Plant Archives Vol. 26, No. 2, 2026 pp. 1793-1800 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.no.2.246

ISOLATION, MORPHOLOGICAL CHARACTERIZATION AND PATHOGENICITY OF *Metarhizium anisopliae* AGAINST *Spodoptera litura* AND THEIR EFFICACY AGAINST *Spodoptera frugiperda*

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(Date of Receiving-24-07-2026; Date of Revision-16-09-2026; Date of Acceptance-24-09-2026)

ABSTRACT

The present study was conducted to collect, isolate, identify and morphologically characterize the entomopathogenic fungus from soil samples and insect cadavers, collected from different agro-climatic parts of Chhattisgarh. During the survey 35 soil samples were collected from various crops such as redgram, greengram, blackgram, groundnut, soybean and sugarcane, along with 12 insect cadavers. One isolate of *Metarhizium anisopliae* was obtained and characterized. The colony initially appeared white due to mycelial growth and later turned green with spore production, forming colonies with raised centers. Microscopically, phialides were observed on conidiophores, and the conidia were oval, hyaline, measuring 2.02–2.35 µm in length and 1.30–1.62 µm in width. The pathogenicity of four isolates of *Metarhizium anisopliae* was evaluated against third-instar larvae of *Spodoptera litura* under laboratory conditions using four conidial concentrations (10^5 –10^8 conidia mL^{-1}). Isolate Ma4 recorded the highest mean larval mortality (89.78%). Larval mortality increased significantly with increasing conidial concentration, reaching a maximum of 89.73% at 10^8 conidia mL^{-1}. The results indicate that Ma4 at higher conidial concentrations is a promising eco-friendly biological control agent for the sustainable management of *S. litura*. *In vivo* evaluation against fall armyworm, *Spodoptera frugiperda*, revealed maximum reduction in larval population (76.74%) with the combined application of *Beauveria bassiana* and *Bacillus thuringiensis* on the 7th day after spraying. The results highlight the potential of entomopathogenic fungi as effective components of integrated pest management.

Key words : *Metarhizium anisopliae*, Entomopathogenic fungi, Morphological characterization, Compatibility studies, *Spodoptera frugiperda*, Biological control, integrated pest management (IPM).

Introduction

Entomopathogenic fungi (EPF) are bioinsecticides capable of infecting and assassinating arthropods. Although they are mainly isolated from arthropod carcasses, their natural habitat is soil. These fungi produce lipase, an enzyme that breaks the wax layer (Porte *et al*., 2025). *Metarhizium anisopliae* occurs naturally in soil, as a saprophyte within parasitized hosts. It was the first entomopathogenic fungi to be mass - produced for its use as a pest control agent because of its pathogenic effect towards seven different orders of insects (Roberts and St. Leger, 2004). Variability among *Metarhizium* isolates can also be expressed in morphological characteristics such as size and pigmentation of the conidia (Schrank and Vainstein, 2010) and in virulence factors such as germination, vegetative growth and formation of conidia, which are influenced by both the fungal strain and nutritional factors of the growth media (Shah *et al*., 2005).

Metarhizium anisopliae is also known as green muscardine fungus due to green colour of its sporulating colonies. The colony of *M. anisopliae* initially appear white when young, but as the conidia gets matured, they turn dark green. The mycelium is composed of hyaline, septate structure that are either simple or branched, terminating in single or clustered phialides. The conidia

are single celled, hyaline, smooth and long avoid to cylindrical in shape (4.8 × 1.6um), formed at the tips of the phialides and arranged in long basipetal chains. The mode of infection shows that the germ tube of entomopathogenic fungi penetrates the cuticle, leading to endocuticle lysis and the formation of hyphal bodies in the hemocoel, which thereafter transforms into invasive mycelia and causes the host death. At the final stage of the infection cycle, the mycelia emerge through the cuticle and form conidiophores (Srisukchayakul *et al*., 2005; Porte *et al*., 2025).

The tobacco cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), is an important polyphagous pest species. Recognized as a significant pest globally, *Spodoptera litura* inflicts substantial economic losses on key crops such as fruits, soybeans and vegetables (Otuka *et al*., 2020). It has been reported that the larvae of *S. litura* feed on more than 380 plant species. However, the chemical control of *S. litura* causes problems such as insecticide resistance and environmental residues (Wu *et al*., 2019). Consequently, there is a need for alternative strategies to manage this pest. The leaf eating caterpillar, *Spodoptera litura* Fabricius (Noctuidae; Lepidoptera) is one of the polyphagous and cosmopolitan pests. The defoliator caused serious economic damage to number of field crops and polyhouse crops.

The fall armyworm (*Spodoptera frugiperda*, Lepidoptera: Noctuidae) is one of the most extensively studied pests of maize, particularly following its recent spread from the Americas to Africa and Asia (Goergen *et al*., 2016; Ward and Kim, 2019). The caterpillars of this *Spodoptera* species are considerably more voracious than many other noctuid maize pests (Day *et al*., 2017).

Due to very limited studies in the state, there is need to be identify new indigenous isolates, pathogenicity and efficacy the present investigation is taken.

Materials and Methods

Collection and isolation of *M. anisopliae* from soil samples and insect cadavers

Isolation from soil : Soil samples were collected from various fields, preferably from the rhizosphere region or areas where insects are abundant. About 1 g of soil is transferred into 9 ml of sterile distilled water to prepare a soil suspension and mixed thoroughly. Serial dilutions are then prepared up to 10^{-3} to 10^{-5}. A small quantity (about

Table 1 : Details of soil sampling collected for isolation of entomopathogenic fungi *Metarhizium anisopliae.*

S. no.	Month	Locations	Latitude and longitude	Standing Crop	Soil type	Sample code
1.	August	Belgahana Bilaspur	22.437900°N 82.037800°E	Green gram	Sandy loam	S_1-S_{10}
2.	August	Karwa Raipur	22.494577°N 82.000794°E	Soyabean	Sandy loam	S_{11}-S_{14}
3.	August	Alesur Bhatapara	21.729558°N 81.987413°E	Green gram	Loamy	S_{15}-S_{18}
4.	August	Khunta Mungeli	22.115616°N 82.518716°E	Sugarcane	Loamy	S_{19}-S_{20}
5.	August	Pendri Kalan Kawardha	22.120551°N 81.443864°E	Sugarcane	Loamy	S_{22}-S_{25}
6.	August	Jeodan Khurd Kawardha	22.059028°N 81.296237°E	Redgram	Sandy loam	S_{26}
7.	August	Dholiya Bemetara	21.773089°N 81.545342°E	Green gram	Loamy	S_{27}
8.	August	Jhal Bemetara	21.807380°N 81.558912°E	Soyabean	Sandy loam	S_{28}
9.	August	BTC CARS Farm Bilaspur	22.105576°N 82.138553°E	Green gram	Sandy loam	S_{29}
10.	August	Umarkhohi Bilaspur	22.601389°N 82.062455°E	Groundnut	Sandy	S_{30}
11.	August	Gaurela-Pendra-Marwahi	22.718084°N 81.897714°E	Black gram, Green gram	Loamy	S_{31}-S_{35}

Table 2 : Details of collection of insect cadavers for isolation of entomopathogenic fungi *Metarhizium anisopliae*.

S. no.	Month	Locations	Lattitude and longitude	Standing Crop	Insect	No. of cadavers collected	Sample code
1	October	BTC CARS Bilaspur	21.105576°N 82.138553°E	Moong	*Helicoverpa armigera*	3	Ma_1-Ma_3
2	October	IGKV Field	21.241389°N 81.719444°E	Soyabean	*Spodoptera litura*	3	Ma_4-Ma_6
3	October	IGKV Field	21.238611°N 81.716389°E	Moong	*Spodoptera litura*	6	Ma_7-Ma_{12}

0.1 ml) of the diluted suspension is spread on sterile Petri plates containing selective media such as Sabouraud Dextrose Agar or Potato Dextrose Agar supplemented with antibiotics to suppress bacterial growth. The plates are incubated at 25–28°C for about 5–7 days. Colonies of *M. anisopliae* initially appear white and later develop a characteristic green to dark olive-green powdery appearance due to conidia formation. Suspected colonies are then transferred to fresh PDA plates to obtain pure cultures (Nirmalkar *et al*., 2020a).

Isolation from insect cadavers : Infected insects showing green fungal growth are selected. The insect cadavers are first surface sterilized by dipping them in 70% Ethanol for about 30–60 seconds, followed by treatment with 1% Sodium hypochlorite for 1–2 minutes, and then rinsed several times with sterile distilled water to remove surface contaminants. The sterilized insects are dried on sterile filter paper and placed directly onto PDA or SDA medium plates. The plates are incubated at 25–28°C for 4–7 days. Fungal growth emerging from the insect body is observed, and colonies showing the characteristic green sporulation of *M. anisopliae* are sub-cultured onto fresh medium to obtain pure isolates. These cultures can then be used for identification, pathogenicity studies, and further research (Nirmalkar *et al*., 2019).

To study the pathogenicity of *Metarhizium anisopliae* against *Spodoptera litura*

The pathogenicity of *Metarhizium anisopliae* against *Spodoptera litura* larvae was tested under laboratory conditions using spore concentrations of 10^3, 10^5, 10^7 and 10^9 CFU/ml. The fungus was grown on Potato Dextrose Agar at 25 ± 2°C for 10–15 days, and conidia were harvested in sterile water with 0.01% Tween 80 and filtered. Second or third instar larvae, reared on pesticide-free host leaves, were dipped in the respective suspensions, while controls were treated with sterile water containing Tween 80. Larvae were maintained under controlled conditions and mortality was recorded after 5 days and percent mortality was calculated to assess fungal pathogenicity (Santosh *et al*., 2026).

In-vivo Bio-efficacy of entompathogenic fungi against *Spodoptera frugiperda* of maize (*Zea mays*)

The *in-vivo* bio-efficacy of entomopathogenic fungi against *Spodoptera frugiperda* was evaluated under field conditions on *Zea mays*. The experiment was conducted using a randomized block design consisting of twelve treatments including an untreated control, with three replications. Treatments included different formulations and concentrations of entomopathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana*. The fungal suspensions were prepared in water to obtain the required spore concentration (1×10^8 CFU ml^{-1}) and applied as foliar spray using a hand sprayer when the pest infestation appeared in the crop. Recommended agronomic practices were followed throughout the crop period. Observations on larval population were recorded from five randomly selected plants in each plot before treatment and at regular intervals after spraying. The number of larvae per five plants was counted and the mean larval population was calculated. The percent population reduction over control was determined to evaluate the effectiveness of the treatments. The bio-efficacy of the entomopathogenic fungi was assessed based on reduction in larval population compared to the untreated control. Percentage of population reduction over control will be calculated using the modified Abbots formula given by (Fleming and Retnakaran, 1985; Urkude *et al*., 2024; Khemchand *et al*., 2026).

$$PROC = [1-\{(Ta \times Cb)/(Tb \times Ca)\}] \times 100$$

Where, Tb = Population in treatment before spray.

Ta = Population in treatment after spray.

Cb = Population in control before spray.

Ca = Population in control after spray.

Treatments Details

S. no.	Treatments	CFU ml^{-1}	Dose ml/li. of water
1	T_1 - *Beauvaria bassiana*	1×10^8	10
2	T_2 - *Beauveria bassiana* + *Bacillus thuringiensis*	1×10^8	10
3	T_3 -*Bacillus thuringiensis*	1×10^8	10
4	T_4 - *Metarhizium anisopilae*	1×10^8	10
5	T_5 - *Paecilomyces lilacinus*	1×10^8	10
6	T_6 - *Metarhizium. anisopilae* + *Paecilomyces lilacinus*	1×10^8	10
7	T_7 - *Verticillium lecanii*	1×10^8	10
8	T_8- *Verticilium lecanii* + *Paecilomyces lilacinus*	1×10^8	10
9	T_9 - *Bacillus thuringiensis* + *Paecilomyces lilacinus*	1×10^8	10
10	T_{10} - Thiamethoxam 75% SG	-	0.4
11	T_{11} - Novaluron 5.25%+Indoxacarb 4.5% SC	-	0.75
12	T_{12} - Untreated control	-	-

Results and Discussion

Isolation and identification of entomopathogens

The morphological characteristics of the *Metarhizium anisopliae* isolate were examined on potato dextrose agar (PDA) medium (Table 3). The colonies were initially white due to active mycelial growth and gradually turned green with the onset of conidial production. Mature colonies were circular, exhibiting a dense, powdery texture with a slightly raised centre. Microscopic examination revealed that the mycelium consisted of hyaline, septate hyphae with branched conidiophores arranged in clusters. The conidiophores bore cylindrical to flask-shaped phialides, which produced smooth, cylindrical to oval conidia in basipetal chains. The conidia were green in colour and imparted the characteristic green appearance to the mature colonies. Similar findings were reported by Nirmalkar *et al*. (2020), Shanker *et al*. (2023) green mycelium developed on the outer surface and intersegmental surface of deceased larvae. Circular ring colonies were formed with green powdery appearance; conidiospores were short, one celled, smooth, hyaline and densely grouped in whorls. Sun *et al*. (2016) hyphae up to 2.0–3.2 µm wide. Conidiophores were podgy, simple or highly branched, with 2–5 sterigma at the branch top. Conidia were colorless, elliptic and cylindrical, with obtuse ends. Most conidia were 4.8–5.5 µm × 2.0–2.5 µm in length.

Pathogenicity of *M. anisopliae* against *Spodoptera litura*

The pathogenicity of four isolates of *Metarhizium anisopliae* (Ma_1, Ma_2, Ma_3 and Ma_4) was evaluated against third-instar larvae of *Spodoptera litura* at four conidial concentrations (1×10^5, 1×10^6, 1×10^7 and 1×10^8 conidia mL^{-1}). Larval mortality increased with increasing conidial concentration in all the isolates. At the concentration of 1×10^5 conidia mL^{-1}, the highest larval mortality was recorded in isolate Ma_4 (75.34%), followed by Ma_2 (74.60%) and Ma_1 (49.00%), whereas the lowest mortality was observed in isolate Ma3 (41.90%). The differences among the isolates were statistically non-significant (Table 4).

At 1×10^6 conidia mL^{-1}, isolate Ma4 produced the highest mortality (90.00%), followed by Ma_2 (83.63%) and Ma_1 (80.00%), while the lowest mortality was recorded in Ma_3 (45.16%). The mortality recorded in Ma_4, Ma_2 and Ma_1 did not differ significantly from one another. At 1×10^7 conidia mL^{-1}, the maximum larval mortality was observed in isolate Ma_4 (95.67%), followed by Ma_1 (90.00%) and Ma_2 (86.42%). The mortality caused by these three isolates was statistically at par, whereas isolate Ma_3 recorded the lowest mortality (48.89%). At the highest concentration, 1×10^8 conidia mL^{-1}, isolate Ma_4 exhibited the greatest pathogenicity, resulting in 98.10% larval mortality, followed by Ma_2 (96.24%) and Ma_1 (94.57%). These three isolates were statistically non-significant with respect to one another. The lowest mortality at this concentration was recorded in isolate Ma_3 (70.00%).

Overall, isolate Ma4 consistently exhibited the highest virulence against third-instar larvae of *S. litura* across all conidial concentrations, whereas isolate Ma3 showed the lowest pathogenicity. An increase in conidial concentration resulted in a corresponding increase in larval mortality, demonstrating a concentration-dependent pathogenic response of *M. anisopliae*. Neethu *et al*. (2024) demonstrated that the results indicated a

Table 3 : Morphological characterization of *Metarhizium anisopliae* isolate.

Isolates	Growth pattern	Colour	Texture	Conidial Shape	Size of conidia	
					Length (µm)	Width (µm)
Ma_1	Green velvety growth	Green	Powdery	Ellipsoidal	2.09	1.55

Table 4 : Mortality percent of third instar larvae of *Spodoptera litura* at 96 HAT against isolates of *Metarhizium anisopliae*.

Isolates	Mortality % at 96 HAT				
	% concentration (CFU ml^{-1})				
	10^5(5%)	10^6(10%)	10^7(15%)	10^8(20%)	Mean
Metarhizium anisopliae (Ma_1)	49.00	80.00	90.00	94.57	**78.39**
M. anisopliae (Ma_2)	74.60	83.63	86.42	96.24	**85.22**
M. anisopliae (Ma_3)	41.90	45.16	48.89	70.00	**51.49**
M. anisopliae (Ma_4)	75.34	90.00	95.67	98.10	**89.78**
Mean	60.21	74.70	80.25	89.73	
		CD (5%)	SEm±	CV	
Factor A (Isolates)		2.27	0.78	1.19	
Factor B (Concentration)		2.27	0.78		
Factor A×B		4.57	1.57		

CFUs- Colony forming units, **HAT-** Hours after treatment.

concentration- dependent increase in mortality, with the highest concentration (1×10^8 conidia/ml) resulting in 91.01%.

In-vivo Bio-efficacy of entompathogenic fungi against *Spodoptera frugiperda* of maize (*Zea mays*)

Among all the treatments, T_{11} (Novaluron 5.25% + Indoxacarb 4.5% SC) was the most effective, recording the highest mean reduction in *Spodoptera frugiperda* population (88.23%) with the lowest mean larval count (0.31 larvae per five plants). This was followed by T_2 (*Beauveria bassiana + Bacillus thuringiensis*) (69.67%) and T_3 (*Bacillus thuringiensis*) (64.64%). The lowest mean reduction was observed in T_7 (*Verticillium lecanii*) (31.13%), followed by T_8 (*Verticillium lecanii + Paecilomyces lilacinus*) (42.88%). Across the observation periods, the highest mean reduction was recorded at 7 DAS (63.31%), followed by 5 DAS (56.57%) and 3 DAS (52.54%) (Table 5).

At 3 DAS, T_{11} (Novaluron 5.25% + Indoxacarb 4.5% SC) recorded the highest reduction (87.00%), followed by T_2 (*Beauveria bassiana + Bacillus thuringiensis*) (69.73%) and T_3 (*Bacillus thuringiensis*) (61.43%). Moderate efficacy was observed with T_1 (*Beauveria bassiana*), T_4 (*Metarhizium anisopliae*) and T_9 (*Bacillus thuringiensis + Paecilomyces lilacinus*), whereas the lowest reduction was recorded in T_7 (*Verticillium lecanii*) (33.42%) and T_8 (*Verticillium lecanii + Paecilomyces lilacinus*) (39.67%).

At 5 DAS, T_{11} (Novaluron 5.25% + Indoxacarb 4.5% SC) remained the most effective (89.20%), followed by T_2 (*Beauveria bassiana + Bacillus thuringiensis*) (78.04%), T_1 (*Beauveria bassiana*) (71.44%) and T_3 (*Bacillus thuringiensis*) (68.91%). Moderate reductions were recorded with T_4 (*Metarhizium anisopliae*), T_6 (*Metarhizium anisopliae + Paecilomyces lilacinus*), and T_9 (*Bacillus thuringiensis + Paecilomyces lilacinus*), while T_7 (*Verticillium lecanii*) (32.07%), T_{10} (Thiamethoxam 75% SG) (39.51%) and T_8 (*Verticillium lecanii + Paecilomyces lilacinus*) (43.25%) were the least effective.

At 7 DAS, T_{11} (Novaluron 5.25% + Indoxacarb 4.5% SC) again recorded the highest reduction (90.20%), followed by T_2 (*Beauveria bassiana + Bacillus thuringiensis*) (82.44%), T_3 (*Bacillus thuringiensis*) (77.48%) and T_1 (*Beauveria bassiana*) (73.62%). Moderate efficacy was observed with T_9 (*Bacillus thuringiensis + Paecilomyces lilacinus*), T_5 (*Paecilomyces lilacinus*) and T_6 (*Metarhizium anisopliae + Paecilomyces lilacinus*), whereas T_7 (*Verticillium lecanii*) (28.62%), T_{10} (Thiamethoxam 75% SG) (44.05%) and T_8 (*Verticillium lecanii + Paecilomyces lilacinus*) (45.71%) recorded the lowest reduction in larval population.

Similar finding was also reported by Patil *et al.* (2023) evaluated the comparative efficacy of selected bio-pesticides against the fall army worm (*Spodoptera frugiperda*) under field condition. The treatments with *Beauveria bassiana* was the most effective (59.72%) followed by *Metarhizium anisopliae* (54.98%). The results indicated that *Beauveria bassiana* was the most effective and economically viable option for managing the *Spodoptera frugiperda* of maize.

Table 5 : *In-vivo* efficacy of entomopathogenic fungi against fall army worm (*Spodoptera frugiperda*).

Treatments	CFUs ml^{-1}	Dose ml-lit	No. of larvae/ 5 plants				Mean	% reduction over control			Mean
			BS	3DAS	5DAS	7DAS		3DAS	5DAS	7DAS	
T_1 - *Beauveria bassiana*	1×10^8	10	0.65 (1.26)	0.71 (1.30)	0.53 (1.23)	0.51 (1.22)	**0.62**	56.59	71.44	73.62	**67.22**
T_2- *Beauveria bassiana* +*Bacillus thuringiensis*	1×10^8	10	0.63 (1.25)	0.49 (1.22)	0.39 (1.17)	0.35 (1.15)	**0.47**	69.73	78.04	82.44	**76.74**
T_3 - *Bacillus thuringiensis*	1×10^8	10	0.77 (1.31)	0.62 (1.27)	0.56 (1.24)	0.44 (1.19)	**0.60**	61.43	68.91	77.48	**69.27**
T_4 - *Metarhizium anisopliae*	1×10^8	10	0.87 (1.34)	0.84 (1.35)	0.82 (1.34)	0.64 (1.27)	**0.79**	46.49	53.72	72.52	**57.58**
T_5 - *Paecilomyces lilacinus*	1×10^8	10	0.85 (1.39)	1.09 (1.44)	1.08 (1.44)	0.78 (1.33)	**0.95**	32.84	39.51	59.11	**43.82**
T_6 - *Metarhizium anisopliae* + *Paecilomyces lilacinus*	1×10^8	10	1.31 (1.47)	0.90 (1.37)	0.85 (1.35)	0.84 (1.35)	**0.98**	44.47	52.41	55.80	**50.89**
T_7 - *Verticillium lecanii*	1×10^8	10	0.78 (1.31)	1.08 (1.44)	1.21 (1.48)	1.34 (1.53)	**1.10**	33.42	32.07	28.62	**31.37**
T_8 - *Verticillium lecanii* + *Paecilomyces lilacinus*	1×10^8	10	0.95 (1.37)	0.98 (1.40)	1.01 (1.41)	1.03 (1.42)	**0.99**	39.67	43.25	45.71	**42.88**
T_9 - *Bacillus thuringiensis* + *Paecilomyces lilacinus*	1×10^8	10	0.87 (1.34)	0.84 (1.35)	0.82 (1.34)	0.64 (1.27)	**0.72**	48.03	54.24	66.83	**56.37**
T_{10} -Thiomethoxam 75% SG	-	0.40	1.36 (1.27)	0.68 (1.29)	1.08 (1.44)	1.06 (1.43)	**1.05**	58.28	39.51	44.05	**47.28**
T_{11} - Indoxacarb 5.25% + Novaluron 4.5% SC	-	0.75	0.64 (1.31)	0.30 (1.10)	0.20 (1.09)	0.20 (1.09)	**0.31**	87.00	89.20	90.20	**88.80**
T_{12} - Untreated control	-	-	1.25 (1.40)	1.63 (1.62)	1.77 (1.66)	1.87 (1.70)	**1.63**	-	-	-	-
Mean			0.89	0.86	0.84	0.80		52.54	56.57	63.31	
CD (5%)			NS	0.15	0.12	0.11		7.72	6.97	5.91	
CV			NS	10.92	8.78	8.33		8.99	6.75	5.52	
SEm(±)				0.05	0.04	0.03		2.60	2.34	1.99	

Data given in parenthesis square root transformation. BS- Before Spray, DAS- Day After Spray.

Conclusion

The fungal isolate showed as white to green powdery colonies, hyaline septate hyphae, clustered conidiophores, flask-shaped phialides and cylindrical to oval conidia, were consistent with the typical features of *Metarhizium anisopliae*, confirming the identity of the isolate. *Metarhizium anisopliae* was highly pathogenic against third-instar larvae of *Spodoptera litura* under laboratory conditions. Among the isolates, Ma_4 was the most virulent. Larval mortality increased with increasing conidial concentration, with the highest mortality at 1×10^8 conidia mL^{-1}. These findings highlight the potential of Ma_4 as an effective biological control agent for the sustainable management of *S. litura*. The study revealed significant differences among the treatments in controlling *Spodoptera frugiperda*. T_{11} (Novaluron 5.25% + Indoxacarb 4.5% SC) was the most effective treatment, among the bio-pesticides combination T_2 (*Beauveria*

bassiana + *Bacillus thuringiensis*) and T_3 (*Bacillus thuringiensis*) were found superior.

Acknowledgements

Authors are thankful to Section of Plant Pathology, BTC, College of Agriculture and Research Station, Bilaspur (IGKV), Chhattisgarh for support to conducting investigation.

Declaration : Conflict of interest, author declare no conflict of interest

Statistical analysis : To compare different numerical observations, data was statistically analysed using appropriate design *i.e.*, factorial CRD with desired transformation as applicable.

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