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Pathogenicity of *Metarrhizium anisopliae* (Metschn.) and *Beauveria bassiana* (Bals.) against *Spodoptera litura* (Fabricius)

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ABSTRACT

The results of present investigation revealed that both the entomopathogenic fungi viz., *M. anisopliae* and *B. bassiana* were found effective against 2nd and 4th instar larvae of *S. litura*. The mortality in infected larvae increased progressively with increase in conidial concentration and exposure period and appeared to be directly proportional to the dose applied indicating a clear dose dependent pathogenic effect of both the fungi under evaluation. Among the tested concentrations, the concentration of 1.5×10^9 conidia/ml reported highest mortality (99.33 and 53.33 % for *M. anisopliae*; 86.66 and 46.66 % for *B. bassiana*) in 2nd and 4th instar larvae of *S. litura*, respectively. The younger larvae (2nd instar) were more susceptible to fungal infection than the older larvae (4th instar). *M. anisopliae* proved slightly more virulent than *B. bassiana* particularly at higher conidial concentrations. The characteristic fungal growth (greenish growth of fungal spores for *M. anisopliae* and white cottony growth for *B. bassiana*) observed on infected larvae confirmed the pathogenic nature of both the entomopathogenic fungi. The fungal pathogenicity and increased larval mortality with increasing dose and exposure may be attributed to greater conidial adhesion, germination, penetration and colonization of the insect body by both the fungal pathogens.

Keywords: Pathogenicity, *M. anisopliae*, *B. bassiana*, *S. litura*

1. Introduction

The extensive use of conventional insecticides for decades has disrupted biological control systems by natural enemies, led to the development of resistance and fostered concern over possible environmental and health hazards. Therefore, in recent years, various researchers have been concentrating their efforts on the search of natural products derived from plants and microorganisms as an alternative to conventional chemical insecticides for insect control. A common feature of some microorganisms, especially fungi, is their natural ability to produce metabolic compounds that may be toxic against insect pests. These bio-control agents kill wide range of insect pests, spread quickly, safe to non-target organisms including man, compatible with other means of pest control, amenable for mass multiplication on readily available cheap substrate. Because most of the fungi invade the host insect through the exoskeleton, they are the only significant naturally occurring entomopathogens of phytophagous insects.

Among several fungal pathogens, *Metarrhizium anisopliae* (Metschn.) Sorokin and *Beauveria bassiana* (Bals.) Vuill. have been found to be promising in the control of several agricultural pests (Lingappa *et al.*, 2005) [1]. *M. anisopliae* is a potential entomopathogenic fungus belonging to the class Hyphomycetes, which reproduce by spores. The diseases caused by this fungus is called green muscardine disease because of green colour of its spores (conidia). It is known to attack a wide range of arthropods, greater than 200 species in over 50 families. It is used to control a number of pests viz., grasshoppers, termites, thrips, caterpillars, beetles, aphids, mealy bugs, scale insects, hoppers and mosquitoes (Chaudhari, 2012; Gurav, 2012) [2, 3]. *B. bassiana* is a hyphomycete insect-pathogenic fungus in the subdivision Deuteromycotina which occurs worldwide. Over 200 species of insects in nine orders, mainly Lepidoptera and Coleoptera have since been recorded as hosts (Li *et al.*, 1988) [4]. It is found naturally on some plants and in soils as is regarded as a safe biopesticide (Uma Devi *et al.*, 2008) [5]. It was found effective against tobacco leaf eating caterpillar, *Spodoptera litura* in green house and in the field proving that the fungal spores significantly affected larval survival, indicating the potential for using the entomopathogen against this pest (Gopalakrishnan and Narayanan, 1989; Vandenberg *et al.*, 1998) [6, 7].

The tobacco leaf eating caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is widely distributed and extremely polyphagous pest with a high mobility and reproductive capacity which causes considerable damage to different host plants (Holloway, 1989) [8]. It is distributed throughout tropical and temperate Asia, Australasia and Pacific Islands (Monobullah *et al.*, 2008) [9]. It devastates a large host range of more

than 120 host plants (Ramana *et al.*, 1988) [10]. The major one includes tobacco, cotton, groundnut, jute, lucerne, maize, rice, soybean, tea, cauliflower, cabbage, capsicum, potato and castor etc. (Sharma *et al.*, 2008) [11]. The extent of damage caused by it varies from 60 to 100 per cent (Chari, 1987) [12] and 25.8 to 100 per cent (Dhir *et al.*, 1992) [13], respectively. Pest is sporadic in occurrence but when left unattended causes complete failure of the crop. It is resistant against almost all the insecticide groups (Kranthi *et al.*, 2002) [14]. Hence, microbial pesticides are alternative to tackle insecticide resistance populations of *S. litura* (Pawar *et al.*, 2005) [15].

2. Materials and methods

2.1 Sources of fungal inoculum

The laboratory cultures of both the test fungi viz., *M. anisopliae* and *B. bassiana* were procured from Bio-control Laboratory, Department of Agricultural Entomology, College of Agriculture, Dapoli. *M. anisopliae* and *B. bassiana* and were isolated from previously maintained lab cultures on agar plates and multiplied on Potato Dextrose Agar (PDA) media in petriplates at Post graduate Laboratory, Department of Plant Pathology, College of Agriculture, Dapoli.

2.2 Preparation of solution of entomopathogenic fungi

The spores of the laboratory cultured fungus viz., *M. anisopliae* and *B. bassiana* were taken in 10 ml water. One milliliter solution of the desired fungus were taken on haemocytometer, cover slip were placed over it for measuring the spores. Five squares were randomly selected for the spore count and then average numbers of spores were calculated for each square. The values obtained were put in following formula given by Knuckles *et al.* (1990) [16] to know the concentration of solution.

$$\frac{\text{No. of spores / ml} = D \times X}{N \times K}$$

Where,

D = Dilution factor

X = Total number of spore counted

N = Number of small squares counted

K = Volume above one small square in cm³

2.3 Culture of *S. litura*

The initial culture of *S. litura* was obtained by collecting a few larvae from field which were reared in the laboratory in a 25 cm high and 17 cm diameter glass jar containing 5 cm thick layer of soil at the bottom to facilitate pupation (Plate II). Fresh castor leaves were supplied daily as food to the larvae after removing the excreta and uneaten parts of leaves till pupation. The newly formed pupae were collected and placed in a glass jar (25 cm x 17 cm). The open end of the jar was closed with muslin cloth, which was secured firmly in position with a rubber band. Freshly emerged males and females were released in another glass jar (25 cm x 17 cm). The open end of the jar was closed with muslin cloth secured firmly with rubber band. A cotton swab soaked in 5 per cent honey solution was kept hanging in the jar as food for the moths. Food was changed daily. Crumpled pieces of blotting paper were kept inside the jar for egg laying. The blotting paper was removed and cut into pieces each containing egg mass and such cut pieces carrying eggs were placed for hatching in a 10 cm diameter petridish containing a round piece of blotting paper at the bottom. Freshly hatched larvae were reared on castor leaves. Thus the mass culture of pest was prepared and maintained in the laboratory (Surve, 2003). A precaution was taken to disinfect the rearing jars and petridishes with 40 per cent formalin to avoid infection. The 2nd and 4th instar larvae of same age were selected randomly from the culture and were used for testing the pathogenicity and efficacy of *M. anisopliae* and *B. bassiana* along with botanicals.

2.4 Methodology

A laboratory experiments were conducted in the Department of Agricultural Entomology, College of Agriculture, Dapoli with a view to study the pathogenicity of *M. anisopliae* and *B. bassiana* at different doses against two different instars (2nd and 4th) of *S. litura* larvae during the year 2025-26. The details of the experiments were as follows.

The culture of *M. anisopliae* and *B. bassiana* developed on PDA media were used for testing the pathogenicity of both the fungi at different doses viz., 1.5×10^8 , 1.5×10^7 , 1.5×10^6 , 1.5×10^5 and 1.5×10^4 conidia/ml prepared after serial dilution from stock suspension of 1.5×10^9 conidia/ml. The larvae along with fresh castor leaves as their food were released in petridish of 20 cm diameter and were directly sprayed with spore suspension with a hand atomizer (Kulkarni *et al.*, 2002) [17]. Then, they were air dried and transferred into petridishes. The larvae in control treatment received a spray of sterilized distilled water. The larvae were provided with untreated fresh castor leaves daily after 48 hours of treatment (Vimala Devi, 1994) [18].

2.5 Experimental details

Design	: Completely Randomized Design (CRD)
Replication	: Three
Treatments	: Seven
	1) 1.5×10^9 conidia/ml
	2) 1.5×10^8 conidia/ml
	3) 1.5×10^7 conidia/ml
	4) 1.5×10^6 conidia/ml
	5) 1.5×10^5 conidia/ml
	6) 1.5×10^4 conidia/ml
	7) Control (Sterilized water spray)
Instars to be tested	: 1) Second instar
	2) Fourth instar
Number of larvae/ treatment/replication	: 10

2.6 Observations recorded

The larvae were observed daily for the symptoms of fungal infection and mortality. In case of second instar, four observations on larval mortality i.e. on 5th, 7th, 9th and 11th DAT were recorded. While in case of fourth instar, three observations were recorded i.e. on 6th, 8th and 10th DAT, respectively, for both the fungal pathogens. Based on healthy and dead larvae, per cent mortality were worked out and subjected to statistical analysis after following arc sine transformation. The dead larvae were removed on the day of observation and maintained in a humid chamber prepared with a petriplate covered with moist blotting paper from all the sides to allow the outgrowth of fungus for confirming its infection.

3. Results and discussion

3.1. Pathogenicity of *M. anisopliae* and *B. bassiana* against second instar larvae of *S. litura*

Observations on pathogenicity of *M. anisopliae* at various doses against second instar larvae of *S. litura* recorded on 5th, 7th, 9th and 11th Day after treatment (DAT) are presented in Table 3.1. The observations recorded on 5th DAT revealed that the lower doses of 1.5×10^4 , 1.5×10^5 and 1.5×10^6 conidia/ml did not appear to be pathogenic as no larval mortality was observed in these treatments against second instar larvae of *S. litura*. However, the higher doses of 1.5×10^7 , 1.5×10^8 and 1.5×10^9 conidia/ml recorded 6.66, 13.33 and 16.66 per cent larval mortality respectively, thereby indicating pathogenic effect of the fungus. Among these treatments, the highest dose of 1.5×10^9 conidia/ml recorded comparatively higher mortality

though the differences among higher doses were not pronounced during the initial stage of observation.

The data recorded on 7th DAT showed that *M. anisopliae* exhibited pathogenicity at all the doses by causing significantly higher larval mortality in second instar larvae than untreated control. The observations clearly indicated that the pathogenicity increased with increase in conidial concentration. The highest dose of 1.5×10^9 conidia/ml was found to be the most pathogenic as it recorded maximum larval mortality of 56.66 per cent. However, it was at par with the mortality caused by 1.5×10^8 . The dose of 1.5×10^8 was found to be significantly more pathogenic than 1.5×10^7 and 1.5×10^6 with 40.00 and 26.66 per cent mortality, respectively. The lower doses of 1.5×10^5 and 1.5×10^4 conidia/ml were observed to be least pathogenic as they recorded significantly the least per cent larval mortality (16.66 and

10.00). Thus, the observations indicated that increase in the dose of conidia per ml of water increased the fungal pathogenicity.

On 9th DAT, *M. anisopliae* at all the doses was found to be more pathogenic than observed on 7th DAT as the larval mortality was ranged from 16.66 to 80.00 per cent as against 10.00 to 56.66 per cent mortality on 7th DAT. The highest dose of 1.5×10^9 was found to be most pathogenic as it caused significantly highest larval mortality (80.00 %), however, it was at par with 1.5×10^8 (73.33 %) followed by 1.5×10^7 (60.00 %). Each higher dose appeared to be significantly more pathogenic than its lower dose with an exception of 1.5×10^6 and 1.5×10^5 which remain at par with each other. The lowest dose of 1.5×10^4 conidia/ml was observed to be the least pathogenic as it caused significantly the least mortality of 16.66 per cent.

Table 3.1. Pathogenicity of *M. anisopliae* and *B. bassiana* against second instar larvae of *S. litura*

Sr. No.	Dose (Conidia/ml)	Mean per cent larval mortality							
		<i>M. anisopliae</i>				<i>B. bassiana</i>			
		5 DAT*	7 DAT	9 DAT	11 DAT	5 DAT*	7 DAT	9 DAT	11 DAT
1	1.5×10^9	16.66 (23.83)**	56.66 (48.78)	80.00 (63.43)	93.33 (75.59)	13.33 (21.12)**	46.66 (43.03)	76.66 (61.15)	86.66 (68.73)
2	1.5×10^8	13.33 (21.12)	46.66 (43.04)	73.33 (58.85)	86.67 (68.74)	6.66 (12.28)	30.00 (32.97)	60.00 (50.74)	80.00 (63.45)
3	1.5×10^7	6.66 (12.28)	40.00 (39.18)	60.00 (50.72)	73.33 (58.94)	3.33 (6.14)	26.66 (30.96)	56.66 (48.78)	73.33 (58.85)
4	1.5×10^6	0.00 (0.00)	26.66 (31.06)	46.66 (43.04)	60.00 (50.74)	0.00 (0.00)	16.66 (23.83)	36.66 (37.21)	50.00 (44.94)
5	1.5×10^5	0.00 (00.00)	16.66 (24.05)	36.66 (37.22)	43.33 (41.11)	0.00 (0.00)	6.66 (12.28)	16.66 (24.02)	26.66 (31.01)
6	1.5×10^4	0.00 (0.00)	10.00 (15.17)	16.66 (24.04)	20.00 (26.52)	0.00 (0.00)	0.00 (0.00)	10.00 (18.41)	16.66 (24.02)
7	Control (Sterilized water spray)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S. Em. (±)	2.18	2.06	1.43	2.56	2.18	2.03	1.85	2.44
	C.D. at 1%	9.18	8.68	6.04	10.80	9.18	8.58	7.81	10.23

*Days after treatment

**Figures in parentheses are arcsin transformed values.

Further observations revealed that *M. anisopliae* remained pathogenic even on 11th DAT as significantly higher larval mortality was observed at all the doses than untreated control. Again, the highest dose of 1.5×10^9 conidia/ml was observed to be the most pathogenic as it produced significantly the highest mortality of 93.33 per cent, however, it was at par with 1.5×10^8 (86.67 %). The doses 1.5×10^8 and 1.5×10^7 , 1.5×10^7 and 1.5×10^6 , 1.5×10^6 and 1.5×10^5 remained equally pathogenic, as there were no significant differences in the mortality. However, all these doses were appeared to be significantly more pathogenic to 1.5×10^4 (20.00 %). The observations also indicated that pathogenicity of the fungus increased as the period after treatment extended and appeared to be directly proportional to the dose applied.

3.2 Pathogenicity of *B. bassiana* against second instar larvae of *S. litura*

Observations on pathogenicity of *B. bassiana* at various doses against second instar larvae of *S. litura* recorded on 5th, 7th, 9th and 11th Day after treatment (DAT) are presented in Table 3.1. The data on 5th DAT indicated that the fungus *B. bassiana* showed its pathogenic activity only at higher doses viz., 1.5×10^7 , 1.5×10^8 and 1.5×10^9 showing 3.33, 6.66 and 13.33 per cent mortality in second instar larvae of *S. litura*, respectively, without any significant differences. However, the mortality was not very high. The highest dose of 1.5×10^9 caused significantly higher mortality than control. The fungus at lower doses

viz., 1.5×10^4 , 1.5×10^5 and 1.5×10^6 showed no pathogenic activity, as it caused zero per cent mortality.

On 7th DAT, it was appeared that the fungus at all the doses, except 1.5×10^4 was pathogenic, however, it caused significantly higher mortality only at 1.5×10^9 and 1.5×10^8 over rest of the doses. The highest dose of 1.5×10^9 produced significantly highest mortality of 46.66 per cent. It was followed by 1.5×10^8 (30.00 %) and 1.5×10^7 (26.66 %) which were at par with each other. There were no significant differences observed between doses of 1.5×10^8 and 1.5×10^7 , 1.5×10^7 and 1.5×10^6 were at par with each other. The lowest dose of 1.5×10^4 appeared to be non-pathogenic as it recorded 0.00 per cent larval mortality along with untreated control. Further, on 7th DAT, the fungal pathogenicity found to be increased than that on 5th DAT as the cumulative mortality caused by the fungus at various doses ranged from 6.66 to 46.66 on 7th DAT as against 3.33 to 13.33 per cent on 5th DAT. Besides this, increase in dose increased the pathogenicity of the fungus.

Observations of 9th DAT revealed that the fungus at all the doses was found to be more pathogenic as the larval mortality in second instar ranged from 10.00 to 76.66 per cent as compared to 0.00 to 46.66 per cent observed on 7th DAT. The highest dose of 1.5×10^9 appeared to be the most pathogenic as it caused significantly highest cumulative mortality of 76.66 per cent followed by 1.5×10^8 (60.00 %), 1.5×10^7 (56.66 %) and 1.5×10^6 (36.66 %). There were no significant differences between the fungal doses of 1.5×10^8 and 1.5×10^7 , $1.5 \times$

10^5 and 1.5×10^4 . The fungus at 1.5×10^6 was also found to be significantly superior to 1.5×10^5 and 1.5×10^4 . Whereas, the control showed zero per cent larval mortality.

Further observations indicated that the fungus, *B. bassiana* continued to show its pathogenicity even on 11th DAT as all the treatments recorded significantly higher larval mortality than untreated control. The highest dose of 1.5×10^9 was found to be most pathogenic and recorded maximum larval mortality of 86.66 per cent followed by 1.5×10^8 (80.00 %) and 1.5×10^7 (73.33 %) and all these three treatments were at par and equally effective in showing their high pathogenicity against second instar larvae of *S. litura*. Among remaining treatments, the treatment of 1.5×10^6 (50.00) was found significantly superior to 1.5×10^5 (26.66 %) and 1.5×10^4 (16.66 %) in causing larval mortality.

It is evident from the observations recorded on 5th DAT, 7th DAT, 9th DAT and 11th DAT that the pathogenicity of the fungus increased as the period after treatment advanced and the pathogenicity was directly proportional to the dose.

3.3 Pathogenicity of *M. anisopliae* against fourth instar larvae of *S. litura*

The observations on pathogenicity of *M. anisopliae* at various doses against fourth instar larvae of *S. litura* recorded on 6th, 8th and 10th Day after treatment (DAT) are presented in Table 3.2. The results indicated that *M. anisopliae* started to exhibit its pathogenic activity against fourth instar larvae of *S. litura* at 6th Day after treatment (DAT). However, the pathogenicity was very low as the fungus at the highest dose of 1.5×10^9 caused 13.33 per cent mortality and it was significantly superior to control and rest of the doses except 1.5×10^8 (10.00 %) which was at par with it. More or less similar trend in the pathogenicity of the fungus was also observed on 8th DAT and 10th DAT.

Again it was appeared that the fungal pathogenicity increased as the period after treatment extended, since the cumulative mortality caused

by the fungus at highest dose of 1.5×10^9 on 8th DAT was 36.66 per cent was more than that observed on 6th DAT (13.33 %). Moreover, the doses of 1.5×10^9 , 1.5×10^8 and 1.5×10^7 remained at par with each other. Lower doses of 1.5×10^6 and 1.5×10^5 recorded per cent larval mortality of 13.33 and 6.66, respectively, whereas, dose of 1.5×10^4 and control showed 0.00 per cent mortality. Further, it was also noticed that as the dose of fungus increased, its pathogenicity also increased.

At 10th DAT the per cent larval mortality ranged from 3.33 to 53.33 per cent showing an increase in the pathogenicity of the fungus as compared to observation on 8th DAT where the mortality ranged from 0.00 to 36.66 per cent. Among the treatments, the highest concentration of 1.5×10^9 was found to be the most effective treatment causing 53.33 per cent larval mortality followed by 1.5×10^8 (46.67 %), 1.5×10^7 (33.33 %). However, doses of 1.5×10^9 and 1.5×10^8 , 1.5×10^8 and 1.5×10^7 were found at par with each and equally effective in their pathogenicity against the test insect. The doses of 1.5×10^6 and 1.5×10^5 recorded per cent larval mortality of 20.00 and 13.33 per cent, respectively and were at par and superior to lowest dose 1.5×10^4 (3.33 %). The untreated control showed zero per cent larval mortality.

3.4 Pathogenicity of *B. bassiana* against fourth instar larvae of *S. litura*

The observations on pathogenicity of *B. bassiana* at various doses against fourth instar larvae of *S. litura* recorded on 6th, 8th and 10th Day after treatment (DAT) are presented in Table 3.2. Observations recorded on the 6th DAT revealed that the lower doses of 1.5×10^6 , 1.5×10^5 and 1.5×10^4 did not show any pathogenic effect, as no larval mortality was observed in these treatments. However, the higher doses of fungus viz., 1.5×10^9 , 1.5×10^8 and 1.5×10^7 caused 10.00, 3.33 and 3.33 per cent larval mortality respectively, indicating the starting of initial pathogenic effect of the entomopathogenic fungus, *B. bassiana*. Among these treatments, the

Table 3.2. Pathogenicity of *M. anisopliae* and *B. bassiana* against fourth instar larvae of *S. litura*

Sr. No.	Dose (Conidia/ml)	Mean per cent larval mortality					
		<i>M. anisopliae</i>			<i>B. bassiana</i>		
		6 DAT*	8 DAT	10 DAT	6 DAT*	8 DAT	10 DAT
1	1.5×10^9	13.33 (21.12)**	36.66 (37.22)	53.33 (46.87)	10.00 (18.41)**	30.00 (33.97)	46.67 (43.03)
2	1.5×10^8	10.00 (18.41)	30.00 (33.08)	46.67 (43.03)	3.33 (6.14)	20.00 (26.04)	36.67 (37.18)
3	1.5×10^7	3.33 (6.14)	23.33 (28.68)	33.33 (35.18)	3.33 (6.14)	16.66 (23.83)	26.66 (31.03)
4	1.5×10^6	0.00 (0.00)	13.33 (21.12)	20.00 (26.37)	0.00 (0.00)	10.00 (18.41)	16.67 (23.83)
5	1.5×10^5	0.00 (0.00)	6.66 (12.28)	13.33 (21.12)	0.00 (0.00)	6.66 (12.28)	10.00 (18.41)
6	1.5×10^4	0.00 (0.00)	0.00 (0.00)	3.33 (8.56)	0.00 (0.00)	3.33 (6.14)	3.33 (6.14)
7	Control (Sterilized water spray)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S. Em. (±)	1.78	2.91	2.18	1.78	2.26	2.48
	C.D. at 1%	7.50	12.26	9.20	7.50	9.54	10.46

*Days after treatment

**Figures in parentheses are arcsin transformed values

highest dose of 1.5×10^9 recorded a higher mortality as compared to other treatments.

Data collected on 8th DAT indicated that the fungus exhibited progressive pathogenicity across all the tested doses by causing higher larval mortality than the untreated control where the per cent larval

mortality ranged from 3.33 to 30.00. The highest dose of 1.5×10^9 proved to be the most lethal, recording a maximum larval mortality of 30.00 per cent, however, it was at par and equally effective with 1.5×10^8 inciting 20.00 per cent mortality. However, there were no significant differences between 1.5×10^8 , 1.5×10^7 and 1.5×10^6 . The

lower doses of 1.5×10^6 , 1.5×10^5 and 1.5×10^4 recorded comparatively low mortality of 10.00, 6.66 and 3.33 per cent, respectively. These results clearly indicated that the pest mortality peaks directly with an increase in conidial concentration.

Further observations on 10th DAT confirmed a substantial increase in fungal pathogenicity at all doses as larval mortality among these different doses ranged from 3.33 to 46.67 as compared to the lower mortality ranges seen during previous intervals of 6th DAT and 8th DAT. The highest dose of 1.5×10^9 proved to be the most pathogenic, resulting in a highest larval mortality of 46.67 per cent but was at par and equally effective in its pathogenicity with 1.5×10^8 (36.67 %) followed by 1.5×10^7 (26.66 %). However, 1.5×10^8 and 1.5×10^7 were at par and equally effective. The lower doses of 1.5×10^5 and 1.5×10^4 resulted in comparatively lower mortality of 10.00 and 3.33 per cent, respectively as compared to 0.00 in control.

The present results on pathogenicity of *M. anisopliae* and *B. bassiana* against *S. litura* are in close agreement with Bekheit *et al.* (2002) [19] who reported that the number of days taken for mummification to occur in treated 2nd instar larvae was shorter than that obtained with the 4th instar larvae and the mortality of *S. littoralis* larvae increased with increasing dosage of *M. anisopliae*. Dayakar *et al.* (2003) [20] showed that the LC₅₀ values of *B. bassiana* and *M. anisopliae* against second instar larvae of *S. litura* were 6.4 and 13.97 conidia/ml, respectively. Wraight *et al.* (2010) [21] found that elevated concentrations of *B. bassiana* resulted in higher mortality of lepidopteran larvae and also accelerated the infection process. Moorthi *et al.* (2011) [22] observed that the mortality of 66.67, 73.33 and 80.0 per cent with respect to the *B. bassiana* isolates Bb₀₂, Bb₀₉ and Bb₁₀, respectively, in larvae of *S. litura* was obtained four days after treatment. The LC₅₀ and LT₅₀ values for Bb₀₂, Bb₀₉ and Bb₁₀ isolates were 2.1×10^6 , 3.6×10^7 , 1.2×10^7 conidia ml⁻¹ and 4.8, 4.8 and 4.0 days @ 10^8 spore ml⁻¹, respectively. Bb₁₀ strain was the most virulent with potential for the management of *S. litura*. Petlamul *et al.* (2012) [23] revealed that all five strains each of *M. anisopliae* and *B. bassiana* were pathogenic to *S. litura* larvae. The mortality of larvae started after 2 days incubation and reached over 50 per cent at 4th day and total death (100.0 %) was occurred at 5th day from *M. anisopliae* (M33, M36, M6 strains) and *B. bassiana* (BNBCRC, BPMC) and 6th days for *M. anisopliae* (M8, MNBCRC) and *B. bassiana* (B14841, B16041, B14532). Asi *et al.* (2013) [24] reported that the virulence potential of the entomopathogenic fungi varied with different biological stages of *S. litura*. The early larval instars were found to be more susceptible than older larvae and the susceptibility of the pest to entomopathogenic fungi decreased with the advancement in age of larvae. The highest mortality was caused by *B. bassiana* 25 followed by *M. anisopliae* L6 whereas, the median lethal concentration (LC₅₀) for 3rd instar larvae was 1.11×10^7 conidia ml⁻¹ in *B. bassiana* 25. Mortality of larvae increased with increase in conidial concentrations and time elapsed after treatment. Ummidi *et al.* (2013) [25] observed that all the strains of *M. anisopliae* (M19, M20 and M45) and *B. bassiana* (B51, B52 and B55) at a concentration of 1×10^8 conidia/ml were found to be pathogenic to 2nd instar larvae of *S. litura*. The mean cumulative mortality due to *B. bassiana* and *M. anisopliae* strains ranged between 71.0 to 99.0 per cent. Mycosis was observed in all the strains where the mycosis percentage ranged from 30.0 to 98.0 per cent. B55 strain showing rapid germination also depicted higher mycosis value (98 %) and maximum value of relative virulence index (1.42). Agrawal *et al.* (2017) [26] revealed that the pathogenicity of *B. bassiana* varied among different larval stages of tobacco caterpillar, *S. litura*, with all treatments causing significantly higher mortality than the control. The earlier instars were more susceptible as compared to later instars. The highest dose of 5 per cent (2.3×10^6 conidia/ml) resulted in mortality rates of 91.66, 90.00, 88.33, 78.77, 66.11, and 49.99 per cent, respectively in all the six instars, as compared to 11.66, 7.21, 1.10, 0.00, 1.11 and 0.00 mortality, respectively in the untreated control. Overall, the larval mortality in *S. litura* increased with increasing fungal spore concentration. Indriyanti *et al.* (2017) [27] showed that the larval mortality in *S. litura* larvae increased with increasing fungal dose of *B.*

bassiana, with the highest concentration (8g 100 ml⁻¹) causing 100 per cent mortality within 10 to 12 days. Zulfritri *et al.* (2018) [28] found that the fungus *B. bassiana* (B2) caused highest mortality (70 %) against larval stages of *S. litura*. Dhar *et al.* (2019) [29] recorded significantly higher mortality rates of tobacco caterpillar, *S. litura* at all the tested concentrations of conidial suspensions (10^3 to 10^7 spores/ml) of *B. bassiana*. The mortality rate increased with the increasing number of conidial concentration used. Sonune *et al.* (2019) [30] reported that the spore suspension of *M. anisopliae* @ 1×10^9 spore ml⁻¹ recorded highest mortality of 91.66 per cent, whereas, the spore suspension of 1×10^5 spore ml⁻¹ recorded minimum mortality of 33.33 per cent in larvae of *S. litura*. Ullah *et al.* (2019) [31] observed that the entomopathogenic fungi *B. bassiana* significantly ($P < 0.001$) reduced relative growth rate (RGR), relative consumption rate (RCR) and efficiency of conversion of ingested food (ECI) in 3rd and 4th instar larvae of *S. litura*. Ramos *et al.* (2020) [32] found that *B. bassiana* and *M. anisopliae* caused 100 per cent mortality in second instar larvae and resulted in 87.0 and 75.0 per cent mortality, respectively in fourth instar larvae of *S. frugiperda*. Wargane *et al.* (2020) [33] observed a dose-dependent increase in larval mortality in second instar larvae of *S. litura* due to *B. bassiana*. The highest mortality (92.33 %) was recorded at 1×10^7 spores/ml. Vivekanandhan *et al.* (2022) [34] reported that *M. flavoviridae* conidia caused dose-dependent mortality in third and fourth instar larvae of tobacco caterpillar, *S. litura*. *M. flavoviridae* estimated LC₅₀ and LC₉₀ values against third instar larvae of *S. litura* were 3.0×10^5 and 1.1×10^{13} conidia/ml, respectively. Larvae had an 80.0 per cent mortality rate at highest concentration (1×10^9 conidia/ml) which was statistically different compared to control. The infection by entomopathogenic fungi significantly reduced total hemocyte count and phenoloxidase activity, indicating suppression of the insect immune system. Antioxidant and detoxifying enzymes such as SOD, CAT, POX, ACP, and ALP were markedly altered following infection, leading to physiological and biochemical imbalance in larvae. Gunjan *et al.* (2024) [35] showed that among the seven isolates of *B. bassiana* and *M. anisopliae* evaluated against *S. litura*, the isolates UKBSPC Bb 1 and UKMFC Ma showed a highest mortality of 93.33 per cent each at 10^8 conidia/ml. Sharma *et al.* (2024) [36] observed that the highest concentration of 1.5 per cent of *B. bassiana* and *M. anisopliae* against the 3rd instar larvae of *S. litura* caused 72 ± 0.21 and 64 ± 0.20 per cent larval mortality after 120 h exposure. Whereas, in 4th instar larvae of *S. litura*, the highest concentration of *B. bassiana* and *M. anisopliae* (1.5 % each) recorded maximum 70 ± 0.21 and 64 ± 0.20 per cent larval mortality, respectively.

4. Conclusion

The pathogenicity of *M. anisopliae* against second instar larvae of *S. litura* indicated that the fungus started to cause second instar mortality from 5th DAT and that too only at the higher doses of 1.5×10^7 , 1.5×10^8 and 1.5×10^9 only and the pathogenicity was also very low with mortality ranging from 6.66 to 16.66 per cent. The higher dose of 1.5×10^9 appeared to be the most pathogenic giving significantly the highest mortality (56.66 %) except 1.5×10^8 . The dose of 1.5×10^8 appeared to be significantly more pathogenic than 1.5×10^4 , 1.5×10^5 and 1.5×10^6 , while the lower dose of 1.5×10^4 was found to be the least pathogenic. Similar pathogenicity trend was also observed on 7th, 9th and 11th DAT. The pathogenicity test against fourth instar larvae indicated that *M. anisopliae* appeared to be pathogenic to 3rd instar larvae only from 6th DAT as against 5th DAT in second instar. The fungus showed its pathogenicity only at the higher doses of 1.5×10^7 , 1.5×10^8 and 1.5×10^9 causing 3.33, 10.00 and 13.33 per cent mortality. However, at 8th DAT the fungus at all the doses except 1.5×10^4 were found to be pathogenic but it caused significant higher mortality only at 1.5×10^8 and 1.5×10^9 than control. The fungal pathogenicity was found to be increased on 8th DAT than on 6th DAT and on 10th DAT than on 8th DAT. The fungus was found to be pathogenic to 4th instar even on 10th DAT and that too at all the doses.

Data on pathogenicity of *B. bassiana* against second instar larvae of *S. litura* revealed that the fungus started to cause mortality in second instar from 5th DAT and that too only at the higher doses of 1.5×10^7 , 1.5×10^8 and 1.5×10^9 only and the pathogenicity was also very low with mortality ranging from 3.33 to 13.33 per cent. However, the fungus was found to be pathogenic against 2nd instar at lower doses, only from 7th DAT with significantly higher mortality than the control. The higher dose of 1.5×10^9 appeared to be the most pathogenic giving significantly the highest mortality (46.66 %). The dose of 1.5×10^9 appeared to be significantly more pathogenic than 1.5×10^4 , 1.5×10^5 and 1.5×10^6 , 1.5×10^7 and 1.5×10^8 while the lower dose of 1.5×10^4 was found to be the least pathogenic. Similar pathogenicity trend was also observed on 9th and 11th DAT. *B. bassiana* also showed its pathogenic activity against fourth instar on 6th DAT and remained pathogenic upto 8th DAT. However, pathogenicity was very low as the fungus at the highest dose of 1.5×10^9 caused only 10.00 and 30.00 per cent mortality on 6th DAT and 8th DAT, which was very marginal and 46.67 per cent mortality on 10 DAT which was acceptable.

5. Limitation

The efficacy of entomopathogenic fungi is highly influenced by environmental factors such as temperature, relative humidity and light intensity, which may affect spore viability, infection rate and persistence. As a result, the performance observed under laboratory or controlled conditions may vary under field conditions. The speed of action of entomopathogenic fungi and botanicals is generally slower compared to chemical insecticides, which may limit their immediate effectiveness against severe pest outbreaks. Mass production, storage and shelf life of entomopathogenic fungi can pose practical challenges, potentially limiting their large-scale adoption by farmers. These limitations highlight the need for further field validation, formulation improvement and long-term studies to fully assess the practical applicability of entomopathogenic fungi and botanicals in integrated pest management programs.

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Declaration of AI use

The manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results and scholarly content. The author(s) accept full responsibility for the accuracy, integrity and final contents of the manuscript.

Competing interests

Authors have declared that no competing interests exist.

References

- Lingappa S, Saxena H, Devi Vimala PS. Role of biocontrol agents in management of *Helicoverpa armigera* (Hubner). In: Saxena H, Rai AB, Ahmad R, Gupta S, eds. Recent advances in *Helicoverpa armigera* Management. Kanpur: Indian Society of Pulses Research and Development, IIPR; 2005:159-184.
- Chaudhari VV. Evaluation of entomopathogenic fungi and botanicals for the management of mealy bug and scale insect. M.Sc. (Agri.) Thesis submitted to Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli - 415 712 (M.S.). 2012:1-50 (Unpublished).
- Gurav PK. Evaluation of entomopathogenic fungi and botanicals for the management of mango hoppers and thrips. M.Sc. (Agri.) Thesis submitted to Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli - 415 712 (M.S.). 2012:1-53 (Unpublished).
- Li YW, Yang JH. Prospects for the use of entomogenous fungus against forest pests. In: Li YW, Li ZZ, Liang ZQ, Wu JW, Wu ZK, Xu QF, eds. Study and application of entomogenous fungus in China. Beijing: Periodical Press; 1988:10-14.
- Uma Devi K, Padmavathi J, Uma Maheshwara Rao C, Akbar Ali Khan P, Murali Mohan C. A study of host specificity in the entomopathogenic fungus, *Beauveria bassiana* (Hypocreales: Clavicipitaceae). Biocontrol Science and Technology. 2008;18:975-989.
- Gopalakrishnan C, Narayanan K. Occurrence of two entomofungal pathogen, *Metarrhizium anisopliae* (Metschnikoff) Sorokin var. minor Tulloch against red plum weevil, *Rhynchophorus ferrugineus*. Phytoparasitica. 1989;34:370-379.
- Vandenberg JD, Shelton AM, Wilsey WT, Ramos M. Assessment of *Beauveria bassiana* sprays for the control of diamondback moth (Lepidoptera: Plutellidae) on crucifers. Journal of Economic Entomology. 1998;90:624-630.
- Holloway JD. The moths of Borneo: Family Noctuidae, triline subfamilies: Noctuinae, Heliethinae, Heninae, Acronictinae, Amphipyriinae, Agaristinae. Malayan Nature Journal. 1989;42:57-226.
- Monobrullah M, Uma Shankar. Sub-lethal effects of SpltNPV infection on developmental stages of *Spodoptera litura* (Lepidoptera: Noctuidae). Bio-control Science and Technology. 2008;18:431-437.
- Ramana VV, Reddy GPV, Krishnamurthy MM. Synthetic pyrethroids and other bait formulation in the control of *Spodoptera litura* (Fab.) attacking rabi groundnut. Pesticides. 1988;1:522-524.
- Sharma RK, Bisht RS. Antifeedant activity of indigenous plant extracts against *Spodoptera litura* Fabricius. Journal of Insect Science. 2008;21:56-60.
- Chari MS. Insect pests of tobacco and their management. Plant protection in field crops. National Seminar on plant protection in field crops. 29-31 January, 1986. CPTII, Hyderabad. Published by Plant Protection Association of India, Hyderabad. 1987:349-368.
- Dhir BC, Mohapatra HK, Senapati B. Assessment of crop loss in groundnut due to tobacco caterpillar, *Spodoptera litura* (F.). Indian Journal of Plant Protection. 1992;20:215-217.
- Kranthi KR, Jadhav DR, Kranthi S, Wanjari RR, Ali SS, Russel DA. Insecticide resistance in five major insect pest of cotton in India. Crop Protection. 2002;21:449-460.
- Pawar VM, Borikar PS. Microbial options for the management of *Helicoverpa armigera* (Hubner). In: Saxena H, Rai AB, Ahmad R, Gupta S, eds. Recent advances in *Helicoverpa armigera* Management. Kanpur: Indian Society of Pulses Research and Development, IIPR; 2005:193-231.
- Knuckles EM, Cuk J. In: Laboratory Exercises in Plant Pathology: An Industrial Kit (Eds. A.B.A.H. Baudoin, G.R. Hopper, D.E. Mathre, R.B. Carroil). Scientific Publishers (India), Jodhpur. 1990:112.
- Kulkarni NS, Lingappa S. Pathogenicity of entomopathogenic fungus, *Nomuraea rileyi* (Farlow) Samson on lepidopterous pests. Karnataka J Agric. Sci. 2002;15(2):293-298.
- Vimala Devi PS. Conidia production of the entomopathogenic fungus *Nomuraea rileyi* and its evaluation for control of *Spodoptera litura* (Fab.) on *Ricinus communis*. J Invertebr Pathol. 1994;63:145-150.
- Bekheik HKM, El Abbas FA. Pathogenicity of entomopathogenic fungi (Hyphomycetes) to larvae of the cotton leaf worm, *Spodoptera* sp. (Lepidoptera: Noctuidae). Arab Univ J Agric Sci. 2002;10(1):405-406.
- Dayakar S, Kanaujia KR. Evaluation of the pathogenicity of *Beauveria bassiana*, *Metarrhizium anisopliae* and *Nomuraea rileyi* on different larval stages of tobacco caterpillar, *Spodoptera litura* (F.). Indian J Pl Protec. 2003;31(2):9-12.

21. Wraight SP, Ramos ME, Avery PB, Jaronski ST, Vandenberg JD. Comparative virulence of *Beauveria bassiana* isolates against lepidopteran pests. *Biological control*. 2010;52(3):202-210.
22. Moorthi PV, 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.
23. Petlamul W, Prasertsan P. Evaluation of strains of *Metarrhizium 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.
24. Asi MR, Bashir MH, Afzal M, Zia K, Akram M. Potential of entomopathogenic fungi for biocontrol of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). *The Journal of Animal and Plant Sciences*. 2013;23(3):913-918.
25. Ummidi VRS, Josyula U, Vadlamani P. Germination rates of *Beauveria bassiana* and *Metarrhizium anisopliae* it possible correlation with virulence against *Spodoptera litura* larvae. *International Journal of Advanced Research*. 2013;2(1):625-630.
26. Agrawal S, Simon S. Efficacy of *Beauveria bassiana* on different larval instars of tobacco caterpillar (*Spodoptera litura* Fab.). *International Journal of Current Microbiology and Applied Sciences*. 2017;6:1992-1996.
27. Indriyanti DR, Mahnuda S, Saimet M. Effect of *Beauveria bassiana* doses on *Spodoptera litura* mortality. *International Journal of Scientific and Technology Research*. 2017;6(9):206-210.
28. Zulfitri A, Lestari AS, Krishanti NPRA, Zulfiana D. Laboratory evaluation of the selected entomopathogenic fungi and bacteria against larval and pupal stages of *Spodoptera litura* L. *IOP. Conf. Series: Earth and Environmental Science*. 2018;166:012099.
29. 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):1-9.
30. Sonune BD, Patil MJ, Kothikar RB, Sawai HR, Wargane VS, Mane KK. Pathogenicity of *Metarrhizium anisopliae* against *Spodoptera litura* and its compatibility with insecticides. *International Journal of Chemical Studies*. 2019;7(5):1637-1640.
31. Ullah MI, Altaf N, Afzal M, Arshad M, Mehmood N, Riaz M, Abdullah A. Effects of entomopathogenic fungi on the biology of *Spodoptera litura* (Lepidoptera: Noctuidae) and its reduviid predator, *Rhynocoris marginatus* (Heteroptera: Reduviidae). *International Journal of Insect Science*. 2019;11:1-7.
32. Ramos Y, Taibo AD, Jiménez JA, Portal O. Endophytic establishment of *Beauveria bassiana* and *Metarrhizium anisopliae* in maize plants and its effect against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) larvae. *Egyptian Journal of Biological Pest Control*. 2020;30(20):1-7.
33. Wargane VS, Parate RL, Lavhe NV, Sonune BD, Mane KK, Sharath CHR. Pathogenicity of *Beauveria bassiana* against second instar larvae of *Spodoptera litura* and compatibility with insecticides. *Journal of Pharmacognosy and Phytochemistry*. 2020;9(1):576-578.
34. Vivekanandhan P, Swathy K, Alford L, Pittarate S, Subala SPRR, Mekchay S, Elangovan D, Krutmuang P. Toxicity of *Metarrhizium flavoviride* conidia virulence against *Spodoptera litura* (Lepidoptera: Noctuidae) and its impact on physiological and biochemical activities. *Scientific Reports*. 2022;12:1-10.
35. Gunjan, Srivastava RM. Efficacy of indigenous isolates of entomopathogenic fungi *Beauveria bassiana* and *Metarrhizium anisopliae* on the tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). *International Journal of Advanced Biochemistry Research*. 2024;SP-8(8):1019-1023.
36. Sharma A, Thakur N, Jhamta S, Tomar P, Singh S, Rai AK, Chowdhury S, Kapoor M, Sheikh S, Rustagi S, Yadav AN. Entomopathogenic bacteria and fungi as eco-safe substitutes to chemical insecticides against leaf eating caterpillars of *Spodoptera litura*. *Journal of Applied Biology and Biotechnology*. 2024;12(4):172-176.

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