BEAUVERIA BASSIANA AS A BIOLOGICAL CONTROL AGENT AGAINST FOREST PESTS *POECILOCERUS PICTUS* (ACRIDIDAE, ORTHOPTERA, AND *BRACHYTRYPES PORTENTOSUS* (GRYLLIDAE,ORTHOPTERA)

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ABSTRACT

Entomopathogenic fungus *Beauveria bassiana* was tested as biological control agent against forest nursery pests *Poecilocerus pictus*, and *Brachytrypes portentosus* in the laboratory at the Pakistan Forest Institute, Peshawar under the controlled temperature of 25 - 35°C and 55-65% relative humidity, for evolving their best possible biological control. *B. bassiana* fungus was tested in the doses of 0.8×10^4 spores/ml, 0.6×10^4 spores/ml in the study. The data showed that *B. bassiana* has given a non significant result against the pests even after 72 hours but results were significant after 96 hours upto 168 hours of treatment. The study showed that after treatment the fungus first established and then caused the mortality of *Poecilocerus pictus* within 2-3 days while in case of *Brachytrypes portentosus* within 7 days. The fungal hyphae developed in the treated insect pests first in their soft body parts like thorax and abdomen covering the whole body within a period of two weeks. The study showed the susceptibility of these insects to the fungus *B.bassiana* which could be used as biological control agent in the field.

INTRODUCTION

Bassi (1935) was first to demonstrate *Beauveria bassiana* as a pathogenic fungus. Since then a large number of researchers have found it effective against a wide range of agriculture and forest crop insect pests. However in a few cases the attempts on biological control using *B.bassiana* did not succeed very much (Tournock and Muldrew, 1971). Vancy and conte (1903) cultivated *B.bassiana* on silkworm and claimed that by contaminating the leaves of vine with a spore emulsion a considerable degree of infection could be achieved. The laboratory studies on some fungi including *B.bassiana* against *Hypsipyla granela* should that with immersion of the larvae in a suspension of conidiospores of *B.bassiana* and 1.4 x 10 viable spores per ml resulted in 14% mortality (Berrios and Hidalagga Salvatirra, 1971).

Yendol (1973) explained the details of observations on the histopathology of *Reticulitermes flavipes* (Koll) infected with the fungus *A.bassiana.* The initial symptoms of infection were sluggishness, anal discharge, passivity and failure to respond to tactile stimuli.

Goettel (1992) stated a brief review of the attributes and limitations of the fungi as potential microbial control agents and of several species of fungi commonly associated with grasshoppers.

To enhance the natural forests and plantations for boosting up timber and fuel wood production, better environment and balanced ecosystem, the forest departments are carrying out the afforestation programmes. For the successful afforestation programme the

raising of nurseries are very much essential. Nursery raising are carried out both in hills and plains by adopting the most recent nursery raising techniques. Unfortunately the nurseries fall prey to the attack of different insect pests. Among the insect pests the ak grass hopper *Poecilocerus pictus* and *Brachytrypes portentosus* are the most distructive insects.

Present every where in small numbers, these insects some times become abundant enough to cause serious losses by cutting off seedlings just above the ground or just below the apical bud and cutting off many leaves that drop to the ground.

Poecilocerus pictus cause defoliation of bamboo forests in dry regions and destroy the seedlings of *Pinus longifolia.* It is also pest of cultivated plants and vegetables. There is normally one generation in a year with hibernation in egg stage.

Cricket (*Brachytrypes portentosus)* cut off primarily young seedlings and low shoots at night and dragging the pieces into the tunnel for feeding. The pests remain very active during March-April and September causing severe damage. It is injurious in nurseries of *Casuarina equisetifolia, Dalbergia sissoo, Eucalyptus* spp., *Ficus elastica, Tectona grandis* tea and agricultural crops. These insects have been controlled by physical, chemical and Biological control methods. Among all the above methods chemical control method has been applied extensively. Initially many chemical insecticides as Aldrin 5%, Paris green mixed with wheat flour, DDT, Lead arsenate and lime mixture etc. were brought into field. Now a days biological control methods are taking the place of chemical control methods. Since chemical insecticides treatment does not improve forest conditions in respect to insect susceptibility. Biological control has preference over the chemical control also the fact that due to sustained application of pesticides over the years has not only polluted the environment but has made pest more resistant giving rise to kind of pests which are difficult to control.

MATERIALS AND METHODS

The fungus *Beauveria bassiana* (Bals). Vull commonly found in galleries of poplar borer was collected from the infected larvae of *Inderbela quadrinotata* (a bark borer) from the field.

The nymphs *Poecilocerus pictus* (ak grasshopper) and *(Brachytrypes portentosus* (cricket) were collected from Peshawar, Rawalpindi from *Dalbergia sissoo* nurseries and agricultural fields during June, July.

The trials were conducted under controlled laboratory conditions at the Pakistan Forest Institute, Peshawar. The entomo-pathagenic fungus *B. bassiana* was tried against nymphs of *P. pictus* and *B. portentosus* for inoculation of *B. bassiana* a dead larva of *I. quadrinotata* (size 2x1.5 x 2.5 cm) was crushed into 400 ml of distilled water to make a fungal suspension from which three dilutions were prepared. For the highest concentration T1 the stock suspension was taken as such. From the stock suspension 200 ml were taken in another beaker to which 200 ml more distilled water was added to make a second dose (T2). From T2 200 ml were taken in another beaker and by adding 200 ml of distilled water the lowest dose was prepared. Samples from each dose were taken in the glass tubes and per ml number of spores were determined under the microscope.

There were four treatments including check and each treatment was replicated four

times applying a randomized complete block design. In each treatment there were twelve nymphs.

The nymphs were kept in completely sterilized chimneys. *Dalbergia sissoo* roots and grasses were provided to cricket as food while Chinar leaves were given to grasshopper as food. Observations were recorded 24 hours after each treatment. Fresh roots and shoots were provided as food after every two days. The in oculated food was not removed from the chimneys.

The experiment was conducted under controlled conditions of temperature ranging from 25 to 35°C with 55-56% relative humidity. Sponger hemocytometer was used to count the fungal spores. A sample of 5 ml of each dose was taken in separate glass tubes and was used for counting down per ml number of spores.

The hemocytometer is a single piece of glass with an 'H' shaped through forming two counting areas. It has supports to hold the glass cover at proper distance above these areas. The counting number was cleared with alcohol and wiped dried with a clean dry cloth. After this the clean cover glass was placed over the counting chambers. A drop of the fungal suspension was placed at the edge of the cover glass and the preparation was allowed to stand for one or two minutes so that it could occupy the space between the cover glass and ruled area of the slide and spore could settle at the bottom. The spores were counted in 5 one square mm rulings designated as A, B,. C, D and E. The number of spores counted in 5 one square mm rulings was summed and multiplied with 2000 as follows, to get the number of spores per ml.

Formula:

$(A+B+C+D+E) \times 2000 =$ No. of spores/ml.

where A, B, C, D and E are five large grid regions of Hemocytometer.

RESULTS AND DISCUSSIONS

Mortality of *Poecilocerus pictus* nymphs treated with *Beauveria bassiana* in four replications in different treatments out of 12 nymphs after hours.

B. bassiana	Observation time after treatment chours						
Spores/ml	24	48	72	96	120	144	168
$0.8x10^4$ T_1	2	5	8	9	12	-	-
$0.6x10^4$ T_2	2	5	6	-	8	11	12
$0.4x10^4$ T_3	0	1	2	2	4	7	7
Control T_0	0	1	1	1	2	3	5

The mortality of *Poeoilocerus pictus* treated with *B. bassiana* after 24 to 72 hours is non-significant which show that *B. bassiana* does not have knock down effect like chemical insecticides. After 96 hours of treatment the test entomopathogenic fungus gave significant results. It has been observed that first *B. bassiana* established itself and then caused mortality. After 120 hours *B. bassiana* has established itself. It shows that treatment effects are significant. As in treatments (T1), (T2) and (T3), (12), (8) and (4) *P. pictus* nymphs had died respectively.

ANOVA TABLE

Sources of variation	df	S.S	M.S.	F.ratio (calculated)	P
Treatments	3	50.89	16.96	8.18*	0.039
Replications	2	4.22	2.11	1.02	0.558
Error	6	12.44	2.07	-	-
Total	11	67.55			

* Significant at 5% level

The table 1 shows significant mortality of *P. pictus* to *B. bassiana* treatment. It is clear from the data that entomopathogenic fungus in the dose of 0.8×10^4 and 0.4×10^4 spores/ml in 7 and 11 days respectively has gained cut percent mortality.

Observations on mortality of *P. pictus* nymphs due to *B. bassiana* infection revealed that within 2-3 days of infection the colour of nymphs first changed from yellowish colour to pink and later on white due to fungal growth.

The fungal hyphae were observed on the head, thorax and abdomen regions first followed by on the appendages within a period of 8-10 days after the mortality of the *P. pictus* nymphs. The overall results show that *B. bassiana* gives positive response to experimental hypothesis under consideration but initially the establishment process was slow. *B. bassiana* can be used for control purpose for *P. pictus* in higher dose.

B.bassian spores/ml	Observation time after treatment (hours)							
	24	48	72	96	120	144	168	192
0.8×10^4 T_1	4	5	6	8	11	11	11	12
0.6×10^4 T_2	1	6	4	5	10	10	11	11
0.4×10^4 T_3	4	4	66	9	9	9	10	11
Control T_0	0	1	2	3	5	5	6	6

B.bassiana did not give significant mortality even after 72 hours as only 8 crickets were found dead in the highest dose indicating the commencement of the fungal infection. In a time period of 120 hours the fungus showed the significant results. Table 2.

CONCLUSION

The study showed the susceptibility of *Poecilocerus pictus* and *Brachytrypes portentosus* to the fungus *Beauveria bassiana.* It also showed that the fungus first established itself and then causaed the mortality. Hence *B. bassiana* could be used as biological control agent in the field.

REFERENCES

Bassi, A. (1835) Del mal sengo calcinaccio 0 mascardino malttia the offlige i bachi da. I. Teorica Tip. Orcesi, Lodi.

Berrios, F. and Hidlago-Salvatierra, 0. (1971) Estudias sobre el barrenader, *Hypsipyla grandella* (Zeller) VIII susceptibilidad de la larva a los honqos *Beauveria bassiana* (Bals) *Beauveria tenella* (Dels) Siemafko. Turrialba 21 (4): 251-254.

Goettel, M.S. and Johnson, D.L. (1992) Environmental impact and safety of fungal biocontrol agents. Biological control of locusts and grasshoppers; proceedings of a workshop held at the International Institute of Tropical Agriculture, Cotonou, Republic of Benin, 29 April to 1 May 1991. 356-361.

Turnocock, W.J. and Muldrew, J.A (1971) *Pristiphora erichsonii* Harting. Larch Sawfly. C.I.B.C. Tech. Blull., 4: 175-180.

Vaney, 0. and Conte, A. (1903) Infection of the eastern substerranean termite, *Reticulitermes flavipes* (Kollar) with the fungus *Beauveria bassiana* (Bals) Vuill. Entomophaga (1971) 16 (3): 343-352.