International Journal of Microbiological Research 6 (1): 54-60, 2015
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2015.6.1.9311

Effect of Native Soil Borne Isolate of *Paecilomyces fumosoroseus* on Food Consumption, Productivity and Enzyme Activity of *Spodoptera litura* Fabricus

[1]*S. Muthukrishnan,* [2]*K.P. Sanjayan and* [3]*Jahir Hussain*

[1]Department of Biotechnology, Ayyya Nadar Janaki Ammal College, Sivakasi- 626124, Tamilnadu, India
[2]Advanced Zoology and Biotechnology, Guru Nanak College, Chennai 600042, Tamilnadu, India
[3]Department of Zoology, C. Abdul Hakeem College, Melvisharam 632509 Tamilnadu, India

Abstract: The fungal isolate of *Paecilomyces fumosoroseus* obtained from the agricultural soils of Kanchipuram district of Tamil Nadu, India. Fungal spores were tested for its effect on the feeding of *Spodoptera litura* at concentration of 10^3, 10^5 and 10^7. Exposure of higher concentration of spore significantly decreases the consumption of leaf. The production efficiency of *S. litura* decreased gradually in relation to exposure to the fungal spores concentrations. Insects infected by the fungus showed reduction in the activity of enzymes such as amylase and trehalase and this could account for the reduced weight gain of infected insects and 58.32 % mortality recorded in infected *S. Litura*.

Key words: *Paecilomyces fumosoroseus* · *Spodoptera litura* · Pathogenecity · Gut Enzymes

INTRODUCTION

Entomopathogenic fungi have an important position in the biological control of insect pests, due to the fact that they are responsible of causing diseases and the death of the insects [1]. Entomopathogenic fungi infect the insect through the cuticle. On account of this, there is no need to be ingested as they act as contact insecticides [2]. The development of the infection is divided into three stages. I) Conidia adhesion and germination in the epicuticle of the insect [3]; germination is activated by carbohydrates present in the cuticle. Entomopathogenic fungi develop an aspersorium with the purpose of beginning the penetration stage through the germ tube formation [4]. II) The cuticle penetration is the result of combined action of mechanical force and the enzymatic action of those enzymes secreted by the fungus. Such enzymes include proteases, chitinases, lipases and lipoxygenases which break down the cuticle, offering nutrients to the fungus [1, 5, 6]. III) Insect's propagation and death; when the fungus reaches the hemocoel, it presents a cellular hyphae differentiation to yeast-like blastospores to evade the insect immune system [7]. The importance of the production of *Paecilomyces fumosoroseus* blastospores resides in their toxic effectiveness as a control agent in combating pests that affect various agricultural crops [8]. Studies on the occurrence of entomopathogenic fungi populations in soils from fields differing in the level of farming intensity can help to monitor the functioning of the agri-environmental packages. Knowledge of the species composition of entomopathogenic fungi in different regions of the country can be also useful in assessing the potential of individual fungal species to regulate the populations of crop pests [9]. *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) is the most notorious chewing insect pest that causes heavy losses in cotton, thus, deprives the farmers from getting high yield. It is difficult to control because of its cryptic habitat and high rate of infestation post- heavy rains [10]. Plant protection measures with frequent applications of synthetic chemical insecticides to protect cotton crop from this pest are typical of intensive cultivation. Insecticides of synthetic origin have been used to manage insect pests for more than 50 years [11]. However, due to adverse effects of insecticides on environment, their rational use is being advocated. *Paecilomyces fumosoroseus* microfungal species insect-borne filamentous fungus belongs to

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

Hyphomycetes of Deuteromycota and have been reported to cause diseases in a wide species of insects [12-15]. The present study was undertaken to evaluate the efficacy of a locally isolated strain of *Paecilomyces fumosoroseus* from the agricultural field at Kanchipuram district of Tamil Nadu against one of the most notorious lepidopteran pest namely *Spodoptera litura*.

MATERIALS AND METHODS

Fungal Strain: *Paecilomyces fumosoroseus* 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 [16]. 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, 4 8 and 72 h after exposure. A suitable control was maintained and the test conducted with four replicates Fig. 1.

Fig. 1: Experimental setup for Entomopathogenic fungal studies

A -Experimental set up for Entomopathogenic study against *Spodoptera litura*

B -Laboratory rearing cage for *S.litura*

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 *B. bassiana* at concentrations of 10^3, 10^5 and 10^7 spore/ml. After 72 h 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 [17].

Assay for Amylase Activity: Amylase activity was determined as described by Ishaaya [18]. 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 [18]. 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

The effect of different concentrations of fungal spores on consumption of *R. communis* by *S. litura* is summarised in Table 1. There was a significant decrease in the consumption in all the experimental treatments compared to the control. The maximum reduction was recorded in the treatment with spore concentration of 10^7/ml. Also, within each spore concentration tested, the consumption of leaves decreased with time after exposure. However, the quantum of leaves consumed up to 72 h was not statistically different from that at 24 h. Compare to concentration of spore 10^7 vs 10^3 and 10^5 vs 10^3 significantly differs the consumption rate. Production efficiency calculated in terms of weight gain was also impaired due to exposure to the spores of *P. fumosoroseus*. Consequent to decreased food consumption, the production efficiency also decreased when exposed to different concentrations of the spores (Table 2). The production efficiency no significantly different between the 24, 48 and 72 h regimes. However concentration of spore such as 10^7 vs. 10^5, 10^7 vs. 10^3 and 10^5 vs. 10^3 significantly differ the production. The activity of two digestive enzymes, namely, amylase and trehalase, was studied in relation to infection by the fungal species. There was a significant reduction in the activities of both these enzyme in comparison to the control. The general trend was a reduction in enzyme activity with increase in spore concentration (Fig. 2). Exposure to *P. fumosoroseus* spores also brought about mortality during the larval, prepupal and pupal stages (Table 3). A cumulative mortality of 58% was obtained with 10^7 concentrations of spores.

Table 1: Effect of different concentration of *P. fumosoroseus* spores on the food consumption of *S. litura*

	Concentration of *P. fumosoroscus*			
Duration (hours)	10^7	10^5	10^3	Control
24	0.1787 ± 0.0107	0.1973 ± 0.0071	0.2229 ± 0.0091	0.3200 ± 0.0196
48	0.1639 ± 0.0085	0.1856 ± 0.0050	0.2070 ± 0.0059	0.3230 ± 0.0277
72	0.1613 ± 0.0128	0.1663 ± 0.0084	0.1943 ± 0.0212	0.2934 ± 0.0070

Values represent mean ±SD in grams per day

Source of Variation	DF	SS	MS	F	P
Duration	2	0.00249	0.00124	2.287	0.121
Concentrations	2	0.00983	0.00492	9.047	<0.001
Duration x Concentrations	4	0.00034	0.0000841	0.155	0.959
Residual	27	0.0147	0.000544		
Total	35	0.0273	0.000781		

Comparisons for factor: Duration

Comparison	Diff of Means	p	Q	P<0.05
48h vs. 72h	0.0181	3	2.686	No
48h vs. 24h	0.0171	3	2.547	No
24h vs. 72h	0.000933	3	0.139	No

Comparisons for factor: Concentrations

Comparison	Diff of Means	p	Q	P<0.05
10^7 vs. 10^3	0.0401	3	5.955	Yes
10^7 vs. 10^5	0.0151	3	2.239	No
10^5 vs. 10^3	0.025	3	3.716	Yes

p: Degree of freedom, q: Studentised range statistic (Tukey), *P*: Probability.

Table 2: Effect of different concentration of *P. fumosoroseus* spores on the production efficiency in *S. litura*

	Concentration of *P. fumosoroscus*			
Duration (hours)	10^7	10^5	10^3	Control
24	0.1504 ± 0.0187	0.1839 ± 0.0052	0.1991 ± 0.0094	0.2981 ± 0.0055
48	0.1380 ± 0.0079	0.1642 ± 0.0092	0.1842 ± 0.0079	0.2915 ± 0.0147
72	0.1176 ± 0.0120	0.1429 ± 0.0071	0.1766 ± 0.0142	0.2728 ± 0.0137

Values represent mean ±SD of weight gained per day in grams

Source of Variation	DF	SS	MS	F	P
Duration	2	0.00053	0.000267	1.193	0.319
Concentrations	2	0.0158	0.00792	35.373	<0.001
Duration x Concentrations	4	0.00042	0.000105	0.47	0.757
Residual	27	0.00604	0.000224		
Total	35	0.0228	0.000652		

Comparisons for factor: Duration

Comparison	Diff of Means	p	Q	P<0.05
48h vs. 24h	0.00908	3	2.103	No
48h vs. 72h	0.00233	3	0.54	No
72h vs. 24h	0.00675	3	1.563	No

Comparisons for factor: Concentrations

Comparison	Diff of Means	p	Q	P<0.05
10^7 vs. 10^3	0.0513	3	11.873	Yes
10^7 vs. 10^5	0.0283	3	6.557	Yes
10^5 vs. 10^3	0.023	3	5.316	Yes

p: Degree of freedom, q: Studentised range statistic (Tukey), *P*: Probability.

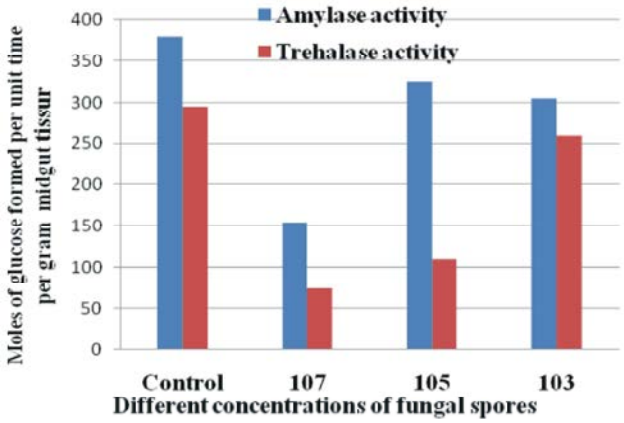

Fig. 2: Activity of amylase and trehalase in the gut of *S. litura* exposed to different concentration of *P. fumosoroseus*

Table 3: Effect of different concentration of fungal spores on the mortality of *S. litura*

	P. fumosoroseus Spore Concentration		
Mortality of *S.litura*	10^7	10^5	10^3
Larval Mortality	33.33	25.00	25
Prepupal	8.33	8.33	0
Pupal Mortality	16.66	16.66	8.33
Cumulative Mortality	58.32	49.99	33.33

DISCUSSION

The infected fungal strain on host insect reduced food intake and therefore the quantity of food consumption is an ideal parameter for assessing the initiation of pathogenicity. Similar results [19] were reported with *B. bassiana* spores, showing a strong negative correlation between inoculums concentrations and food consumption in the tiger moth, *Atteva sciodoxa*. Also, Ekesi [20] reported that leaf consumption by beetles

treated with *B. bassiana* was significantly reduced within 2 days after treatment. Similarly, high concentrations of 10^8 and 10^7 conidia/ml, resulted in greater reduction in food consumption as compared to the lower concentrations (10^6 and 10^5 conidia spores/ml solution) in *Chilo partellus* larvae treated with *B. bassiana* and *Metarhizium anisopliae* [21]. As the fungal infection progresses on the insect host, there is reduced growth and therefore data on the production efficiency appears as a useful additional parameter for evaluating pathogenicity. Since much of the energy obtained from food consumption is diverted towards combating infection, weight gain in infected insects is reduced and consequently the production efficiency dwindles. Production efficiency of *S. litura* decreased on exposure to increased dosage of fungal spores. Moreover, Tefera and Pringle [21] reported similar results with *Chilo partellus* larvae infected with *B. bassiana* and *M. anisopliae*. Another work of Hajek and Anne [22] who infected *Lymantria dispar* larvae with the Entomopathogenic fungus, *Entomophaga maiming* and showe that the weight gain of infected larvae was less than weight gain of healthy larvae. Furthermore [23] observed the highest morality of 2nd instar larvae of *S. litura* at the highest conidial concentration of fungal isolates. In present study also maximum mortality was recorded in higher (10^7) concentration of spores.

Fungal infected Insects showed reduced activities of enzymes such as amylase and trehalase and this could account for the reduced weight gain of infected insects. Abundant amylase activities have been reported during larval development in many insect species in the present study, implying that this enzyme has some physiological role during growth [24]. Trehalases are devoted to utilising intracellular and extracellular trehalose to supply energy and material for the whole body and play a pivotal role in various physiological processes [25]. Infected insects also showed lesions in the alimentary canal, which accounted for the reduced enzyme activity. In the event of the insect succumbing to the infection, percentage mortality reveals the usefulness of the fungus as a biocontrol agent. Sahayaraj and Thomson [26] showed that the amylase activity in *Dysdercus cingulatus* Fabricius was reduced significantly when exposed to infection by fungal spores. 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.

Cumulative mortality in *S. litura* (50%) by 2.4 x 10^6 concentration of *Metarhizum anisopliae* was reported [27]. Meanwhile [28] reported reduced rate of pupation (43.3%) was observed in larvae treated with a high spore concentration of 2.4×10^7 spores/ml of *B. bassiana*. A sequential follow up from this assay was done on the resulting pupae and adults. Further treatment of the resultant pupae caused mortality and adult malformation. Similarly, in the present study also a high concentration of 10^7 spores/ml caused maximum cumulative mortality (58.32%). Also, Kaur *et al*. [29] reported highest percentage of *S. litura* larval mortality at 2.03×10^8 spore concentration

CONCLUSION

Pest management involving biocontrol agents is assuming prominence and have been considered as an important strategy in insect population reduction. 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. Mustafa, U. and G. Kaur, 2009. Extracellular Enzyme Production in Metarhizium anisopliae Isolates. Folia Microbiologica, 54: 499-504. http://dx.doi.org/10.1007/s12223-009-0071-0
2. Charnley, A.K. and S.A. Collins, 2007. Entomopathogenic Fungi and Their Role in Pest Control. In: Kubicek, C.P. and Druzhinina, I.S., Eds., The Mycota IV: Environmental and Microbial Relationships, 2nd Edition, Springer-Verlag, Berlin, pp: 159-187.
3. Wösten, H.A.B., 2001. Hydrophobins: Multipurpose Proteins. Annual Review of Microbiology, 55, 625-646. http://dx.doi.org/10.1146/annurev.micro.55.1.625.
4. Pedrini, N., R. Crespo and M.P. Juárez, 2007. Biochemistry of Insect Epicuticle Degradation by Entomopathogenic Fungi. Comparative Biochemistry and Physiology-Part C: Toxicology & Pharmacology, 146, 124-137. http://dx.doi.org/10.1016/j.cbpc.2006.08.003.

5. Lubeck, I., W. Arrud, B.K. Souza, F. Stanisçuaski, C. R. Carlini and A. Schran, 2008. Evaluation of Metarhizium anisopliae Strains as Potential Biocontrol Agents of the Tick Rhipicephalus (Boophilus) microplus and the Cotton Stainer Dysdercus peruvianus. Fungal Ecology, 1, 78-88. http://dx.doi.org/10.1016/j.funeco.2008.09.002.
6. Boldo, J.T., A. Junges, K.B. Amaral, C.C. Staats, M.H. Vainstein and A. Schrank, 2009. Endochitinase CHI2 of the Biocontrol Fungus Metarhizium anisopliae Affects Its Virulence toward the Cotton Stainer Bug Dysdercus Peruvians. Current Genetics, 55: 551-560. http://dx.doi.org/10.1007/s00294-009-0267-5.
7. Wang, C.S. and R.J. St. Leger, 2006. A Collagenous Protective Coat Enables Metarhizium anisopliae to Evade Insect Immune Responses. Proceedings of the National Academy Sciences USA, 103, 6647-6652. http://dx.doi.org/10.1073/pnas.060195110.
8. Jackson, M.A., A.R. Payne and D.A. Odelson, 2004. Liquid-culture production of blastospores of the bioinsecticidal fungus Paecilomyces fumosoroseus using portable fermentation equipment. J. Ind. Microbiol. Biotechnol, 31: 149-154.
9. Meyling, N.V. and J. Eilenberg, 2006. Occurrence and distribution of soil borne entomopathogenic fungi within a single organic agroecosystem. Agric. Ecosyst. Environ, 113: 336-341.
10. Chen, Y.I., 1979. Some notes on the lepidopteran rice pests at Java, Timur, Indonesia, National Taiwan University. Phytopathol. Entomol., 6: 38-43.
11. Charnley, A.K. and S.A. Collins, 2007. Entomopathogenic fungi and their role in pest control. In: Kubicek, C.P. and I.S. Druzhinina (eds.), Environment and microbial relationships. 2nd Edition, The Mycota iv. Springer-Verlag Berlin Heidelberg, pp: 159-185.
12. Altre, J A., J.D. Vandenberg and F.A. Cantone, 1999. Pathogenicity of Paecilomyces fumosoroseus isolates to Deamondback moth, Plutella xylostella: correlation with spore size, germination speed and attachment to cuticle. J. Invertebr. Pathol, 73: 332-338.
13. Cantone, F.A. and J.D. Vandenberg, 1999. Use of the green fluorescent protein for investigations of Paecilomyces fumosoroseus in insect hosts. J. Invertebr. Pathol., 74: 193-197.
14. Lacey, L.A., A.A. Kirk, L. Millar, G. Mercadier and C. Vidal, 1999. Ovicidal and larvicidal activity of conidia and blastospores of Paecilomyces fumosoroseus (Deuteromycotina: Hyphomycetes) against Bemisia argentifolii (Homoptera: Aleyrodidae) with a description of a bioassay system allowing prolonged survival of control insects. Biocontrol Science and Technology, 9: 9-18.
15. Nam, S.H., S.S. Yoon, K.Y. Kim, S.Y. Cho and M.S. Han, 2000, Report on the muscardine (Paecilomyces fumosoroseus) of silkworm (*Bombyx mori*) in Korea. Korean J. Seric., Sci. 42(1): 28-30.
16. Waldbauer, G.P., 1968, "The Consumption and Utilization of Food by Insects", Advances in Insect Physiology, 5: 229-288.
17. 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.
18. 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.
19. Abood, F., G.A. Bajwa, Y.B. Ibrahim and A.S. Sajap, 2010, "Pathogenicity of Beauveria bassiana Against the Tiger Moth, Atteva sciodoxa (Lepidoptera: Yponomeutidae)", Journal of Entomology, 7(1): 19-32.
20. Ekesi, S., 2001, "Pathogenicity and Antifeedant Activity of Entomopathogenic Hyphomycetes to th Cowpea Leaf Beetle, Ootheca mutabilis", Insect Science and Application, 21(1): 55-60.
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. Hajek, A.E. and Anne, 1989. "Food Consumption by Lymantria dispar (Lepidoptera: Lymantriidae) Larvae Infected with Entomophaga maimaiga (Zygomycetes: Entomophthorales)", Environmental Entomology, 18(4): 723-727.
23. 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.
24. Abraham, E.G., J. Nagaraju and R.K. Datta, 1992. "Biochemical Studies of Amylases in Silkworm, Bombyx mori L: Comparative Analysis in Diapausing and Nondiapausing Strains", Insect Biochemistry Molecular Biology, 22(8): 867-873.

25. Chen, J., B. Tang, H. Chen, Q. Yao, X. Huang, C. Jing, D. Zhang and W. Zhang, 2010. "Different Functions of the Insect Soluble and Membrane-Bound Trehalase Genes in Chitin Biosynthesis Revealed by RNA Interference", PLoS ONE, 5(4): e10133.
26. Sahayaraj, K. and M. Thomson, 2010, "Impact of Two Pathogenic Fungal Crude Metabolites on Mortality, Biology and Enzymes of Dysdercus Cingulatus (Fab.) (Hemiptera: Pyrrhocoridae", Journal of Biopesticides, 3: 163-167.
27. Sachin, K., R.L. Parate, S.S. Mane and V.V. Deshmukh, 2012. Effect of Metarrhizium Anisopliae on Spodeptera Litura and Compatibility with chemicals. International Journal of Science, Environment and Technology, 1(5): 499-505.
28. Malarvannan, S., P.D. Murali, S.P. Shanthakumar, V.R. Prabavathy and S. Nair, 2010. "Laboratory Evaluation of the Entomopathogenic Fungi, Beauveria bassiana Against the Tobacco Caterpillar, Spodoptera litura Fabricius (Noctuidae: Lepidoptera)", Journal of Biopesticides, 3: 126-131.
29. Kaur, S., H.P. Kaur, K. Kaur and K. Kaur, 2011. "Effect of Different Concentrations of Beauveria bassiana on Development and Reproductive Potential of Spodoptera litura (Fabricius)", Journal of Biopesticides, 4(2): 161-168.