World Applied Sciences Journal 34 (1): 71-77, 2016
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2016.34.1.10280

Agricultural Soil Micro-fungal Isolates of *Metarhizium anisopliae* on Food Consumption, Productivity and Enzyme Activity of *Spodoptera litura* Fabricus

[1]S. Muthukrishnan and [2]K.P. Sanjayan

[1]Department of Biotechnology, Ayya Nadar Janaki Ammal College,
Sivakasi- 626124, Tamilnadu, India
[2]Advanced Zoology and Biotechnology, Guru Nanak College,
Chennai 600042, Tamil nadu, India

Abstract: The Entomopathogenic micro-fungal species *Metarhizium anisopliae* was collected from the agricultural soils of Kanchipuram district of Tamil Nadu, India. Micro-fungal spores of *Metarhizium anisopliae* were tested for its effect on the feeding of *Spodoptera litura* at three different concentrations of 10^3, 10^5 and 10^7. The Higher concentration of spore significantly reduce the consumption of leaf. The exposure of fungal spore's concentration decrease the production efficiency. The microfungal infection reduction of *Spodoptera litura* gut enzymes such as amylase and trehalase and reduced weight gain of infected insects and 33.32% mortality recorded in microfungal infected *S.litura*.

Key words: Entomopathogenic micro-fungi · *Metarhizium anisopliae* · *Spodoptera litura* · Mortality

INTRODUCTION

Biopesticides based on bacteria, viruses, entomopathogenic fungi and nematodes are often considerable scope as plant protection agents against several insects. Recent advances in fungal production, stabilization, formulation and application have led the way toward commercialization of a large number of new fungus-based biopesticide products [1, 2].

The biological plant protection with entomopathogenic fungi has key role in sustainable pest management program. Entomopathogens as biocontrol agents have several advantages when compared with conventional insecticides. These include low cost, high efficiency, safety for beneficial organisms, reduction of residues in environment and increased biodiversity in human managed ecosystems [3]. Fungal biocontrol agents have unique mode of infection. In contrast to bacteria and viruses, they do not need to be ingested and can invade their host directly through the cuticle. That is why entomopathogenic fungi are capable of infecting non feeding mesh like eggs [4, 5] and pupae of insects [5, 6]. The use of entomopathogenic fungi, particularly in this strategy, requires a thorough knowledge of the biology and ecology of both pests and their natural enemies. It also requires recognition of factors that may interfere with their effectiveness. Conservation biological control also needs long-term and large-scale researches on multitrophic relationships between natural enemy and their hosts and their impact on natural regulation of serious insect pests [7]. Fungal biological control agents have demonstrated efficacy against a wide range of insect pests including Spodoptera species [9-11]. Fungal pathogen particularly, *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii* and *Nomuraea rileyi* have been found to be promising in the control of several agricultural pests [12]. It is found naturally on some plants and in soils and is regarded as a safe biopesticide [13]. Present study focus was undertaken to evaluate the efficacy of a locally isolated strain of *Metarhizium anisopliae* against one of the most notorious lepidopteran pest namely *Spodoptera litura* in the form of consumption of food, production efficiency, insect gut enzyme and mortality.

MATERIALS AND METHODS

Laboratory Rearing of *Spodoptera litura*: Newly hatched larvae from the field-collected egg masses were reared on the Castor (*Ricinus communis* Linn.) at 28±2°C, 60-70%

Corresponding Author: S. Muthukrishnan Krishnan, Department of Biotechnology, Ayya Nadar Janaki Ammal College, Sivakasi- 626124, Tamilnadu, India. Mob: +9841708316.

relative humidity, with a photoperiod of 14:10 (L:D) in the laboratory. At the prepupal stage, they were transferred into a plastic jar (diameter 7cm, height 10cm), provided with a 2 to 3 inchs thick layer of sterilized moist sand for pupation. Approximately 50 pupae were transferred to wooden cages (25x25x30cm) for adult emergence. The adult moths were provided with a solution mixture containing 20mg sucrose, 1ml multi vitamin mixture (ABDEC), 1 ml honey in 1ml sterile distilled water and reared under the same conditions as the larvae. They were allowed to lay eggs on filter paper which served as oviposition sheets. The oviposition sheets were replaced daily. The egg masses were collected and incubated at room temperature. The young larvae were transferred using a fine Camlin brush into a plastic container (20x30 cm) containing fresh *R. communis* leaves. Further experiments were carried out with individuals from the F1 generation.

Fungal Strain Collection: *Metarhizium anisopliae* was isolated from soil collected from the agricultural fields at Thevariampakkam, Kanchipuram district (12°82' N and 79°89'E), Tamil Nadu, where paddy, groundnut, cotton and vegetables are cultivated in rotation every year. Pure cultures were maintained through the technique of serial dilution in Sabouraud dextrose agar medium.

Preparation of Spore Inoculums: Conidia from cultures that are 2 to 3 weeks old were scraped from the surface of the stock culture plates with a sterile scalpel into sterile distilled water with 0.1% Tween-80 solution. The concentrations of the fungal spores in the culture were adjusted to 10^7, 10^5 and 10^3spores/ml. The spore concentration in the suspension was determined by a haemocytometer slide.

Feeding Bioassay for Pathogenicity Testing: Circular discs (14 cm diameter) were cut from the leaves of *Ricinus communis*. Fungal spores at concentration of 10^3, 10^5, 10^7 per ml was tested by spreading 2 ml of the spore suspension using a fine pipette on the circular discs leaf. After air drying, the disc were placed in a round plastic box and three freshly moulted third instar larvae of *S. litura* were placed at the centre and allowed to feed. After 24 hrs, the insects and remaining leaves were weighed. Subsequently fresh untreated leaves were provided each day till the larvae pupated. Data on the amount of leaf consumed and weight of the insect was recorded daily and indices of consumption and production calculated as per Waldbaur [14].There were four replicates for each spore concentration tested. A Similar experiment was conducted to evaluate the mortality of insects exposed to different concentration of the spores. However, each test had thirty instar larvae exposed to the spores and the larval mortality was recorded at the end of 24, 48 and 72 h after exposure. A suitable control was maintained and the test conducted with four replicates.

Effect of Fungal Infection on the Activity of Gut Enzymes: Third instar larvae of *S. litura* were fed with leaves of *R. communis* smeared with spores of the *Metarhizium anisopliae* at concentrations of 10^3, 10^5 and 10^7 spore/ml. After 72 hous of feeding, the insects were sacrificed and the gut dissected under the dissection microscope (Wild Leitz). The midgets obtained from three individuals were pooled, weighed and homogenised using a tissue homogeniser and centrifuged at 10000 rpm for 20 min at 4°C to avoid denaturing of the enzymes. The supernatant solution was used for the enzyme assay [15].

Assay for Amylase Activity: Amylase activity was determined as described by Ishaaya [16]. The amylase reaction was carried out with 0.4 ml of 0.05 M glycine NaOH buffer at pH 7.0, 0.2 ml of enzyme solution and 2 ml of 2% starch. After 60 min of incubation at 37°C, the reaction was terminated by adding 1.6 ml of 3, 5-dinitrosalicylic acid reagent. The reaction mixture was heated at 100°C for 5 min and was immediately cooled in an ice bath. The colour developed was read spectrophotometrically at 550 nm. The activity of enzyme was expressed as micrograms of glucose per gram of midgut tissue per hour.

Assay for Trehalase Activity: Trehalase activity was determined as described by Ishaaya [16]. The reaction consisted of 0.4 ml of 0.2 M phosphate buffer at pH 7.0, 0.2 ml of 1.5% trehalose and 0.2 ml of enzyme solution. After 60 min of incubation at 37°C, the reaction was terminated by adding 1.6 ml of 3, 5-dinitrosalicylic acid reagent. The reaction mixture was heated at 100°C for 5 min and was immediately cooled in an ice bath. The colour developed was read spectrophotometrically at 550 nm and the enzyme activity expressed as micrograms of glucose per gram of midgut tissue per hour.

RESULTS

Life cycle of *Spodoptera litura:* *Spodoptera litura* is a highly polyphagous multivolatine species Table 1, provides data on the duration of the larval and pupal stages of the insect reared in the laboratory on *Ricinus communis*. The larval stages lasted for 13-14 days with

Table 1: Larval and pupal duration of *S. litura* reared on castor

Larval and Pupal duration	Duration of larval instars (in days)
First instars	2.25±0.22
Second instars	2.10±0.11
Third instars	2.49±0.19
Fourth instars	2.23±0.15
Fifth instars	2.51±0.21
Sixth instars	2.10±0.19
Larval	13.68±0.49
Prepupal	1.49±0.20
Pupal	7.05±0.22

Value represents mean±SD

the third instar having an average span of 2.49 days. The larvae pupated for 7 days before moulting into the adult.

Effect of *Metarhizium Anisopliae* Spores on the Consumption of Food by *Spodoptera Litura*: The effect of different concentration of *Metarhizium anisopliae* spores on the consumption of food by *Spodoptera litura* is provided is provided in (Table 2). There was a significant decrease in the amount of food consumed in the three treatments from that of the control. Among the treatments also, a decrease in consumption was recorded with increase in concentration of the spores. Also after exposure to the fungal spores, the amount of food consumed decreased each day. However, the difference in the mean values among the different levels of "Duration" is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in "Concentrations". There no statistically significant difference (p = 0.080). However, the values of consumption in relation to spore concentration was significantly different (p=0.003). The effect of different levels of "Duration" does not depend on the level of "Concentrations" of spores present. There is no statistically significant interaction between "Duration" and "Concentrations" (P = 0.982).

Effect of Different Concentration of *Metarhizium Anisopliae* Spores on the Production Efficiency in *Spodoptera Litura*: The production efficiency of *S.litura* decreased on exposure to spores of M. *anisopliae* and with the passages of time indicates there was a further decrease in production (Table 3).

Table 2: Effect of different concentration of *Metarhizium anisopliae* spores on the food consumption rate of *Spodoptera litura*

	Concentration of *Metarhizium anisopliae*			
Duration (hours)	10^7	10^5	10^3	Control
24	0.1878±0.0169	0.2113±0.0139	0.2339±0.0145	0.3200±0.0196
48	0.1631±0.0203	0.1868±0.0214	0.2051±0.0202	0.3230±0.0277
72	0.1409±0.0092	0.1634±0.0218	0.1948±0.0305	0.2934±0.0070

Values represent mean±SD in grams/day

Source of Variation	DF	SS	MS	F	P
Duration	2	0.00515	0.00258	2.784	0.08
Concentrations	2	0.0135	0.00673	7.272	0.003
Duration x Concentrations	4	0.00022	0.000054	0.0583	0.993
Residual	27	0.025	0.000925		
Total	35	0.0438	0.00125		

Comparisons for factor: Duration

Comparison	Diff of Means	p	Q	P<0.05
48h vs. 24h	0.029	3	3.303	No
48h vs. 72h	0.0109	3	1.243	No
72h vs. 24h	0.0181	3	2.06	No

Comparisons for factor: Concentrations

Comparison	Diff of Means	P	Q	P<0.05
10^7 vs. 10^3	0.0474	3	5.393	Yes
10^7 vs. 10^5	0.0233	3	2.655	No
10^5 vs. 10^3	0.024	3	2.738	No

Table 3: Effect of different concentration of *Metarhizium anisopliae* spores on the production efficiency in *Spodoptera litura*

	Concentration of *Metarhizium anisopliae*			
Duration (hours)	10^7	10^5	10^3	Control
24	0.1544±0.0107	0.1810±0.0180	0.2089±0.0151	0.2981±0.0055
48	0.1338±0.0130	0.1537±0.0167	0.1792±0.0195	0.2915±0.0147
72	0.1103±0.0078	0.1286±0.0102	0.1575±0.0179	0.2728±0.0137

Values represent mean±SD of weight gain /day in grams

Source of Variation	DF	SS	MS	F	P
Duration	2	0.00388	0.00194	6.247	0.006
Concentrations	2	0.0145	0.00725	23.349	<0.001
Duration x Concentrations	4	0.00012	0.0000308	0.0992	0.982
Residual	27	0.00838	0.000311		
Total	35	0.0269	0.000768		

Comparisons for factor: Duration

Comparison	Diff of Means	P	Q	P<0.05
72h vs. 24h	0.024	3	4.716	Yes
72h vs. 48h	0.0047	3	0.924	No
48h vs. 24h	0.0193	3	3.793	Yes

Comparisons for factor: Concentrations

Comparison	Diff of Means	P	Q	P<0.05
10^7 vs. 10^3	0.049	3	9.641	Yes
10^7 vs. 10^5	0.0216	3	4.241	Yes
10^5 vs. 10^3	0.0275	3	5.4	Yes

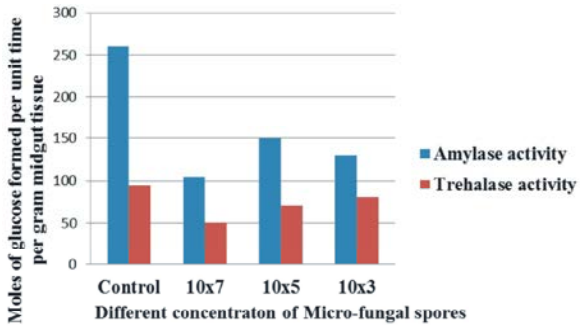

Fig. 1: Actvity of amylase and trehalase in the gut of *Spodoptera litura* exposed to different concentration of *Metarhizium anisopliae* Miro-fungal spores

The difference in the mean values of production efficiency among the different levels of "Duration" is greater than would be expected by chance after allowing for effects of differences in "Concentrations". There is a statistically significant difference (p = 0.006).

The difference in the mean values of production among the different levels of "Concentrations" is greater than would be expected by chance after allowing for effects of differences in "Duration". There is a statistically significant difference (p = <0.001).

The effect of different levels of "Duration" does not depend on the level of "Concentration" of spore present. There is no statistically significant interaction between "Duration" and "Concentrations". (P = 0.982).

Effect of Fungal Infection on Gut Enzyme Activity: The activity of two digestive enzymes namely Amylase and Trehalase was studied in relation to infected fungal species. Insect depend on these enzyme for their immediate energy requirement. There was a significant reduction in the activities of both these enzymes in comparison to the control. The general trend was a reduction in activity with increase in spore concentration (Fig-1).

Mortality of *Spodoptera Litura*: A high concentration of 10^7 spores of *Metarhizium anisopliae* was required for larval and pupal mortality of *Spodoptera litura*. Data on the cumulative mortality indicate that total mortality of *Spodoptera litura* 33.32% recorded at 10^7 spores concentration and followed by 24.99% in 10^5 concentration (Fig-2).

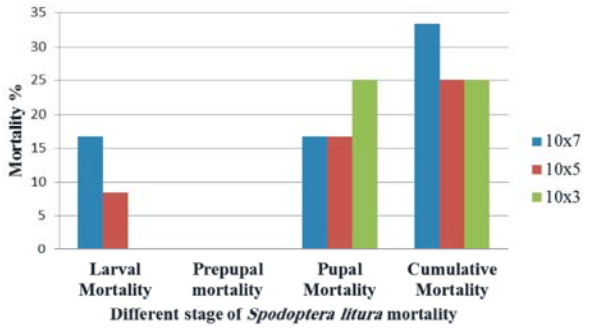

Fig. 2: Effect of different concentration of *Metarhizium anisoplia* Micro-fungal spores on the mortality of *Spodoptera litura*

DISCUSSION

Insect Pest Management programmes have re-enforced the need to search for Biocontrol agent that could facilitate in the control of the pest. Entomopathogenic fungi are important natural regulators of insect populations and have potential as mycoinsecticide agents against diverse insect pests in agriculture. These fungi infect their hosts by penetrating through the cuticle, gaining access to the hemolymph, producing toxins and grow by utilizing nutrients present in the haemocoel to avoid insect immune responses [17]. In India, the polyphagous caterpillar, *Spodoptera litura*, earlier known to be a sporadic pest, has attained major pest status on several crops in the recent years. This is primarily because *S. litura* has developed resistance to most of the insecticides presently being used in agriculture.

The use of fungal biological control agents is a rapidly developing field and is increasingly adopted and accepted worldwide management of agricultural pests [18, 19]. Hence, in the present study, entomopathogenic fungi were isolated from the agricultural soils of Kanchipuram district of Tamil Nadu, India and were evaluated for their spore germination, in vitro assays against *Spodoptera litura* to know the potentiality of entomopathogenic fungi. Entomopathogenic fungi, *M. anisopliae*, *I. fumosorosea*, *B bassiana* and *Lecanicillium* sp. are important natura mortality factors of many noctuid pests worldwide. Their efficacy against *S. litura* has been described by various researchers [5, 9, 10]. Different isolates of same species do not have equal potential for control of the same insect pest [20]. Entomopathogenic fungi have unique ability to infect eggs of insects [17]. Fungal strains show varying degrees of infective potential towards unscaled and scaled eggs of S. litura unscaled eggs being more susceptible to fungal infection [5]. As the fungal infection progresses on the insect host, there is reduced growth and therefore data on the production efficiency appears as an useful additional parameter for evaluating pathogenecity. Production efficiency of S. *litura* decreased on exposure to increased dosage of fungal spores [21]. In the present study, Pathogenicity of the fungus on the insect expressed in terms of reduced food intake and therefore the rate of food consumption is an ideal parameter for assessing the initiation of pathogen city. insects infected by the fungus showed reduced activities of enzymes such as amylase and trehalase and this could account for the reduced weight gain of infected insects. Infected insects also showed lesions in the alimentary canal which accounted for the reduced enzyme activity. St. Leger [22] reported that the insect pathogen *M. anisopliae* in infected insects resulted in a 25% increase in virulence and a concomitant decrease in host food consumption. Ekesi [23] reported leaf consumption by beetles treated with *B. bassiana* significantly reduced within 2 days after treatment in cowpea leaf beetle. As the fungal infection progresses on the insect host, there is reduced growth and therefore data on the production efficiency appears as an useful additional parameter for evaluating pathogenecity. Production efficiency of S. *litura* decreased on exposure to increased dosage of fungal spores. In the event of the insect succumbing to the infection, percentage mortality reveals the usefulness of the fungus as a biocontrol agent.

CONCLUTION

The virulence of fungal entomopathogens involves four steps: adhesion, germination, differentiation and penetration. Each step is influenced by a range of integrated intrinsic and external factors which ultimately determine the pathogenicity. A successful infection is achieved by the attachment or adhesion of spores to the host. There are different groups of entomopathogenic fungi in different habitats. Different insect pathogenic mycofloras could be found in the soil and different in the over ground environment. Fungal pathogenicity and mortality of insect differ for site specific variation. Pathogenicity of fungal on host insect express in terms reduce food intake and reduce production efficiency

followed by reduce intestinal enzyme amylase and trehalase final cause mortality of host. Biopesticides is the better option for the management of *Spodoptera litura* and ecofriendly not harmful to human being.

REFERENCES

1. Faria, M.R. and S.P. Wraight, 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biol. Control, 43: 237-256.
2. Wraight, S.P., M.A. Jackson and S.L. De Kock, 2001. Production, stabilization and formulation of fungal biocontrol agents. In: T.M. Butt, C.W. Jackson & N. Magan (eds.), CAB International, Wallingford, UK, pp: 253-28.
3. Lacey, L.A., R. Frutos, H.K. Kaya and P. Vails, 2001. Insect pathogens as biological control agents: Do they have future? Biol. Contr., 21(3): 230- 248.
4. Ujian, A.A. and S. Shahzad, 2007. Pathogenicity of Metarhizium anisopliaeVar. Acridum strains on Pink Hibiscus Mealy bug (Maconellicoccus hirsutus) affecting cotton crop. Pakistan J. Bot., 39(3): 967-973.
5. Anand, R. and B.N. Tiwary, 2009. Pathogenicity of entomopathogenic fungi to eggs and larvae of Spodoptera litura, the common cutworm. Biocontrol Sci. Technol., 19(9): 919-929.
6. Nguyen, N., C. Borgemeister, H. Poehling and G. Zimmermann, 2007. Laboratory investigations on the potential of entomopathogenic fungi for biocontrol of Helicoverpa armigera (Lepidoptera: Noctuidae) larvae and pupae. Biocontrol Sci. Technol., 17(12): 853-864.
7. Anand, R., B. Prasad and B.N. Tiwary, 2008. Relative susceptibility of Spodoptera litura pupae to selected entomopathogenic fungi. Biocontrol, 54(1): 85-92.
8. Tscharntke, T., R. Bommarco, Y. Clough, T.O. Crist, D. Kleijn, T.A. Rand, J.M. Tylianakis, S. van Nouhuys and S. Vidal, 2008. Conservation biological control and enemy diversity on a landscape scale. *Biological Control*, Vol. 45, No. 2, (May 2008), pp: 238–253, ISSN 1049-9644.
9. Purwar, J.P. and G.C. Sachan, 2005. Biotoxicity of Beauveria bassiana and Metarhizium anisopliae against Spodoptera litura and Spilarctia oblique. Ann. Pl. Protec. Sci., 13(2): 360-364.
10. Lin, H.P., X.J. Yang, Y.B. Gao and S.G. Li, 2007. Pathogenicity of several fungal species on Spodoptera litura. Chinse J. Appl. Ecol., 18(4): 937-940.
11. Amer, M.M., T.I. El-Sayed, H.K. Bakheit, S.A. Moustafa and Y.A. El-Sayed, 2008. Pathogenicity and genetic variability of five entomopathogenic fungi against Spodoptera littoralis. Res. J. Agric. Sci., 4(5): 354-367.
12. Lingappa, S., H. Saxena and P.S. Devi Vimala, 2005. Role of biocontrol agents in management of Helicoverpa armigera (Hubner). In: Recent advances in Helicoverpa armigera Management (Hem Saxena, A. B. Rai, R. Ahmad and Sanjeev Gupta eds.). Indian Society of Pulses Research and development, IIPR, Kanpur, pp: 159-184.
13. Uma devi, K., A. Reineke, N. Nageswara rao reddy, C. Uma maheswara rao and J. Padmavathi, 2006. Genetic diversity, reproductive biology and speciation in the entomopathogenic fungus Beauveria bassiana (Balsamo) Vuillemin. Genome, 49: 495-504.
14. Waldbauer, G.P., 1968. “The Consumption and Utilization of Food by Insects”, Advances in Insect Physiology, 5: 229-288.
15. Neubauer, I., N. Ishaaya, N. Aharonson and B. Raccah, 1980. “Acitivity of Soluble and Membranebound Trehalase in Apterous and Alate Morphs of Aphis citriola”, Comp. Biochemistry and Physiolology, 66: 505-510.
16. Ishaaya, I., 1986. “Nutritional and Allelochemic Insect-plant Interaction”, In Insect-plant Interaction, Miller, J.R. and Miller, T.A. (Eds.), Springer-verlag, New York, pp: 191-224.
17. Hajek, A.E. and R.J. St. Leger, 1994. Interactions between fungal pathogens and insect hosts. Ann. Rev. Entomol., 39: 293-322.
18. Jaronski, S.T., 2010. Ecological factors in the inundative use of fungal entomopathogens. Biocontrol, 55: 159-185.
19. Hajek A.E. and I. Delalibera Jr, 2010. Fungal pathogens as classical biological control agents against arthropods. Biocontrol, 55: 147-158.
20. Altre, J.A., J.D. Vandenberg, and F.A. Cantone, 1999. Pathogenicity of *Paecilomyces fumosoroseus* isolates to Deamondback moth, *Plutella xylostella*: correlation w ith spore size, germination speed and attachment to cuticle. J. Invertebr. Pathol., 73: 332-338.

21. Tefera, T. and K.L. Pringle, 2003. "Food Consumption by *Chilo partellus* (Lepidoptera: Pyralidae) Larvae Infected with *Beauveria bassiana* and *Metarhizium anisopliae* and Effects of Feeding Natural Versus Artificial Diets on Mortality and Mycosis", Journal of Invertebrate Pathology, 84(3): 220-225.
22. St. Leger, R.J., L. Joshi, M.J. Bidochka and D.W. Roberts, 1996. Construction of an improved mycoinsecticide overexpressing a toxic protease. Proc. Natl. Acad. Sci. USA, 93: 6349-6354.
23. Ekesi, S., 2001. Pathogenicity and Antifeedant activity of entomopathogenic hyphomycetes to the cowpea leaf beetle, Ootheca mutabilis shalberg. Insect Sci. Applic., 21(1): 55-60.