Field Evaluation of Three Entomopathogenic Fungi on Groundnut Pests

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Keywords: Biological control- Groundnut pests- *Beauveria bassiana- Paecilomyces fumosoroseus- Verticillium lecanii*- Persistence- India

Summary

Entomopathogenic fungi Beauveria bassiana *(Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes),* Paecilomyces fumosoroseus *(Wize) (Deuteromycotina: Hyphomycetes),* Verticillium lecani Viegas *(Deuteromycotina: Hyphomycetes) were tested against groundnut pests,* Aphis craccivora *(Koch) (Homoptera: Aphididae),* Aproarema modicella *(Deventer) (Lepidoptera: Gelechiidae),* Mylabris pustulata *Faust (Coleoptera: Meloidae) and* Spodoptera litura *(Fabricius) (Lepidoptera: Noctuidae) in field conditions. Among the tested fungi,* V. lecanii *suppressed 62% of* A. craccivora *population at 39 Days After Seedling Emergence (DASE). During the same period,* B. bassiana *reduced 72% of* S. litura *larval population (0.73 larvae). The infestation of* S. litura *and* A. modicella *were greatly reduced after the treatment of* B. bassiana*; subsequently the yield (1721.31 kg/ha^{-1}) and cost benefit ratio (1: 1.93) were increased.* P. fumosoroseus *and* V. lecanii *were less effective than* B. bassiana*. The persistence of fungal pathogens was found to be higher in soil than the phyllosphere indicating that they can be naturally favored for the control of pests in groundnut.*

Résumé

Champignons entomopathogènes dans la gestion des ravageurs de l'arachide

L'utilisation éventuelle de Beauveria bassiana *(Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes),* Paecilomyces fumosoroseus *(Wize) (Deuteromycotina: Hyphomycetes),* Verticillium lecani Viegas *(Deuteromycotina: Hyphomycetes) ont été testés contre les ravageurs de l'arachide,* Aphis craccivora *(Koch) (Homoptera: Aphididae),* Aproarema modicella *(Deventer) (Lepidoptera: Gelechiidae),* Mylabris pustulata *Faust (Coleoptera: Meloidae) et* Spodoptera litura *(Fabricius) (Lepidoptera: Noctuidae) dans les conditions de terrain. Parmi les champignons testés,* V. lecanii *a supprimé 62% de la population de* A. craccivora *à 39 jours après la levée. L'infestation de* S. litura *et* A. modicella *ont été considérablement réduit après le traitement de* B. bassiana*, ce qui a entraîné une augmentation du rendement et le rapport coûts-avantages.* P. fumosoroseus *et* V. lecanii *se sont montrés moins efficaces que* B. bassiana*. La persistance des agents pathogènes fongiques a été trouvé plus élevée dans le sol que dans la phyllosphère ce qui indique qu'ils peuvent être naturellement favorisés pour le contrôle des ravageurs de l'arachide.*

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 (5, 30). Many workers extensively investigated the field bioefficacy of the entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) (24, 31), *Paecilomyces fumosoroseus* (Wize) (Deuteromycotina: Hyphomycetes) (6, 26) and *Verticillium lecanii* Viegas (Deuteromycotina: Hyphomycetes) (7, 12, 13). Information obtained from such studies is the fundamental for testing the success of a biological control agent; provide information on the persistence and epidemiological development. Since simple, viable and cheapest mass production technology of these fungi (22) is available in the literature it is worthwhile to test their bioefficacy and possible utilization of these fungi in groundnut IPM is imperative. Furthermore, monitoring of introduced control agent is essential for understanding the interactions with their living environment and needs to be the part of ecological impact and safety assessment. Persistence of the entomopathogenic fungi on field was investigated (2, 21) in different fields. However, the information about the persistence of these entomopathogenic fungi on groundnut pest is not available in the literature. We hypothesized that application of the neem oil-based *B. bassiana*, *P. fumosoroseus* and *V. lecanii* control the chosen insect pests, increase the groundnut production and the persistence of spores in the field.

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Received on 28.01.11 and accepted for publication on 07.06.11.

The present study was undertaken to evaluate the field bioefficacy of *B. bassiana, P. fumosoroseus* and *V. lecanii* on the groundnut pests and the persistence of these fungi.

Materials and methods

1. Plot description

Field experiment was conducted to evaluate the bio-efficacy of three entomopathogenic fungi on groundnut (TMV 7 variety) in 43,600 square feet area at Vallam (76^0 24' E 7^0 96' N) Tirunelveli District, Tamil Nadu, India. Groundnut was cultivated under recommended irrigation method. All the other recommended management practices of groundnut were applied except the insecticide application. The trial was carried out in a randomized block design with five treatments including untreated control and standard with three replications for each treatment. The total area was divided into 5 plots having 8, 720 square feet each [A - *B. bassiana*, B - *P. fumosoroseus*, C - *V. lecanii*, D - Endosulfan (standard) and E - Untreated Control]. Each plot was again divided into three sub-plots. Each sub-plot was separated from the adjacent plot by providing one feet soil.

2. Preparation and application of fungal conidia

The fungal conidia were obtained from well sporulated cereals (*B. bassiana* from Wheat, *P. fumosoroseus* and *V. lecanii* from Sorghum). The number of conidia/g was counted using a haemocytometer. Immediately prior to application, the conidia were suspended in 0.5% neem oil (*Azadirachta indica*). The treatments were applied with knapsack sprayer (India) using a spray fluid of 625 litre/ha. Spraying was carried out in the evening hours mainly during low wind velocity (> 4 m/s). Target concentration of conidia in oil formulation was 1.0 x 10^{10} spores/ml. First and second spray was carried out at 30 and 59 Days After Seedlings Emergence (DASE). The pest populations (no/plants) were counted one day before and 7 days after the treatment from 30 randomly selected plants in each plot. Leaf damage (both defoliation and infestation) was assessed by visual method (29).

3. Persistence of fungi on leaves and soil

Five grams of the leaves per sub-plot was collected randomly from the top and middle of the plants 10 and 30 days after the application of conidia separately. Within one hour after collection, the leaves were transported to the laboratory in sterilized plastic bags kept in ice bags and stored at 5 °C for a maximum of 12 hours, phyllosphere populations were recorded. The phyllosphere is a term used in microbiology to refer to leaf surfaces or total above-ground surfaces of a plant as a habitat for microorganism. Soil samples were also taken for this study from 20 different sites of each treated plots separately. Using a sterilize spoon, these samples were taken within the top 2 cm of soil, because the majority of fungal inocula are usually concentrated in this soil layer. Samples were collected in sterile polythene bags and kept in ice bag. Persistence of the fungal pathogens in the field was also recorded by collecting the dead insects after the subsequent spray. The dead insects were kept under observation for a period of 20 days at 25 ± 1 °C temperature and relative humidity 70 ± 10% percent to observe mycosis. Collected leaves were suspended in 100 ml of sterile distilled water with 0.01% Tween-80 for 1 hour on a rotatory shaker at 100 rpm. The suspension was serially diluted. One gram aliquots of soil (sample from 20 different sites were pooled) in each treated plot collected from different sites were suspended for 1 hour at 100 rpm in 250 ml of conical flask containing 100 ml of sterile distilled water along with Tween 80 at 0.01%. The resulting suspension was serially diluted. 0.1 ml of aliquots of leaves and soil sample plated on iodine based modified wheat meal agar media (DBMWA) and potato dextrose agar (PDA). After three weeks of incubation at 26 °C in darkness, the fungal growth was assessed to quantify the number of colony forming units (CFU). Temperature, mean relative humidity and rainfall were recorded from the nearest weather station (Courtallam, Tirunelveli District, Tamil Nadu, India) (Table 1).

Table 1
Weather data recorded during the crop age from first to 15th week

Crop age in week	Maximum Temperature	Minimum Temperature	Rainfall (in mm)	Relative humidity (%)
1	24.34	21.82	21.50	81.3
2	23.71	21.17	7.21	86.7
3	26.17	23.12	4.12	79.8
4	24.12	21.24	11.13	81.1
5	24.17	21.19	4.23	81.6
6	27.17	24.12	3.17	78.2
7	26.11	22.17	-	79.1
8	23.12	21.11	6.17	86.1
9	24.12	21.34	3.19	81.1
10	26.17	23.14	-	78.6
11	25.12	22.17	4.12	80.1
12	24.17	21.19	-	80.9
13	22.12	19.17	11.13	90.1
14	22.17	19.21	7.12	89.4
15	24.12	20.17	4.12	80.2

4. Cost benefit ratio

On the harvest day, the production of groundnut from each subplot was recorded and expressed as kg/ha^{-1}. From the data the cost benefit ratio was worked out. The data was subjected to Duncan Multiple Range Test (DMRT) to assess the significance of fungal treatment with untreated control and standard (pesticide treatment) using the statistical software SPSS version 11.5.

Results

1. Pest population

Pre-treatment observations showed that *A. craccivora* was found to be predominant pest, followed by *S.*

litura, *A. modicella* and *M. pustulata*. From the table 1 it was very clear that incidence of all pests was reduced greatly after the application of fungal pathogens as well as endosulfan sprayed groundnut field. Among the four treatments, *V. lecanii* completely reduced *A. craccivora* populations than by endosulfan treatment at 46 Days After Seedling Emergence (DASE). *S. litura* populations was significantly (P< 0.05) reduced by endosulfan compare with entomopathenic sprays with *B. bassiana*, *P. fumosoroseus*, and *V. lecanii*. A similar observation was also observed for *A. modicella* (Table 2). Except the comparison between the *V. lecanii* and control, all other comparisons were statistically significant (df=1,58; F= 2.40; P< 0.05). The reduction of *M. pustulata* population was 0.51, 0.52, 0.52 and 0.10 for *B. bassiana, P. fumosoroseus*, *V. lecanii* and endosulfan treated plots respectively. Statistical comparison between *B. bassiana* and endosulfan separately with control were also significant at 5% level (df= 1,58; F= 2.44; P< 0.05).

2. Pest infestation

The infestation of two groundnut pests such as *S. litura* and *A. modicella* were greatly reduced after the treatment of the fungal pathogens. Between the two defoliators recorded during the study period the infestation due to *S. litura* was the highest (7.3/plant) followed by *A. modicella* (5.1/plant). The infestation due to *S. litura* was reduced to 0.6 and 0.3 in first and second spray of *B. bassiana*. Similarly the infestation decreased to 4.9 and 0.9 during the first and second spray of *P. fumosoroseus*. A similar observation was also observed in *V. lecanii* treated plot (4.8 and 1.1 for first and second spray respectively). In the case of *A. modicella*, the infestation reduced from 5.1 to 1.9 and 0.5, 2.7 to 0.9 and 3.1 to 1.4 during first and second spray for *B. bassiana*, *P. fumosoroseus* and *V. lecanii* treated plot respectively. Since both *A. craccivora* and *M. pustulata* caused minor infestation, their infestation levels were not recorded.

3. Persistence of entomopathogenic fungi

In general the persistance of all the three entomopathogenic fungal population was higher in the soil than in the phyllosphere. For instance, the persistence of *B. bassiana* was found to the maximum in soil (22.1 x 10^{11} CFU/g) followed by *P. fumosoroseus* (7.1 x 10^{11} CFU/g) and *V. lecanii* (5.4 x 10^{11} CFU/g). In the phyllosphere, the population was found to be 26.4 x 10^{11}, 17.3 x10^{10} and 3.2 x 10^{10} CFU/g for *B. bassiana, P. fumosoroseus* and *V. lecanii* respectively.

4. Yield and cost benefit ratio

High production of groundnut was recorded in

Table 2
Effect of three entomopathogenic fungi on *A. cracivora, A. modicella* and *M. pustulata* populations (all data presented here are mean values)

Pests	Treatment	I spray			II spray		
		31	39	46	52	59	66
A. craccivora	Control (A)	44.76	59.12	62.17	77.19	84.13	89.19
	B. bassiana (B)	44.46	27.49	19.19	12.47	10.64	10.64*
	P. fumosoroseus (C)	44.70	26.12	19.17	12.20	10.10	8.17*
	V. lecanii (D)	44.96	17.13	0.0	0.00	0.00	0.00*
	Endosulfan (E)	44.06	10.7	0.07	0.07	0.07	0.07*
A. modicella	Control (A)	1.39	1.76	1.91	1.97	2.01	2.01
	B. bassiana (B)	1.89	0.77	0.61	0.37	0.37	0.11*
	P. fumosoroseus (C)	1.22	0.91	0.81	0.49	0.41	0.23*
	V. lecanii (D)	1.30	0.94	0.87	0.67	0.42	0.34
	Endosulfan (E)	1.32	0.62	0.57	0.22	0.19	0.08*
M. pustulata	Control (A)	0.71	0.77	0.77	0.75	0.77	0.76
	B. bassiana (B)	0.74	0.71	0.67	0.67	0.55	0.51*
	P. fumosoroseus (C)	0.70	0.71	0.70	0.69	0.57	0.52
	V. lecanii (D)	0.72	0.71	0.71	0.69	0.58	0.52
	Endosulfan (E)	0.71	0.46	0.31	0.27	0.27	0.10*
S. litura	Control (A)	2.11	2.18	2.37	3.11	3.17	3.17
	B. bassiana (B)	2.62	0.73	0.61	0.35	0.21	0.10*
	P. fumosoroseus (C)	2.71	1.54	1.17	0.75	0.35	0.19*
	V. lecanii (D)	2.44	1.57	1.24	0.79	0.56	0.37
	Endosulfan (E)	2.01	0.57	0.49	0.27	0.27	0.07

A, B, C, D and E denotes plots name;
*Values are statistically significant when compared to the control. Statistical analysis (Duncan Multiple Range Test) was made between control with fungal pathogens and expressed at 5% level

B. bassiana treated plot (1721.31 kg/ha^{-1}) than *P. fumosoroseus* (1682.43 kg/ha^{-1}) and *V. lecanii* (1298.67 kg/ha^{-1}). Total of 1096.4 and 1117.2 kg/ha^{-1} was recorded in untreated control and endosulfan treated plot, respectively. Similarly, the cost benefit ratio was higher in *B. bassiana* treatment (1: 1.93) followed by *P. fumosoroseus* (1: 1.86) and *V. lecanii* (1: 1.73). The cost benefit ratio was found to be lower in untreated control (1: 1.10) and in endosulfan treated plot it was found to be 1: 1.11.

Discussion

Field study revealed that all the entomopathogenic fungi effectively control the groundnut pest populations and their incidence. Environmental factors play a vital role on entomopathogenic fungi. Higher humidity and frequent rainfall observed in the study area favors the activity of the fungal pathogens as reported in other studies (15, 18, 21). Generally, entomopathogenic fungi survive well in nature between 10 ˚C and 30 ˚C (15). Temperature dependent processes such as rate of disease development and level of disease are higher at the optimum temperature (18). Observations revealed that in the study area, the temperature and humidity ranged from 19.21 to 24.12 ˚C and 78.6 to 84.4% respectively (Table 1). The dead caterpillars of *S. litura* and *A. modicella* could be observed within 5 days after the application of fungal pathogens. Fully mycosed larvae were observed after 15 to 20 days after the treatment of the fungi. The spores from this dead cadavers might be carried away by the wind and caused further infection and enhanced the fungal activity.

Apart from the environment factors, fungi have considerable epizootic potential and spread quickly through the insect populations (10, 27). Our results showed that the pest population except *M. pustulata* was found to be higher. A critical factor that appears to influence the fungal pathogenicity is the population densities of the insects. Uneven population density on different leaves may modify the microenvironment in which the interaction between the host and pathogen takes place. For instance, high host population densities may enhance the pathogen fitness by facilitating the development and dissemination among insects due to the increased abundance of honeydew (8). This may enhance the fungal activity. The successful use of biological control agents depends on the suitable formulation technique. Moreover, the fungal pathogens applied with oil formulation increases their persistence and their dependability on the prevailing environment (28). They also revealed that the infective propagules of fungus either in the form of blastospores or conidiospores showed better activity than they were mixed with suitable ingredients, which may act as nutrients, additives or wetting agents. It was also revealed that oil formulation was found to be highly effective compare to other agents tested in their study (19). The lipophilic conidia of deuteromycete fungi readily suspend in oil uniformly and dispersed its conidia might favor uniform coverage of plant surfaces. Oil based formulation effect of *Nomuraea rileyi* and *B. bassiana* on *S. litura* and *Myzus persicae* was reported respectively (28). It was reported earlier that one spray of *V. lecanii* caused 100% inhibition of aphid's population after two weeks of application in cucumber (9). In the present study, 100% eradication of *A. craccivora* was observed and this might be due to the favorable environmental condition and higher aphid population, which directly or indirectly increased the fungal activity. However, application of talc-based formulation of *B. bassiana* and *Pseudomonas fluorescens* strains mixture through seed, soil and foliar spray effectively reduced the incidence of leaf miner and collar rot in groundnut compared to individual bio-formulation and control treatments both under glasshouse and field conditions (25).

Studying the persistence of the entomopathogenic fungal pathogen is essential for their successful application and efficacy in the soil (4). In the present study, the pathogens density was found to be higher in soil than leaves except, *B. bassiana*. To dominate both the *V. lecanii* and *P. fumosoroseus, B. bassiana* established its population more as phyllosphere. Fungi in soil are generally protected from desiccation, UV radiation and extreme temperature. This may enhance the more number of CFU of fungal pathogens. Padmaja and Gurvinder Kaur (17) found that *B. bassiana* fungal spores can be viable upto 30 days after treatment. Conidia of *B. bassiana* (21) survive in or on the surface of the soil for a longer period. In the present study, all entomopathogenic fungi density was found to be high in soil than phyllosphere. Soil is a natural reservoir for the entomopathogenic fungi and kills the pest subsequently and falls to the ground (14). Our results revealed that at each test site all the fungi were recovered one month after the application of fungi. This indicated that when the larvae or beetle comes in contact with the soil, the infective propagules bind readily to the insect epicuticle upon the contact. Moreover, they also infect insects pupae present in the soil. The pupae may be highly susceptible to these viable infectious fungal propagules in the soil. In phyllosphere the numbers of CFU of all entomopathogens were found to be lower than the soil. There are several factors such as sunlight, nature of leaves and normal antagonistic microflora that may inactivate the conidia. In general, the half-life of spores of most fungi when exposed to stimulated sunlight ranged from one to four hours (3, 11). Density of hairs, waxiness, veins, size and shape angle of leaves of the stem the leaf, number of stomata and density of the canopy also influence the microclimate of the leaf. The phyllosphere pH may also exert some impact on the pathogens (32). The hyphomycetous entomopathogenic fungi *M. anisopliae, V. lecanii* and *B. bassiana* have been used to target pest insects for over a century.

This field study clearly revealed that the

entomopathogenic fungal treatment distinctly decreased the pest population and their infestation, cost benefit ratio and yield were higher in *B. bassiana* treated plot than *P. fumosoroseus* and *V. lecanii* treatments. We then concluded that the use of *B. bassiana* in groundnut increased the groundnut production and showed persistence in the field for the next cultivation.

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Acknowledgement

The first author K. Sahayaraj highly thankful to the management of St. Xavier's College (Autonomous), Palayamkottai for providing necessary laboratory facilities and the encouragements.

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