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Potential of entomopathogenic fungi for biocontrol of *Spodoptera litura* Fab. (Lepidoptera: Noctuidae)

Kumari Pragya and SB Das

Abstract
Glycine max 'The Miracle Crop' is occupying an eminent position in world agriculture. Among major defoliators, infesting soybean crop, Tobacco caterpillar, *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) damages the crop during the vegetative stage as well as reproductive stage. Scientists are speculating an increased outbreak of this pest as the increased resistance level against various insecticides had been reported. Therefore, an effective alternative and an environmentally safe pest management strategy is the demand of day and use of biocontrol agents is undoubtedly a boon to be harnessed. Efficacy of entomopathogenic fungi i.e. *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecanii* to 2nd instar larval stage of *S. litura* at concentrations 1×10^{8}, 1×10^{10} and 1×10^{12} spores/ml were tested in-vitro at Biocontrol Research and Production Center, JNKVV, Jabalpur. Highest larval mortality (2nd instar) was observed with *B. bassiana*, followed by *M. anisopliae* and lowest in *L. lecanii*, at all the three concentrations *i.e.* 1×10^{12}, 1×10^{10} and 1×10^{8} spores / ml, which were significantly superior than the control. Therefore, in the present study *B. bassiana* at highest spore concentration (1×10^{12} spores / ml) was found to be highly effective as it registered maximum larval mortality at 120 HAT. It can be inferred that larval mortality was dependent on dose, time and pathogenicity of EPF used.

Keywords: *Entomopathogenic fungi*, soybean, *Spodoptera litura*, efficacy, biopesticides, biocontrol

Introduction
One of the major aim of green revolution that occurred in 1960's was to reach the maximum productivity level and to maximize the agricultural production, thus ensuring food security in India. Insect pests have always been a nuisance element prohibiting us from reaching this goal. In India every year approximately 16.80% crop loss has been attributed to damage due to insect pests, which results into annual revenue loss of about 36 billion USD (Dhaliwal *et al*. 2015) [4].

Soybean (*Glycine max*) is a very crucial crop which had helped India to achieve self-sufficiency in oilseeds by being a part of yellow revolution. (Deccan Herald). It belongs to family leguminaceae and also known as "miracle bean, golden bean and crop of the planet". It occupied 42% of India's total oilseeds and 25% of edible oil production (Brahman *et al*. 2018) [3]. Its luxurious crop growth, soft and succulent foliage, unlimited source of food, space and shelter makes it more preferable for insects and pests. The soybean defoliators mainly includes tobacco caterpillar and green semilooper. Immature stage of Tobacco caterpillar (*Spodoptera litura*; Lepidoptera: Noctuidae) harm the crop by feeding on vegetative parts and in severe cases completely defoliates the crop. It even damages to soybean pods, leading to dramatic yield loss (Patil 2002 and Sastawa *et al*., 2004) [14, 20]. To overcome this situation, the strategy of excessive use of pesticides has been adopted which resulted into a number of hazardous consequences like high levels of pesticides resistances, environmental toxicity, fishery losses, ground and surface water contamination, depletion of rhizospheric microflora, food safety hazards and human health concerns (Dhar *et al*. 2019) [5]. Hong *et al*. (2013) [7] reported an increased resistance level in *S. litura* against organophosphates, carbamates, synthetic pyrethroids and abamectin. An increased resistance of 3921 folds in *Spodoptera* against spinosad after eleven generations of selection have been noticed by Rehan and Freed (2014) [19]. Therefore, our research work is primarily aimed at exploring the potential of entomopathogenic fungi as an effective biopesticide for its management. The entomopathogenic fungi (EPF) have a significant role among all the biocontrol agents because of its mode of pathogenicity, broad host range, easy delivery and ability to cause epizootics (Reddy *et al*. 2013) [18]. The potential of EPF often vary among fungal species and strains. Therefore, highly virulent fungal species against a particular insect can be identified and

manipulated (Asi *et al*. 2013) [2]. Among the EPF's, *Beauveria bassiana* and *Metarhizium anisopliae* acts by depositing spores on insect cuticle and forming a germ tube, which through enzymatic and mechanical action penetrates the cuticle. Another EPF, *Lecanicillium lecanii* is also significant for degradation of insect cuticle, saprophytic growth of the fungi, activation of prophenoloxidase in the haemolymph, thus acting as a virulent factor (Reddy *et al.* 2013) [18].

Materials and Methods

Media preparation

For mass culturing of entomopathogenic fungi, Potato dextrose agar (PDA) media was prepared. For this purpose 250g of potato was washed, peeled, sliced into small pieces, 500 ml distilled water and 20g agar was added and boiled for 30 minutes. The potato extract thus obtained, was filtered through a muslin cloth. 20g dextrose was then added to the strained potato extract and the volume was made up to 1 liter with distilled water. Thereafter, the media was poured into 250ml conical flask, plugged with non-absorbent cotton wool, covered it with paper sheet and tied tightly with rubber band, and placed them in autoclave for sterilization at 15 lbs pressure and 121 ºC for 15 minutes (Shah, 2018 and www.agritech.tnau.ac.in) [22].

Entomopathogenic fungi

Three fungi *viz*. *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecani* isolated from infested larvae collected from JNKVV farm were used for bioassay studies.

Culturing of Entomopathogenic Fungi

Pure mother culture of all the three fungus were maintained on PDA slants at 4 ºC under refrigerated conditions till further use. Regular maintenance was done for further multiplication at 25±2 ºC and 70±10% RH (Shah, 2018) [22].

Materials used

The materials used to conduct the efficacy studies were zoom binocular microscope, "0" size camel hair brush, muslin cloth, conical flask, needle, pipette, filter paper, marking tags, petridishes, soybean cultivar JS 335 (Susceptible check) (Sasane *et al*. 2018) [20] atomizer, Tween-80 (0.02%), 20 mega pixel camera, BOD incubator, *S. litura* second instar larvae and three entomopathogenic fungus.

Preparation of fungal suspension

Aqueous conidial suspensions (10 ml) were made from conidia harvested from the slants prepared in conical flasks (250ml) after 14 days of inoculation. Tween-80 (0.02%) was used to disperse the conidia, it was then filtered through a double layered muslin cloth. The number of conidia per ml was enumerated using plate count method (Reddy *et al*. 2016) [17]. Initially highest required concentration (1×10^{12} spores/ml) of the fungal suspension was prepared. This filtrate was the stock solution and further lower concentrations (up to 1×10^{8} spores/ml) were prepared from it by serial dilution technique (Geroh *et al*. 2015) [6].

Bioassay against *S. litura* second instar larval stage

The virulence test was conducted against second instar larvae of *S. litura* as per the methodology proposed by Kaur *et al*. (2011) [10]. The second instar larvae were treated with three concentration (1×10^{12}, 1×10^{10} and 1×10^{8} spores/ml) of the different entomopathogenic fungi (*B. bassiana*, *M. anisopliae* and *L. lecanii*) by dipping them for 30 seconds in 10 ml of suspension with a dilution factor of 1ml of different spore concentration. In case of control, larvae were treated with distilled water having a drop of Tween- 80 (0.02%). Treated larvae were allowed to crawl freely on blotting paper to remove excess moisture. Thereafter, the larvae were placed individually in insect rearing box. All treated larvae were incubated at 27±1 ºC, 65±5% relative humidity and photophase of 14:10 hours. Fresh leaves of soybean variety JS 335 were provided as food source for the larvae which were replaced regularly at an interval of 24 hours (Asi *et al*., 2013) [2]. Larval mortality data was recorded daily and continued till their mortality or the emergence of the adult, whichever was earlier. The corrected mortality was calculated by using Abbott's formula (Prasad, 2014) [16].

$$\text{Corrected Mortality (CM)} = (T - C/100 - C) \times 100$$

Where,
T = Mortality in treatment (%)
C = Mortality in the control (%)

Statistical Analysis

Analysis of the different variables was carried out to know the degree of variation amongst all the treatments. The data was statistically analysed by applying Factorial CRD.

Result

a) Spore concentration: 1×10^{12} spores / ml

Perusal of data in Table 1 revealed that at 24 HAT, the differences in the mean *Spodoptera litura* larval mortality among different entomopathogenic fungi (EPF) were non-significant and it ranged from 0.00 (*Lecanicilium lecanii* and control) to 6.67% (*Beauveria bassiana*). As there was no mortality in control, hence the corrected mortality due to different EPF remained the same.

At 48, 72 and 96 HAT, the differences in the mean *S. litura* larval mortality among different EPF were significant. Among the EPF, *B. bassiana* recorded highest larval mortality (33.33, 73.33 and 96.67%, respectively), followed by *Metarhizium anisopliae* (30.00, 66.67 and 80.00%, respectively) and *L. lecanii* (26.67, 60.00 and 70%, respectively), but they were statistically at par with each other but were significantly superior to control (3.33, 6.67 and 10%, respectively).

No significant differences were observed among the EPF for corrected larval mortality at 48 and 72 HAT and it ranged from 24.07 (*L. lecanii*) to 30.74% (*B. bassiana*) and 56.67 (*L. lecanii*) to 71.48% (*B. bassiana*), respectively, whereas it was significant at 96 HAT. Highest corrected mortality was recorded in *B. bassiana* (96.30%), followed by *M. anisopliae* (77.78%) and *L. lecanii* (66.67%), but the later two did not differ significantly from each other.

At 120, 144 and 168 HAT, the differences in the mean *S. litura* larval mortality among different EPF were significant. Among the EPF, *B. bassiana* recorded highest larval mortality (100%), which was statistically at par with *M. anisopliae* (93.33 and 96.67%, respectively), but were significantly superior to *L. lecanii* (76.67 and 83.33%, respectively). Lowest larval mortality was recorded in control (10.00%). Computation of corrected larval mortality at 120, 144 and 168 HAT, revealed that there was significant difference among the EPF. Highest corrected mortality was recorded in *B. bassiana* (100% at 120, 144 and 168 HAT), followed by *M. anisopliae*

(92.59% at 120 HAT and 96.30% at 144 and 168 HAT) but were at par with each other. However, these were significantly superior to *L. lecanii* (74.07% at 120 HAT and 81.48% at 144 and 168 HAT).

Table 1: Efficacy of entomopathogenic fungi (EPF) (1×10^{12} spores ml^{-1}) on *S. litura* (2^{nd} instar larvae) at different intervals after treatment

EPF	Mean mortality (M) and corrected mortality (CM) of *S. litura* larvae (%) at different HAT													
	24*		48		72		96		120		144		168	
	M	CM	M*	CM	M*	CM	M	CM	M	CM	M	CM	M	CM
Beauveria bassiana	6.67 (13.96)	6.67 (13.96)	33.33 (35.52)	30.74 (33.84)	73.33 (59.33)	71.48 (57.80)	96.67 (83.86)	96.30 (83.51)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Metarhizium anisopliae	3.33 (9.01)	3.33 (9.01)	30.00 (33.32)	27.04 (30.99)	66.67 (55.09)	64.07 (53.24)	80.00 (63.93)	77.78 (62.38)	93.33 (77.71)	92.59 (77.02)	96.67 (83.86)	96.30 (83.51)	96.67 (83.86)	96.30 (83.51)
Lecanicillium lecanii	0.00 (4.05)	0.00 (4.05)	26.67 (31.32)	24.07 (29.64)	60.00 (51.15)	56.67 (48.93)	70.00 (57.00)	66.67 (54.93)	76.67 (61.71)	74.07 (60.00)	83.33 (66.14)	81.48 (64.76)	83.33 (66.14)	81.48 (64.76)
Control	0.00 (4.05)	-	3.33 (9.01)	-	6.67 (13.96)	-	10.00 (18.43)	-	10.00 (18.43)	-	10.00 (18.43)	-	10.00 (18.43)	-
SEm±	3.50	4.04	3.41	3.99	3.36	3.17	4.17	5.12	3.94	4.82	3.36	4.10	3.36	4.10
CD@5%	NS	NS	11.31	NS	11.13	NS	13.79	18.05	13.04	17.02	11.12	14.47	11.12	14.47

* = Figures in parentheses are (x+0.5) arcsin transformed values
() = Figures in parentheses are arcsin transformed values
HAT = Hours after treatment M = Mortality CM = Corrected mortality NS = Non significant

b) Spore concentration: 1× 10^{10} spores / ml

Perusal of data in Table 2 revealed that at 24 HAT, the differences in the mean *S. litura* larval mortality among different EPF were non-significant and it ranged from 0.00% (*L. lecanii* and control) to 6.67% (*B. bassiana*), as there was no mortality in control hence the corrected mortality due to different EPF remained the same.

At 48 HAT, the differences in the mean *S. litura* larval mortality among different EPF were significant. Among the EPF, *M. anisopliae* recorded highest larval mortality (30.00%), followed by *B. bassiana* (26.67%) and *L. lecanii* (23.33%), but three were statistically at par with each other, whereas in control it was 3.33%. Computation of corrected larval mortality at 48 HAT, revealed that there was no significant difference among the EPF and it ranged from 23.50 (*L. lecanii*) to 31.84% (*M. anisopliae*).

At 72, 96 and 120 HAT, the differences in the mean *S. litura* larval mortality among different EPF were significant. Among the EPF, *B. bassiana* recorded highest larval mortality (70.00, 96.67 and 100%, respectively), and was significantly superior to *M. anisopliae* (60.00, 83.33 and 93.33%, respectively) and *L. lecanii* (56.67, 70.00 and 80.00%, respectively), but the later two were statistically at par with each other, whereas in control it was 6.67% at 72 HAT and 10.00% at 96 and 120 HAT. Computation of corrected larval mortality at 72 HAT, revealed that there was no significant difference among the EPF and it ranged from 46.92 (*L. lecanii*) to 55.45% (*B. bassiana*) whereas at 96 and 120 HAT, revealed that there was significant difference among the EPF. Highest corrected mortality was recorded in *B. bassiana* (96.30 and 100%, respectively), which was significantly superior to *M. anisopliae* (81.48 and 92.59%, respectively) and *L. lecanii* (66.67 and 77.78%, respectively).

At 144 and 168 HAT, the differences in the mean *S. litura* larval mortality among different EPF were significant. Among the EPF, *B. bassiana* recorded highest larval mortality (100%), which was statistically at par with *M. anisopliae* (96.67%), but were significantly superior to *L. lecanii* (83.33%), while in control the mortality was 10.00%. Computation of corrected larval mortality at 144 and 168 HAT revealed that there was significant difference among the EPF. Highest corrected mortality was recorded in *B. bassiana* (100%), but was at par with *M. anisopliae* (96.30%). These were found significantly superior to *L. lecanii* (81.48%).

Table 2: Efficacy of entomopathogenic fungi (EPF) (1×10^{10} spores ml^{-1}) on *S. litura* (2^{nd} instar larvae) at different intervals after treatment

EPF	Mean mortality (M) and corrected mortality (CM) of *S. litura* larvae (%) at different HAT													
	24*		48*		72		96		120		144		168	
	M	CM	M	CM	M*	CM	M	CM	M	CM	M	CM	M	CM
B. bassiana	3.33 (9.01)	3.33 (9.01)	26.67 (31.32)	23.70 (28.99)	70.00 (57.32)	67.41 (55.45)	96.67 (83.86)	96.30 (83.51)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
M. anisopliae	3.33 (9.01)	3.33 (9.01)	30.00 (33.52)	27.41 (31.84)	60.00 (51.15)	56.67 (48.93)	83.33 (66.14)	81.48 (64.76)	93.33 (81.14)	92.59 (80.62)	96.67 (83.86)	96.30 (83.51)	96.67 (83.86)	96.30 (83.51)
L. lecanii	0.00 (4.05)	0.00 (4.05)	23.33 (28.45)	20.00 (23.50)	56.67 (49.14)	53.33 (46.92)	70.00 (57.00)	66.67 (54.93)	80.00 (63.43)	77.78 (61.87)	83.33 (66.14)	81.48 (64.76)	83.33 (66.14)	81.48 (64.76)
Control	0.00 (4.05)	-	3.33 (9.01)	-	6.67 (13.96)	-	10.00 (18.43)	-	10.00 (18.43)	-	10.00 (18.43)	-	10.00 (18.43)	-
SEm±	3.50	4.04	4.04	6.61	3.65	3.89	3.82	4.69	4.43	5.41	3.36	4.10	3.36	4.10
CD@5%	NS	NS	13.39	NS	12.10	NS	12.66	16.55	14.67	19.10	11.12	14.47	11.12	14.47

* = Figures in parentheses are (x+0.5) arcsin transformed values
() = Figures in parentheses are arcsin transformed values
HAT = Hours after treatment M = Mortality CM = Corrected mortality NS = Non significant

c) Spore concentration: 1× 10^{8} spores / ml

Perusal of data in Table 3 revealed that at 24 HAT, no larval mortality was observed in all EPF, including control, hence, it was not possible to compute the mortality and corrected mortality.

At 48 HAT, the differences in the mean *S. litura* larval mortality among different EPF were non-significant and it ranged from 13.33% (*M. anisopliae*) to 16.67% (*B. bassiana*

and *L. lecanii*). Computation of corrected larval mortality at 48 HAT, revealed that there was no significant difference among the EPF and it ranged from 10.00% (*M. anisopliae*) to 14.44% (*B. bassiana*).

At 72 and 96 HAT, the differences in the mean *S. litura* larval mortality among different EPF were significant. Among the EPF, *B. bassiana* recorded highest larval mortality (43.33 and 66.67%, respectively), followed by *M. anisopliae* and *L. lecanii* (40.00 and 56.67%, respectively), which were statistically at par with each other, whereas in control it was 6.67 and 10%, respectively. Computation of corrected larval mortality at 72 and 96 HAT, revealed that there was no significant difference among the EPF and it ranged from 35.93 and 51.85% (*M. anisopliae* and *L. lecanii*) to 39.63 and 62.96% (*B. bassiana*).

At 120 HAT, the differences in the mean *S. litura* larval mortality among different EPF were significant. Among the EPF, *B. bassiana* recorded highest larval mortality (76.67, 80.00 and 83.33%, respectively), which was statistically at par with *M. anisopliae* (73.33 and 76.67%, respectively). These were significantly superior to *L. lecanii* (60.00 and 66.67%). Lowest larval mortality was recorded in control (10.00%). Computation of corrected larval mortality at 120 HAT, revealed that there was no significant difference among the EPF and it ranged from 55.56 (*L. lecanii*) to 74.07% (*B. bassiana*). Computation of corrected larval mortality at 144 and 168 HAT, revealed that there was significant difference among the EPF. However, highest corrected mortality was recorded in EPF *B. bassiana* (77.78 and 81.48%, respectively), which was statistically at par with *M anisopliae* (74.07 and 76.67%, respectively). These were found significantly superior to *L. lecanii* (62.96%).

Table 3: Efficacy of entomopathogenic fungi (EPF) (1×10^8 spores ml^{-1}) on *S. litura* (2^{nd} instar larvae) at different intervals after treatment

EPF	Mean mortality (M) and corrected mortality (CM) of *S. litura* larvae (%) at different HAT													
	24*		48*		72		96		120		144		168	
	M	CM	M	CM	M*	CM	M	CM	M	CM	M	CM	M	CM
B. bassiana	0.00 (4.05)	0.00 (4.05)	16.67 (20.83)	14.44 (19.51)	43.33 (41.37)	39.63 (38.94)	66.67 (55.07)	62.96 (52.81)	76.67 (61.22)	74.07 (59.49)	80.00 (63.43)	77.78 (61.87)	83.33 (66.14)	81.48 (64.76)
M. anisopliae	0.00 (4.05)	0.00 (4.05)	13.33 (21.58)	10.00 (16.63)	40.00 (39.44)	35.93 (36.76)	56.67 (48.85)	51.85 (46.06)	73.33 (59.00)	70.37 (57.12)	76.67 (61.22)	74.07 (59.49)	76.67 (61.22)	74.07 (59.49)
L. lecanii	0.00 (4.05)	0.00 (4.05)	16.67 (24.25)	13.33 (19.30)	40.00 (39.44)	35.93 (36.93)	56.67 (48.85)	51.85 (46.06)	60.00 (50.85)	55.56 (48.25)	66.67 (54.78)	62.96 (52.55)	66.67 (54.78)	62.96 (52.55)
Control	0.00 (4.05)	-	3.33 (9.01)	-	6.67 (13.96)	-	10.00 (18.43)	-	10.00 (18.43)	-	10.00 (18.43)	-	10.00 (18.43)	-
SEm±	-	-	6.01	7.87	3.97	2.69	3.02	3.81	2.31	2.90	1.49	1.86	2.02	2.50
CD@5%	-	-	NS	NS	13.15	NS	10.00	NS	7.66	NS	4.95	6.57	6.68	8.82

* = Figures in parentheses are (x+0.5) arcsin transformed values
() = Figures in parentheses are arcsin transformed values
HAT = Hours after treatment M = Mortality CM = Corrected mortality NS = Non significant

d) Impact of Entomopathogenic fungi and spore concentration (Sc) on mortality of 2^{nd} instar *S. litura* larvae.

At 24, 48 and 72 HAT

Entomopathogenic fungi (EPF)

Perusal of data in Table 4 revealed that at 24, 48 and 72 HAT, the differences in the *S. litura* larval mortality among different EPF were non-significant. Highest larval mortality was recorded in *B. bassiana* (3.33, 25.56 and 62.22%, respectively), followed by *M. anisopliae* (2.22, 24.44 and 55.56%, respectively) and lowest moratility in *L. lecanii* (0.00, 22.22 and 52.22%, respectively). The result indicated that among the three EPF, *B. bassiana* was virulent against 2^{nd} instar *S. litura* larvae.

Spore concentration

Different spore concentrations of EPF evaluated for larval mortality at 24 HAT were found to be non-significant. However at 48 and 72 HAT, it was found to be significant and highest larval mortality was registered with spore concentration Sc_1 (30.00 and 66.67%, respectively), followed by Sc_2 (26.67 and 62.22%, respectively) and lowest in Sc_3 (15.56 and 41.11%) (Table 4). The results indicated that the larval mortality was dependent on spore concentration.

Interactions: EPF × Spore concentration

The interaction of EPF and spore concentration had no significant impact on larval mortality (Table 4).

At 96 HAT

EPF

Perusal of data in Table 4 revealed that at 96 HAT, the differences in the *S. litura* larval mortality among different EPF were significant. Among them highest mortality was recorded in *B. bassiana* (86.67%), followed by *M. anisopliae* (73.33%) and *L. lecanii* (67.78%), and they differed significantly from each other.

The results indicated that among the three EPF, the *B. bassiana* was the most virulent EPF against *S. litura* 2^{nd} instar larvae.

Spore concentration

Different spore concentration of EPF evaluated for larval mortality at 96 HAT were found to be significant. Spore concentration Sc_2 recorded highest larval mortality (83.33%), which was followed by Sc_1 (82.22%), but were at par with each other. These were significantly superior to Sc_3 which recorded larval mortality of 62.22% (Table 4). The results indicated that the larval mortality was dependent on spore concentration and period of infection.

Interactions: EPF × Spore concentration

The interaction of EPF and spore concentration had no significant impact on the larval mortality (Table 4).

Table 4: Effect of Entomopathogenic fungi (EPF) and Spore concentration (Sc) on *Spodoptera litura* (2nd instar larvae) at different hours after treatment (24 to 96 hrs.)

EPF	Mean mortality of *S. litura* (2nd instar larvae) (%) at different HAT															
	24*				48				72				96			
	Sc_1	Sc_2	Sc_3	Mean	Sc_1	Sc_2	Sc_3	Mean	Sc_1	Sc_2	Sc_3	Mean	Sc_1	Sc_2	Sc_3	Mean
B. bassiana	6.67 (13.96)	3.33 (9.01)	0.00 (4.05)	3.33 (9.01)	33.33 (35.52)	26.67 (31.32)	16.67 (20.83)	25.56 (29.22)	73.33 (59.00)	70.00 (57.00)	43.33 (41.07)	62.22 (52.36)	96.67 (83.86)	96.67 (83.86)	66.67 (55.07)	86.67 (74.26)
M. anisopliae	3.33 (9.01)	3.33 (9.01)	0.00 (4.05)	2.22 (7.36)	30.00 (33.32)	30.00 (33.52)	13.33 (21.58)	24.44 (29.47)	66.67 (54.78)	60.00 (50.85)	40.00 (39.15)	55.56 (48.26)	80.00 (63.93)	83.33 (66.14)	56.67 (48.85)	73.33 (59.64)
L. lecanii	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	26.67 (31.32)	23.33 (28.45)	16.67 (24.25)	22.22 (28.01)	60.00 (50.85)	56.67 (48.85)	40.00 (39.15)	52.22 (46.28)	70.00 (57.00)	70.00 (57.00)	63.33 (53.07)	67.78 (55.69)
Mean	3.33 (9.01)	2.22 (7.36)	0.00 (4.05)	-	30.00 (33.39)	26.67 (31.10)	15.56 (22.22)	-	66.67 (54.88)	62.22 (52.23)	41.11 (39.79)	-	82.22 (68.26)	83.33 (69.00)	62.22 (52.33)	-
	SEm±		CD (0.05)		SEm±		CD (0.05)		SEm±		CD (0.05)		SEm±		CD (0.05)	
EPF	0.64		NS		0.87		NS		0.60		NS		0.88		2.63	
Sc	0.64		NS		0.87		2.58		0.60		1.79		0.88		2.63	
EPFx Sc	1.91		NS		2.60		NS		1.80		NS		2.65		NS	

$Sc_1 = 1\times 10^{12}$ spores / ml $Sc_2 = 1\times 10^{10}$ spores / ml $Sc_3 = 1\times 10^{8}$ spores / ml
NS = Non-significant HAT = Hours after treatment
* = Figures in parenthesis are (x + 0.5) arcsin transformed values
() = Figures in parenthesis are arcsin transformed values

At 120, 144 and 168 HAT

EPF

Perusal of data in Table 5 revealed that at 120, 144 and 168 HAT, the differences in the *S. litura* larval mortality among different EPF were significant. Highest mortality was recorded in *B. bassiana* (92.22, 93.33 and 94.44%, respectively), followed by *M. anisopliae* (86.67% at 120 HAT and 90.00% at 144 and 168 HAT, respectively) and *L. lecanii* (72.22% at 120 HAT and 77.78% at 144 and 168 HAT, respectively), and they differed significantly from each other. The results indicated that among the three EPF, the *B. bassiana* was the most virulent EPF against *S. litura* 2nd instar larvae.

Spore concentration

Different spore concentration of EPF evaluated for larval mortality at 120 HAT were found to be significant. Spore concentration Sc_2 recorded highest larval mortality (91.11%), which was followed by Sc_1 (90.00%) but were at par with each other. These were significantly superior to Sc_3 with larval mortality of 70.00% (Table 5).

Different spore concentration of EPF evaluated for larval mortality at 144 and 168 HAT were found to be significant. Spore concentration Sc_1 and Sc_2 both recorded highest larval mortality (93.33%) and were significantly superior to Sc_3 (74.44 and 75.56%, respectively) (Table 5).

The results indicated that the larval mortality was dependent on spore concentration and period of infection.

Interactions: EPF × Spore concentration

The interaction of EPF and spore concentration had no significant impact on larval mortality (Table 5).

Table 5: Effect of Entomopathogenic fungi (EPF) and Spore concentration (Sc) on *Spodoptera litura* (2nd instar larvae) at different hours after treatment (120 to 168 hrs.)

EPF	Mean mortality of *S. litura* (2nd instar larvae) (%) at different HAT											
	120*				144				168			
	Sc_1	Sc_2	Sc_3	Mean	Sc_1	Sc_2	Sc_3	Mean	Sc_1	Sc_2	Sc_3	Mean
B. bassiana	100.00 (90.00)	100.00 (90.00)	76.67 (61.22)	92.22 (80.41)	100.00 (90.00)	100.00 (90.00)	80.00 (63.43)	93.33 (81.14)	100.00 (90.00)	100.00 (90.00)	83.33 (66.14)	94.44 (82.05)
M. anisopliae	93.33 (77.71)	93.33 (81.14)	73.33 (59.00)	86.67 (72.62)	96.67 (83.36)	96.67 (83.36)	76.67 (61.22)	90.00 (76.31)	96.67 (83.86)	96.67 (83.86)	76.67 (61.22)	90.00 (76.31)
L. lecanii	76.67 (61.71)	80.00 (63.43)	60.00 (50.85)	72.22 (58.67)	83.33 (66.14)	83.33 (66.14)	66.67 (54.78)	77.78 (62.36)	83.33 (66.14)	83.33 (66.14)	66.67 (54.78)	77.78 (62.36)
Mean	90.00 (76.47)	91.11 (78.19)	70.00 (57.03)	-	93.33 (80.00)	93.33 (80.00)	74.44 (59.81)	-	93.33 (80.00)	93.33 (80.00)	75.56 (60.72)	-
	SEm±		CD (0.05)		SEm±		CD (0.05)		SEm±		CD (0.05)	
EPF	0.82		2.42		0.64		1.90		0.66		1.97	
Sc	0.82		2.42		0.64		1.90		0.66		1.97	
EPF x Sc	2.45		NS		1.92		NS		1.99		NS	

$Sc_1 = 1\times 10^{12}$ spores / ml $Sc_2 = 1\times 10^{10}$ spores / ml $Sc_3 = 1\times 10^{8}$ spores / ml
NS = Non-significant HAT = Hours after treatment
* = Figures in parenthesis are (x + 0.5) arcsin transformed values
() = Figures in parenthesis are arcsin transformed values

Discussion and Conclusion

Highest larval mortality (2nd instar) was observed with *Beauveria bassiana*, followed by *Metarhizium anisopliae* and lowest in *Lecanicillium lecanii*, at all the three concentrations *i.e.* 1×10^{12}, 1×10^{10} and 1×10^{8} spores / ml, which were significantly superior than the control. The present findings confirms the findings of Nandini and Rahman (2018) [13]. The larval mortality were observed to increase with the elapse of time, as the lowest and highest mortalities were recorded at 24 and 168 hours after treatment (HAT), respectively and it

corroborates the findings of Asi *et al.* (2013) [2]. At 144 HAT, maximum *S. litura* larval mortality (100.00%) was observed with *B. bassiana*, followed by *M. anisopliae* (96.67%) and *L. lecanii* (83.33%) at 1×10^{12} and 1×10^{10} spores / ml. However, mortality observed for each entomopathogenic fungus at 24, 48, 72 and 96 HAT at 1×10^{12} spores / ml were higher in comparison to 1×10^{10} spores / ml. In the present study *B. bassiana* at highest spore concentration (1×10^{12} spores / ml) was found to be highly effective as it registered maximum larval mortality at 120 HAT, It can be inferred that larval mortality was dependent on dose, time and pathogenicity of EPF used. Similar findings have been reported by Anand and Tiwary (2009) [1], Kaur *et al.* (2011) [10], Karthikeyan and Selvanarayanan (2011) [9], Moorthi *et al.* (2011) [12], Petlamul and Prasertsan (2012), Indriyanti *et al.* (2017) [8], Swami (2018) [15] and Kumar *et al.* (2018) [11].

References

1. Anand R, Tiwary BN. Pathogenecity of entomopathogenic fungi to eggs and larvae of *Spodoptera litura*, the common cutworm. Biocontrol Science and Technology. 2009;19(9):919-929.
2. Asi MR, Bashir MH, Afzal M, Zia K, Akram M. Potential of entomopathogenic fungi for biocontrol of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Journal of Animal and Plant Sciences. 2013;23(3):913-918.
3. Brahman SK, Awasthi AK and Singh S. Studies on insects-pests of soybean (*Glycine max*) with special reference to seasonal incidence of Lepidoptera defoliators. Journal of Pharmacognosy and Phytochemistry. 2018;7(1):1808-1811.
4. Dhaliwal GS, Jindal V, Mohindru B. Crop losses due to insect pests: global and Indian scenario. Indian J Ent. 2015;77(2):165-168.
5. Dhar S, Jindal V, Jariyal M, Gupta VK. Molecular characterization of new isolates of the entomopathogenic fungus *Beauveria bassiana* and their efficacy against the tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). Egyptian Journal of Biological Pest Control. 2019;29:8
6. Geroh M, Gulati R, Tehri K. Determination of lethal concentration and lethal time of entomopathogen *Beauveria bassiana* (Balsamo) Vuillemin against *Tetranychus urticae* Koch. International Journal of Agricultural Sciences. 2015;7(5):523-528.
7. Hong T, Qi S, Xiaomao Z, Lianyang B. Field resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates and four newer chemistry insecticides in Hunan, China. J Pest Sci. 2013;86(3):599-609.
8. Indriyanti DR, Mahmuda S, Slamet M. Effect of *Beauveria* doses on *Spodoptera litura* mortality. International Journal of Scientific and Technology Research. 2017;6(9):201-210.
9. Karthikeyan A, Selvanarayanan V. *In vitro* efficacy of *Beauveria bassiana* (Bals.) Vuill. and *Verticillium lecanii* (Zimm.) Viegas against selected insect pests of cotton. Recent Research in Science and Technology. 2011;3(2):142-143.
10. Kaur S, Kaur HP, Kaur K, Kaur A. Effect of different concentration of *Beauveria bassiana* on development and reproductive potential of *Spodoptera litura* (Fabricius). Journal of Biopesticides. 2011;4(2):161-168.
11. Kumar DVS, Dhanapal R, Sandhya Rani HC, Kumar VM, Lakshmipathy R. Pathogenecity testing of indigenous isolates of entomopathogenic fungus, *Lecanicillium lecanii* against tobacco caterpillar, *Spodoptera litura*. Presented in "International Conference on Biological Control: Approaches and Applications" held at ICAR-NBAIR, Bengaluru from 27th to 29th September 2018, 99.
12. Moorthi VP, Balasubramanian C, Kubendran T. Efficacy of local isolates of *Beauveria bassiana* against *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Journal of Biological Control. 2011;25(1):22-25.
13. Nandini B, Rahman SJ. Virulence of different microbial pesticides against *Spodoptera litura*. International Journal of Current Microbiology and Applied Sciences. 2018;7(6):1380-1384.
14. Patil RH. Evaluation of insect pest management components in soybean eco-system. Ph.D. Thesis, University of Agricultural Sciences, Dharwad, 2002.
15. Petlamul W, Prasertsan P. 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. Mycobiology. 2012;40(2):111-116.
16. Prasad TV. Handbook of Entomology. Third Edition. New Vishal Publications. New Delhi, 2014, 252.
17. Reddy GB, Vijayavani S, Swarnabala G, Reddy KV. Evaluation of locally available substrates for conidial biomass production of *Beauveria bassiana* MCC0044 employing solid substrate fermentation. Journal of Agriculture and Veterinary Sciences. 2016;9(7):59-65.
18. Reddy KRK, Kumar DP and Reddy KRN. Entomopathogenic fungi: A potential bioinsecticide. KAVAKA. 2013;41(1):23-32.
19. Rehan A, Freed S. Selection, mechanism, cross resistance and stability of spinosad resistance in *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). Crop Protec. 2014;56(1):10-15.
20. Sasane AR, Bhalkare SK, Rathod PK, Undirwade DB. Biophysical basis of resistance in soybean genotypes against defoliators. Journal of Entomology and Zoology Studies. 2018;6(2):01- 07.
21. Sastawa BM, Lawan M, Maina YT. Management of insect pest of soybean. Effects of sowing dates and intercropping on damage and grain yield in the Nigerian Sudan Savanna. Crop Protection. 2004;23(2):155-161.
22. Shah N. Bionomics of whitefly, *Bemisia tabaci* on soybean cultivars and its management with entomopathogenic fungi. M.Sc. Thesis, College of Agriculture, JNKVV, Jabalpur, 2018.