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In-vitro evaluation of combination of microbial bio-pesticides against *Spodoptera litura* in soybean

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Abstract
Tobacco caterpillar, *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae), was reared on soybean leaves and the second instar larvae were selected to conduct bio-assay under *in-vitro* condition. The larvae were treated with different combinations of microbial bio-pesticides under laboratory conditions. Spore suspensions of microbial conidia were imposed on larvae with topical spray and leaf dip method. Results of the investigation revealed that *Metarhizium rileyi* ($2x10^8$ cfu/g) was most effective treatment against *Spodoptera litura* with highest larval mortality (100%) followed by the treatment *M. rileyi* + *Bacillus thuringiensis* var. *kurstaki* (*Btk*) after a week of treatment imposition. The least pupation (8.34%) was observed in larvae treated with *Beauveria bassiana* ($2x10^8$cfu/g). A sequential follow up from this assay was done on the resulting pupae and adults, if any. Further treatment of the resultant pupae caused mortality and adult malformation. The healthy moth emergence and fecundity was least in *B. bassiana* (5%). Thus, the present investigation confirmed the efficacy of different combinations of microbial bio-pesticides against *S. litura* that can be exploited under field condition while planning for pest control strategies in an eco-friendly manner.

Keywords: Tobacco caterpillar, *in-vitro* evaluation, microbial bio-pesticides, soybean

Introduction
Soybean (*Glycine max* (L.) Merill), also known as the "wonder crop" of the twenty-first century, contains 20 per cent oil and 40 per cent high quality protein. It is a major oilseed crop grown primarily for its seeds throughout the world. Soybean is in high demand in the market due to its high protein level and good quality oil, which contains vital fatty acids. It's mostly used for human consumption, cattle feed and industrial applications. This crop is now grown in over 50 countries and is the most widely produced and consumed oil seed crop in the world (Wilcox, 2004) [10]. After introduction of soybean crop to India during nineteen seventies, there was no insect pest problem and the crop could be harvested without any use of chemical pesticides until 1990. The low productivity of soybean both at state and national level is attributed due to biotic and abiotic stresses like drought, weeds, insect pests and diseases (Adimani, 1976 and Thippaiah, 1997) [1, 9]. Among these, insect pests often pose a serious threat to soybean production by increasing cost of cultivation and impairing quality of the produce in many ways.
However, over the last two decades the crop has been invaded by wide range of insect pests. Among them, defoliators *viz.*, tobacco caterpillar (*Spodoptera litura*), semilooper (*Thysanoplusia orichalcea*) and Bihar hairy caterpillar (*Spilosoma obliqua*) play a significant role in reducing the crop yield (Singh and Singh, 1990) [8]. The use of chemical pesticides to control the soybean pests has resulted in a number of health and environmental risks, as well as the development of insecticide resistance, which has amplified the condition, resulting in a pest outbreak. Use of microbial pesticides for the control of insect pests in the recent years offered several advantages over the chemical pesticides *viz.*, safety towards natural enemies (predators), targeted activity to the desired pest and effective at lower quantities thereby provides lower exposure and to quick deposition on leaves, no residues problems on the plant and allowing field re-entry immediately after application and amenability to use in rotation with chemical pesticides as part of IPM programmes. So, the main motto of the study was to evaluating combination of microbial bio-pesticides to minimise pesticide impact in the environment.

Materials and Methods
In order to know the efficacy of combination of microbial bio-pesticides used in pest

management were tested at Department of Agricultural Entomology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka during 2021-22. The experiment was laid out in Completely Randomized Block Design (CRD), having 11 treatments and each treatment was replicated three times.

Maintenance of test insect culture of tobacco caterpillar, *Spodoptera litura*

Egg mass and neonate larvae of *Spodoptera litura* collected from the field were taken as the initial culture. Larvae were maintained in the laboratory at 22±2^{0}C and 70-75% RH and reared up to adult stage on castor leaves, later a pair of freshly emerged adult moths were released into a wooden cage (36x36x36 cm size) for egg laying, where provision was made for 10% honey solution as adult food. Fresh castor leaves were provided inside the cage for oviposition by the mated female. The cut end of the leaf was covered with wet cotton wad to maintain the turgidity and freshness of the leaf.

Freshly laid eggs were kept in rearing boxes and provided with wet blotting paper at the bottom to protect the eggs from desiccation. After two days when eggs were turned to black purple colour, they were provided with fresh castor leaves as food for neonate larvae. The neonate larvae were released with the help of soft hair brush and kept in the rearing box whose cap was covered with muslin cloth in order to facilitate aeration. The food was changed after every 24 hours. Up to the end of first instar, larvae were reared in mass on castor leaves. When larvae were moulted to the second instar, uniform sized larvae were selected for further treatments.

Preparation of insecticidal solution

All the test cultures used for the study were procured from Institute of Organic Farming (IOF), University of Agricultural Sciences, Dharwad, Karnataka. The required solution of test cultures was prepared by using distilled water. The powder formulation of different microbial bio-pesticides was weighed separately with the help of a digital weighing machine and dissolved in water by mixing thoroughly. Bioassay was performed with two methods *viz*., topical application and leaf dip method.

A. Topical application method

The powder formulation of different test cultures weighed individually and dissolved in water by mixing thoroughly. Each test dose was applied on second instar larvae placed in a petri plate using hand atomizer. For each treatment 10 larvae were treated with recommended dose of a microbial bio-pesticides and replicated thrice. Water spray was applied in a similar manner which served as control.

B. Leaf dip method

Fresh soybean leaves were collected from the unsprayed field was washed properly with a clean water and air dried. The solution of each treatment was applied to soybean leaves by dipping separately in different microbial bio-pesticides for ten seconds. The treated leaves were allowed to dry under ceiling fan for ten minutes. One day starved second instar larvae of *Spodoptera litura* were kept in petri plates and then treated leaves will be provided as food for them. The petri plates were also provided with cotton wad and filter paper to maintain the leaf turgor. Ten larvae per treatment in each treatment with three repetitions were kept. The larvae were provided with fresh untreated food after 24 h of feeding on the treated food.

Larval mortality

Larval mortality in each treatment was recorded from first day to tenth day after treatment application. Data on mortality was converted into corrected per cent mortality of the pest in each treatment by using the formula:

$$\text{Corrected mortality} = \frac{\text{Per cent mortality in treatment - Per cent mortality in control}}{\text{100 - Per cent mortality in control}} \times 100$$

Adult emergence

Per cent pupation was calculated by counting total number of pupae formed from the total number of the larvae taken for study and per cent emergence of normal and abnormal adults were recorded by counting the adults emerged from total number of larvae used in the study. Data obtained were subjected to ANOVA after transformation with web-based software, WASP (Web Agri Stat Package) by ICARgoa.res.in.

Results and Discussion

Per cent larval mortality

In order to assess the efficacy of combination of microbial bio-pesticides used in insect pest management were tested under *in-vitro* conditions. Upto three days there was no mortality in any of the treatments. The larval mortality was observed after four days of treatment.

On fourth, fifth, sixth and seventh day, larval mortality was gradually increased and maximum larval mortality was observed after a week of treatment imposition. Seventh day after treatment, recorded data clearly indicated that the treatments T10: *Metarhizium rileyi* and T5: *M. rileyi* + *B. thuringiensis var. kurstaki* (*Btk*) were found equally superior over the all the tested bio-pesticides with 100 per cent larval mortality (Table 1). The treatments T3: *Btk*, T6:*M. rileyi* + *Beauveria bassiana*, T9: *M. anisopliae* + *M. rileyi*, T7:*M. anisopliae* + *Btk*, T4:*M. anisopliae* + *B. bassiana* and T8: *B. bassiana* were found be next effective treatments with 90, 80, 66.67, 53.33, 50 and 40 per cent larval mortality, respectively and least per cent mortality was recorded in the treatment T7: *M. anisopliae* (33.33%). Eighth day after treatment complete larval mortality (100%) was observed in the treatments *Bacillus thuringiensis* var. *kurstaki*, *Metarhizium rileyi* + *Beauveria bassiana* and *M. anisopliae* + *M. rileyi*. The treatments *B. bassiana* + *Btk* (90.00%), *B. bassiana* (63.33%) and *M. anisopliae* (43.33%) were found to be next best treatments. Untreated control recorded the least larval mortality of 3.33 per cent. On ninth day after treatment imposition *Beauveria bassiana* + *Bacillus thuringiensis* var. *kurstaki* (*Btk*) recorded 90.66 per cent larval mortality followed by *Metarhizium anisopliae* + *B. thuringiensis* var. *kurstaki* (87.33%), *M. anisopliae* + *B. bassiana* (77.33%), *B. bassiana* (66.66%), *M. anisopliae* (46.66%) and least mortality was observed in untreated control (6.66%).The same trend was observed on tenth day after treatment.

General observations made during the study indicated that the fungal infection was initiated after four days of treatment imposition and larval mortality significantly increased at six to seven days of post-treatment. The cadavers of dead larvae infected with microbial bio-pesticides were firm and brittle. During mummification, the cadavers were covered with white

(*M. rileyi* and *B. bassiana*) and green (*M. anisopliae*) fungal growth through the intersegmental membranes, whereas *Btk* infected larvae turns blackened colour, later stop feeding and leading to death by starvation. The early instars of larvae were more susceptible than late larval instars, which is may be because of the composition of larval integument that allowed effective penetration of infection peg of the fungus and resulted in higher mortality in early instars than late larval instars. Similar observations on per cent larval mortality were made by Patil and Abhilash (2014) [7] who reported that the mycopathogen, *Nomuraea rileyi* was proved pathogenic to leaf eating caterpillars and non-pathogenic to other beneficial insects. Nandini and Rahman (2018) [6] reported that 12 days after treatment *Beauveria bassiana* showed the highest pathogenicity (96%) against *S. litura* followed by *M. anisopliae* (80%). Up to 100% larval mortality of *Spodoptera frugiperda* was recorded at 7 days after treatment of *B. bassiana* (Montecalvo and Navasero, 2021) [5]. Bosa *et al.* (2004) [3] reported the efficacy of *M. rileyi* isolates in Colombia, causing 73-100% mortality in second instar larvae of *S. frugiperda.*

Table 1: *In-vitro* evaluation of combination of microbial bio-pesticides against *Spodoptera litura*

Tr. No.	Treatment details	Dosage (Per litre)	Per cent mortality recorded at different days after treatment						
			4 DAT	5 DAT	6 DAT	7 DAT	8 DAT	9 DAT	10 DAT
T_1	*Beauveria bassiana* ($2x10^8$ cfu/g)	2 g	3.33 (10.50)^d	10.00 (18.42)^f	23.33 (28.86)^f	40.00 (39.23)^g	63.33 (52.74)^d	66.66 (54.75)^d	66.66 (54.73)^d
T_2	*Metarhizium anisopliae* ($2x10^8$cfu/g)	2 g	0.00 (0.00)^e	6.67 (14.93)^g	16.17 (24.05)^g	33.33 (35.26)^h	43.33 (41.16)^e	46.66 (49.22)^e	46.66 (43.08)^e
T_3	*Bacillus thuringiensis* var. *kurstaki* (*Btk*)	1 ml	10.00 (18.43)^b	30.00 (33.21)^b	56.67 (48.84)^b	90.00 (71.62)^b	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)^a
T_4	*M. anisopliae* ($2x10^8$ cfu/g) + *B. bassiana* ($2x10^8$ cfu/g)	2 g + 2 g	3.33 (10.50)^d	13.33 (21.40)^e	30.00 (33.21)^e	50.00 (45.00)^f	76.67 (61.22)^c	77.33 (61.57)^c	78.00 (62.04)^c
T_5	*M. rileyi* ($2x10^8$cfu/g) + *Btk*	2 g + 1 ml	10.00 (18.43)^b	30.00 (33.21)^b	60.00 (50.77)^b	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)^a
T_6	*M. rileyi* ($2x10^8$ cfu/g) + *B. bassiana* ($2x10^8$ cfu/g)	2 g + 2 g	10.00 (18.43)^b	26.67 (31.08)^c	50.00 (45.00)^c	80.00 (63.45)^c	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)^a
T_7	*M. anisopliae* ($2x10^8$cfu/g) + *Btk*	2 g + 1 ml	3.33 (10.50)^d	13.33 (21.40 ^{e}	30.00 (33.21)^e	53.33 (46.91)^{ef}	86.67 (68.66)^b	87.33 (69.17)^b	88.00 (69.77)^b
T_8	*B. bassiana* ($2x10^8$ cfu/g) + *Btk*	2 g + 1 ml	3.33 (10.50)^d	13.33 (21.40)^e	33.33 (35.25)^e	56.67 (48.84)^e	90.00 (71.57)^b	90.66 (72.22)^b	91.66 (73.23)^b
T_9	*M. anisopliae* ($2x10^8$cfu/g) + *M. rileyi* ($2x10^8$cfu/g)	2 g + 2 g	6.67 (14.95)^c	20.00 (26.56) ^{d}	40.00 (39.23)^d	66.67 (54.75)^d	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)^a
T_{10}	*M. rileyi* ($2x10^8$cfu/g) - Standard check	2 g	13.33 (21.41)^a	36.67 (37.26)^a	70.00 (56.79)^a	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)^a
T_{11}	Untreated control	-	0.00 (0.00) ^{e}	0.00 (0.00) ^{h}	0.00 (0.00)^h	3.33 (10.50)^i	3.33 (10.50)^f	6.66 (14.93)^f	6.66 (14.93)^f
S.Em ±			0.22	0.42	0.66	0.96	0.96	0.51	0.53
CD (1%)			0.88	1.70	2.65	3.84	3.83	2.03	2.09
CV (%)			3.15	3.15	3.21	3.08	3.18	3.03	3.15

Figures in parentheses are angular transformed values; DAT- Days after treatment
Means followed by same letters in the column are not statistically different by DMRT (P=0.05)

Per cent normal and abnormal pupae and adult emergence

From the results, it was found that highest pupation was noticed in untreated control (93.34%) followed by the treatment *Metarhizium anisopliae* (53.34%) and *Beauveria bassiana* (33.34%). Abnormal pupae were more in the treatment *Metarhizium anisopliae* (13.33%). Highest adult emergence was noticed in untreated control (90%) followed by the treatment *M. anisopliae* (40%). Abnormal adult emergence was higher in the treatment *B. bassiana* and *M. anisopliae* (6.67%) (Table 2).

Table 2: Effect of bio-pesticides on pupation and adult emergence of *Spodoptera litura* under *in-vitro* condition

Tr. No.	Treatment details	Pupation (%)			Adult emergence (%)		
		Normal	Abnormal	Total	Normal	Abnormal	Total
T_1	*Beauveria bassiana* ($2x10^8$ cfu/g)	26.67	6.67	33.34	20.00	6.67	26.67
T_2	*Metarhizium anisopliae*($2x10^8$cfu/g)	40.00	13.33	53.34	33.33	6.67	40.00
T_3	*Bacillus thuringiensis* var. *kurstaki* (*Btk*)	0.00	0.00	0.00	0.00	0.00	0.00
T_4	*M. anisopliae* ($2x10^8$ cfu/g) + *B. bassiana* ($2x10^8$ cfu/g)	18.67	3.33	22.00	15.33	3.33	18.67
T_5	*M. rileyi*($2x10^8$cfu/g) + *Btk*	0.00	0.00	0.00	0.00	0.00	0.00
T_6	*M. rileyi* ($2x10^8$ cfu/g) + *B. bassiana* ($2x10^8$ cfu/g)	0.00	0.00	0.00	0.00	0.00	0.00
T_7	*M. anisopliae*($2x10^8$cfu/g) + *Btk*	8.67	3.33	12.00	8.67	0.00	8.67
T_8	*B. bassiana* ($2x10^8$ cfu/g) + *Btk*	8.33	0.00	8.34	5.00	0.00	5.00
T_9	*M. anisopliae* ($2x10^8$cfu/g) + *M. rileyi* ($2x10^8$cfu/g)	0.00	0.00	0.00	0.00	0.00	0.00
T_{10}	*M. rileyi* ($2x10^8$cfu/g) - Standard check	0.00	0.00	0.00	0.00	0.00	0.00
T_{11}	Untreated control	90.00	3.33	93.34	86.67	3.33	90.00

The present findings revealed that, either in combination of *Metarhizium anisopliae* + *Beauveria bassiana* or alone application of *Metarhizium anisopliae* and *Beauveria bassiana* does not cause any deleterious effects on *S. litura* population. Since the larval mortality is very less in these treatments, the survived larvae undergo pupation and finally emerged as adults. Observations made during the study revealed that pupae were very less susceptible to fungal infection. However, the fungal infection caused deformities or abnormalities in adults which may affect its mating and oviposition behavior as well as the flying capacity of the insect.

The present findings are in accordance with Asi *et al.* (2013) [2], who reported that pupae of *S. litura* were less susceptible to entomopathogenic fungi, however, adult emergence was delayed in fungal treated pupae and malformations in emerged adults from treated pupae were observed with reduced body size and wings making them unable to fly and eventually die without mating. Pupae treated with fungal pathogens result in lower adult emergence. Low mortality in pre-pupae and pupae can be attributed to the shorter period for fungal infection to occur (Ekesi *et al*., 2002) [4]. Montecalvo and Navasero (2021) [5] reported that the entomopathogenic fungi at 1×10^8 conidia/ml caused low mortality in pre-pupae with only 3.33% in *B. bassiana* and 20.00% in *M. anisopliae* in *Spodoptera frugiperda.* Abnormalities in adults of *S. frugiperda* were recorded in 11.54 to 24.13% and 7.14 to 27.59% of treated pupae with *B. bassiana* and *M. anisopliae*, respectively.

Conclusion

The treatment *Metarhizium rileyi* was found to be most effective bio-pesticide by recording highest mortality on 4, 5, 6, 7 days after treatment followed by the treatments *M. rileyi* + *Bacillus thuringiensis* var. *kurstaki, Btk*, *M. rileyi* + *Beauveria bassiana* and *M. rileyi* + *M. anisopliae.* Least per cent pupation was observed in the treatment *Beauveria bassiana* + *Bacillus thuringiensis* var. *kurstaki* followed by *Metarhizium anisopliae* + *Bacillus thuringiensis* var. *kurstaki and Metarhizium anisopliae* + *Beauveria bassiana.* Highest per cent adult emergence was noticed in the treatment *M. anisopliae.*

References

1. Adimani BD. Studies on the insects of soybean (*Glycine max* (L.) with special reference to the bionomics and control of pod borer, *Cydia ptychora* (Lepidoptera: Tortricidae). M. Sc. (Agri.) Thesis. University of Agricultural Sciences, Bengaluru, India, 1976.
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 Plant Sciences. 2013;23(3):913-918.
3. Bosa O, Chavez D, Toress L, Paris A, Villamizar L, Cotes AM. Evaluation of native isolates of *Nomuraea rileyi* for the control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Review of Colombia Entomology. 2004;30(1):93-97.
4. Ekesi S, Maniania NK, Lux SA. Mortality in three African tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. Biocontrol Science Technology. 2002;12(1):7-17.
5. Montecalvo MP, Navasero MM. *Metarhizium rileyi* (Farlow) Samson from *Spodoptera exigua* (Hubner) cross infects fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) larvae. Philippians Journal of Science. 2021;150(1):193-199.
6. Nandini B, Rahman SJ. Virulence of the different microbial pesticides against *Spodoptera litura*. International Journal of Current Microbiology and Applied Sciences. 2018;7(6):1380-1384.
7. Patil RH, Abhilash C. *Nomuraea rileyi* (Farlow) Samson, A bio-pesticide IPM component for the management of leaf eating caterpillars in soybean ecosystem. International conference on Biological, Civil and Environmental Engineering (BCEE-2014), Dubai, 2014,131-132.
8. Singh OP, Singh KJ. Insect pests of soybean and their management. Indian Farming. 1990;39(100):9-14.
9. Thippaiah M. Bio-ecology of semilooper, *Thysanoplusia orichalcea* (Fabricius) (Noctuidae: Lepidoptera) with observation on other pest complex of soybean and their management. M.Sc. (Agri.) Thesis. University of Agricultural Sciences, Bangalore, India, 1997.
10. Wilcox JR. World distribution and trade of soybean. Soybeans: Improvement, Production and Uses. 2004;16(3):25-32.