noctuidae) larvae and pupae. Biocontrol Science and Technology, 17(12), 853-864.
27. Petlamul W, Prasertsan P, 2012. Evaluation of strains of Metarhizium anisopliae and Beauveria bassiana against Spodoptera litura on the basis of their virulence, germination rate, conidia production, radial growth and enzyme activity. Microbiology, 40(2),111–116
28. Purwar JP, Sachan GC, 2005. Biotoxicity of Beauveria bassiana and Metarhizium anisopliae against Spodoptera litura and Spilarctia oblique. Annals Plant Protection Science, 13(2), 360-364.
29. Ramzan M, Murtaza G, Javaid M, Iqbal N, Raza T, Arshad A, Awais M, 2019[a]. Comparative Efficacy of Newer Insecticides against Plutella xylostella and Spodoptera litura on Cauliflower under Laboratory Conditions, Indian Journal of Pure and Applied Biosciences, 7(5), 1-7.
30. Ramzan M, Naeem-Ullah U, Bokhari SHM, Saba S, 2019[b]. Pesticides; acute poisoning, suicides and disability in humans. World Journal of Biology and Biotechnology, 4(2), 5-8.
31. Rodriguez-Rueda D, Fargues J, 1980. Pathogenicity of entomopathogenic hyphomycetes, Paecilomyces fumosoroseus and Nomuraea rileyi to eggs of Noctuids, Mamestra brassicae and Spodoptera littoralis. Journal of Invertebrates Pathology, 36, 399-408.
32. Shad S, Sayyed A, Fazal S, Saleem M, Zaka S, Ali M, 2012. Field evolved resistance to carbamates, organophosphates, pyrethroids, and new chemistry insecticides in Spodoptera litura Fab. (Lepidoptera: Noctuidae). Journal of Pest Science, 85, 153–162
33. Tanada Y, Kaya HK, 1993. Insect pathology. Academic Press, Inc., New York, NY
34. Thackar JRM, 2002. An introduction to Arthropod pest control. Cambridge university press, The Edinburg Building Cambridge CB2 2RU, UK, 144.
35. Uma DK, Padmavathi J, Uma MR, Akbar AKGCP, Murali MC, 2008. A study of host specificity in the entomopathogenic fungus, Beauveria bassiana (Hypocreales: Clavicipitaceae). Biocontrol Science Technology, 18, 975 – 989.