EFFICACY OF *BEAUVERIA BASSIANA* AS BIO-CONTROL AGENT AGAINST HADDA BEETLE *EPILACHNA DODECASTIGMA* MULSANT (COCCINELLIDAE; COLEOPTERA) UNDER LABORATORY CONDITIONS

Hanif Gul[1] and Abida Hamid[2]

Abstract

To test the pathogenicity of Entomopathogenic fungus *Beauveria bassiana* (Bals) vuill against all stages of *E. dodecastigma* trials were conducted in the labroatories at the Pakistan Forest Institute, Peshawar. The fungus, *B. bassiana* was tried in the doses of 0.4×10^4 spores/ml 0.6×10^4 spores/ml and 0.8×10^4 spores/ml against all stages of Hadde beetles. The fungus *B. bassiana* proved to be effective against all the stages except eggs. For grubs among the three doses 60% mortality occurred after 120 hours in the dose of 0.8×10^4 spores/ml while 53% and 33% mortality was recorded by other two doses in the same time period respectively. For pupae highest dose gave 57% kill in 168 hours while the other two doses gave 43 and 26% mortality in the same time period respectively. For adults among the three doses 55% mortality occurred after 192 hours in the dose of 0.8×10^4 spores/ml while doses of 0.6×10^4 spores/ml and 0.4×10^4 spores/ml gave only 34 and 22% kill respectively.

Introduction

Hadda beetles *E. dodecastigma* Mulsant (Cocinellidae; Coleoptera) attack different Solanaceous vegetables like brinjal, tomato and potato, with maximum population at the end of April or in early May. During May and June the population declines and rebuild again during August. Beetles are 8-9mm long, 5-6mm wide have six black spots on each elytron with rounded abdomen. Grubs are yellowish in colour and have six rows of long branched spines of 6mm length. Grubs as well as beetles cause damage by feeding on the upper surface of the leaves. They eat up regular area of the leaf tips leaving parallel bands in between the uneaten tissues the damaged leaves thus presents lace like or ragged

---

[1] Forest Entomologist, Pakistan Forest Institute, Peshawar.

[2] NWFP Agriculture University, Peshawar.

appearance. They turn brown dry up and fall off and thus partially or completely defoliate the plants. The defoliated plants hardly survive and therefore a heavy loss due to this beetle is generally experienced. The plants with partially damaged leaves produce low quality and quantity of fruit.

This pest is widely distributed through out the world and has been recorded on various cultivated and wild plants. Both the adults and grubs feed on plant leaves which severely defoliate the host plants throughout the season from March to October, later on it hibernates as adult.

Pesticides gave enormous results in term of enhanced quality and quantity of agricultural production and protecting the health of the people, but as environmental awareness grow up many of the most promising pesticides emerged as threat to global ecosystem. Thus search began for alternative pest control strategies which were not only effective but also environmentally safe. One of these alternative methods is to use the entomopathogenic fungi which are considered as beneficial organisms with potentials as pest suppressive agents. Fungi that causes diseases in insect belong to classes Phycomycetes, Ascomycetes, Basidiomycetes and Deutermycetes. The genus Beauveria of class Deutromycetes contains the species *B. bassiana* and *B. tenalla* which have been applied against insect pests in the field more frequently than any other fungi. *B.bassiana* produces natural toxins such as beauvericin which eventually may be useful in insect suppression programme.

Keeping in view the importance of the pest and fungi, trials were conducted at the Pakistan Forest Institute, Peshawar to achieve the following objectives:

1. To find out efficacy of entomopathogenic fungus *B. bassiana* against various stages of *Epilachna dodecastigma*

2. To work out effective doses of *B. bassiana* against each stage of *E.dodecastigma*

Review of Literature

Mietkiewski (1985) examined a total of 66713 larvae of *Euproctis chrysorrhoea* that died during diapuse in winter webs and isolated seventeen species of fungi from each larva, the most common of which was *Paecilomyces* *farinosus* (from 46.9% of larvae). *Beauveria bassiana* was isolated from 44.2% of dead larvae and *Verticillium lecanii* from 24.6%.

Guerin (1986) reported that granular formulation of *Beauveria bassiana* was 70-90% effective against *Ostrinia nubilalis* with infection ranging from 1.3 to 1.9 larvae/stem.

Rombach *et al.* (1986) tested *Beauveria bassiana, Metarhizium anisopliae* and *Paecilomyces lilacinus* as suspensions of conidia and as dry mycelium on populations of *Seotonophara coaretata* on rice plants in screen cages and reported that the number of hugs were significantly reduced in all treated plots compared to control upto 9 weeks.

Aguda and Rombach (1987) studied the virulence of *Beauveria bassiana* and *Momuraea rileyi* to the rice pest *Cnaphalocrocis medinalis*. Suspension of both fungi were prepared at 0, 10^4, 10^5, 10^6, 10^7 and 10^8 conidia/ml. Thirty (third instar) larvae were dipped in each suspension and placed on filter paper for several minutes. Treaded larvae were incubated in petridishes containing rice leaves and mortality was determined a week later.

Gopalakrishnan and Narayanan (1990) reported *Beauveria bassiana* to be pathogenic to all stages of *(Heliothes armigera)*, causing 60-100% mortality of larvae and 100% mrotality of eggs when individuals of *H. armigera* were dipped into a suspension of 1.0 x 107 conidia/ml.

Pestidge and Willoughby (1990) evaluated the entomophilic nematode *Heterorhabditis bacteriophora* and the entomogenous fungus *Beauveria bassiana* for the control of the curculionid *Phlyctinus callosus*, using larvae and pupae in a suspension of 4.6 x 106 conidia. Spores of *B. bassiana* gage 85% mortality after 72 hours.

Badilla and Alves (1991) tested four dosages of *Beauveria bassiana* isolates against the *Sphenophorus levis* a pest of sugar cane. The LD_{50} was 8.8 x 10^9 conidia/1500 ml flank. In field tests in sao paulo, isolate 447 at 4.5 x 1.11 conidia per piece of treated sugar cane resulted in 92.3% of mortality of *S. levis*.

Materials and Methods

To carry out trials on the efficacy of *Beauveria bassiana* (Bals) vuill on Hadda beetle, *Epilachna dodecastigma*, the test material entomopathogene fungus

B. bassiana commonly found in galleries of poplar borer was obtained from the infected larvae of *Inderbela quadrinotata* a poplar bark borer from the field.

The trials were conducted in the larboratories of the Pakistan Forest Institute, Peshawar. The experiments were conducted under controlled labroatory conditions of temperature (25 to 35°C) and humidity (55 to 65%).

Hadda beetles for the trial were collected from Peshawar area in the month of June. The culture for the trials was developed by releasing pairs of the beetles in the glass chimneys containing leaves of brinjal and tomato. Eggs in batches of 15 to 65 were collected from the underside of leaves and placed in seperate petridishes alongwith fresh leaves of tomato or brinjal. After getting enough culture the actual experiment was started which included different doses of fungus *B. bassiana* for the effective control of Hadda beetles, efficacy of *B. bassiana* against various stages of Hadda beetles.

For inoculation of *B. bassiana, Inderbela quadrinotata* infected larvae with *B. bassiana* infection were crushed in Petridish and fungus suspension was prepared by mixing 400ml of distilled water. From this suspension the following different doses of suspension were then prepared.

For the highest concentration i.e., T1 the stock suspension was taken as such. From the stock suspension 200ml was taken in another beaker to which 200ml more distilled water was added to make second dose T2. From T2 200ml was taken in another beaker and by adding 200ml distilled water the lowest dose T3 was prepared. Sample from each dose were taken in glass tubes and per ml number of spores was determined under the microscope using spencer haemocytometer which were as follows:

T_1 8000 spores/ml or 0.8×10^4 spores/ml.
T_2 6000 spores/ml or 0.6×10^4 spores/ml.
T_3 4000 spores/ml or 0.4×10^4 spores/ml.
T_4 Control.

The trials were laid out in Randomized complete block design with four treatments including control and were replicated five times. In each treatment there were 25 beetles i.e. five in eavh replication.

farinosus (from 46.9% of larvae). *Beauveria bassiana* was isolated from 44.2% of dead larvae and *Verticillium lecanii* from 24.6%.

Guerin (1986) reported that granular formulation of *Beauveria bassiana* was 70-90% effective against *Ostrinia nubilalis* with infection ranging from 1.3 to 1.9 larvae/stem.

Rombach *et al.* (1986) tested *Beauveria bassiana, Metarhizium anisopliae* and *Paecilomyces lilacinus* as suspensions of conidia and as dry mycelium on populations of *Seotonophara coaretata* on rice plants in screen cages and reported that the number of hugs were significantly reduced in all treated plots compared to control upto 9 weeks.

Aguda and Rombach (1987) studied the virulence of *Beauveria bassiana* and *Momuraea rileyi* to the rice pest *Cnaphalocrocis medinalis*. Suspension of both fungi were prepared at 0, 10^4, 10^5, 10^6, 10^7 and 10^8 conidia/ml. Thirty (third instar) larvae were dipped in each suspension and placed on filter paper for several minutes. Treaded larvae were incubated in petridishes containing rice leaves and mortality was determined a week later.

Gopalakrishnan and Narayanan (1990) reported *Beauveria bassiana* to be pathogenic to all stages of *(Heliothes armigera)*, causing 60-100% mortality of larvae and 100% mrotality of eggs when individuals of *H. armigera* were dipped into a suspension of 1.0 x 107 conidia/ml.

Pestidge and Willoughby (1990) evaluated the entomophilic nematode *Heterorhabditis bacteriophora* and the entomogenous fungus *Beauveria bassiana* for the control of the curculionid *Phlyctinus callosus*, using larvae and pupae in a suspension of 4.6 x 106 conidia. Spores of *B. bassiana* gage 85% mortality after 72 hours.

Badilla and Alves (1991) tested four dosages of *Beauveria bassiana* isolates against the *Sphenophorus levis* a pest of sugar cane. The LD_{50} was 8.8 x 10^9 conidia/1500 ml flank. In field tests in sao paulo, isolate 447 at 4.5 x 1.11 conidia per piece of treated sugar cane resulted in 92.3% of mortality of *S. levis*.

Materials and Methods

To carry out trials on the efficacy of *Beauveria bassiana* (Bals) vuill on Hadda beetle, *Epilachna dodecastigma*, the test material entomopathogene fungus

B. bassiana commonly found in galleries of poplar borer was obtained from the infected larvae of *Inderbela quadrinotata* a poplar bark borer from the field.

The trials were conducted in the larboratories of the Pakistan Forest Institute, Peshawar. The experiments were conducted under controlled labroatory conditions of temperature (25 to 35°C) and humidity (55 to 65%).

Hadda beetles for the trial were collected from Peshawar area in the month of June. The culture for the trials was developed by releasing pairs of the beetles in the glass chimneys containing leaves of brinjal and tomato. Eggs in batches of 15 to 65 were collected from the underside of leaves and placed in seperate petridishes alongwith fresh leaves of tomato or brinjal. After getting enough culture the actual experiment was started which included different doses of fungus *B. bassiana* for the effective control of Hadda beetles, efficacy of *B. bassiana* against various stages of Hadda beetles.

For inoculation of *B. bassiana, Inderbela quadrinotata* infected larvae with *B. bassiana* infection were crushed in Petridish and fungus suspension was prepared by mixing 400ml of distilled water. From this suspension the following different doses of suspension were then prepared.

For the highest concentration i.e., T1 the stock suspension was taken as such. From the stock suspension 200ml was taken in another beaker to which 200ml more distilled water was added to make second dose T2. From T2 200ml was taken in another beaker and by adding 200ml distilled water the lowest dose T3 was prepared. Sample from each dose were taken in glass tubes and per ml number of spores was determined under the microscope using spencer haemocytometer which were as follows:

T_1 8000 spores/ml or 0.8×10^4 spores/ml.
T_2 6000 spores/ml or 0.6×10^4 spores/ml.
T_3 4000 spores/ml or 0.4×10^4 spores/ml.
T_4 Control.

The trials were laid out in Randomized complete block design with four treatments including control and were replicated five times. In each treatment there were 25 beetles i.e. five in eavh replication.

Results and Discussion

The observations recorded on the efficacy of *B. bassiana* on different stages of *E. dudecastigma* are:

i. Efficacy of *Beauveria bassiana* against eggs

The experiment conducted against the egg stage of Hadda beetle showed that the egg did not develop fungal infection upto 96 hours after spray. Mortality of eggs due to fungus was negligible as 96% eggs, hatched to grubs in period of 3-5 days.

ii. Efficacy of *Beauveria bassiana* against grubs

Grubs mortality with *B. bassiana* infection is given in the following table:

Table 1. Mortality of Grubs of Hadda beetle, *Epilachna duodecastigma,* after application of the entomopathogenic fungus *Beauveria bassiana*

Treatment	Larval mortality after hours					
	24hrs	48hrs	72hrs	96hrs	120hrs	Means
T_1	24.0 (6) IJ	52.0 (13) EF	72.0 (18) D	84.0 (12) BC	100.0 (25) A	66.400 (83) A
T_2	12.0 (3) KL	32.0 (8) HI	52.0 (13) EF	76.0 (19) CD	92.0 (23) AB	52.800 (66) B
T_3	4.0 (1) LM	12.0 (3) KL	40.0 (10) GH	48.0 (12) FG	60.0 (15) E	32.800 (41) C
T_4	0.0 (0) M	4.0 (1) LM	8.0 (2) KLM	8.0 (2) KLM	16.0 (4) JK	0.200 (9) D
Mean	10.0 (10) A	25.0 (25) B	43.0 (43) C	54.0 (54) D	67.0 (6) E	-

Note:
1 = LSD for treatments at alpha 0.05 = 10.78
2 = LSD for timings at alpha 0.05 = 5.489
3 = LSD treatment x timings interaction at alpha 0.05 = 10.98
4 = Figures followed by the same letters are not significantly different from each other
T_1 = 0.8 x 104 spores/ml
T_2 = 0.6 x 104 spores/ml
T_3 = 0.4 x 104 spores/ml
T_4 = Check

The figures in paranthesis indicate the original data.

The above table shows that all the doses differed significantly from each other. Highest mean mortality of 66.4% was found in T_1 after 120 hours, followed by T_2, T_3 and T_4 having 52.80, 32.8 and 7.2 respectively.

As far as effect of time interval is concerned the average effect was lowest being 10% after 24 hours of treatment followed by 25, 43, 54 and 67% after 48, 72, 96 and 120 hours respectively. The treatment x time interaction is significant and resembles main effect of treatment as well as timing. However, highest percent mortaligy of 100% was found in T_1 after 120 hours of treatment and lowest 16% in control (no treatment).

iii. Efficacy of *Beauveria bassiana* against pupae

The observation recorded on efficacy of *B. bassiana* on pupae are presented in the following table:

Table 2. Mortality of Pupae beetle, Epilachna duodecastigma, after application of the entomopathogenic fungus *Beauveria bassiana*

Treatment	Larval mortality after hours							
	24hrs	48hrs	72hrs	96hrs	120hrs	144 hrs	168 hrs	Means
T_1	16.0 (4) IJ	28.0 (7)G	36.0 (9) F	56.0 (14) D	76.0 (19) C	84.0 (21) B	100.0 (25) A	56.57 (99) A
T_2	8.0 (2) KL	16.0 (4) IJ	24.0 (6) GH	36.0 (9) F	60.0 (15) D	72.0 (18) C	88.0 (22) B	43.429 (76) B
T_3	4.0 (1) LM	8.0 (2) KL	8.0 (2) KI	20.0 (5) HI	36.0 (9) F	48.0 (12) E	56.0 (14) D	25.714 (45) C
T_4	0.0 (0) M	0.0 (0) M	4.0 (1) LM	8.0 (2) KL	120.0 (3) JK	16.0 (4) IJ	20.0 (5) HI	8.571 (15) D
Mean	7.0 (7) F	13.0 (13) E	43.0 (43) C	54.0 (54) D	46.0 (46) C	55.0 (55) B	66.0 (66) A	-

Note:
1 = LSD for treatments at alpha 0.05 = 6.058
2 = LSD for timings at alpha 0.05 = 5.706
3 = LSD treatment x timings interaction at alpha 0.05 = 6.716
4 = Figures followed by the same letters are not significantly different from each other
T_1 = 0.8×10^4 spores/ml
T_2 = 0.6×10^4 spores/ml
T_3 = 0.4×10^4 spores/ml; T_4 = Check

The figures in paranthesis indicate the original data.

The above table shows that different doses differed significantly from each other, in (T_1) the mean mortality recorded was 55.5% after 168 hours of treatment followed by I_2, I_3 and I_4 having 43.43% 25.71% and 8.5% mortality in the same time period. In I_1 the mortality rate of pupae after 24 hours and 48 hours of treatment was very low which slowly increased after 96 and 120 hours and reached to 84% and 100% after 144 and 168 hours, respectively. The same trend was observed in I_2, I_3, I_4.

The average effect of time interval on the mortality or pupae was only 7%, 13% and 18% after 24 hours, 48 hours and 72 hours of treatment, followed by 30%, 46%, 55% and 66% after 96, 120, 144 and 168 hours of treatment, respectively. The results reveal that pupal mortality increased with time.

iv. Efficacy of *Beauveria bassiana* against adults

Observation recorded on efficacy of Beauveria bassiana against adults.

Table 3. Mortality of adults of Hadda beetle, Epilachna duodecastigma, after application of the entomopathogenic fungus *Beauveria bassiana*.

Treatment	Larval mortality after hours								Means
	24hrs	48hrs	72hrs	96hrs	120hrs	144 hrs	168 hrs	192 hrs	
T_1	8.0 (2) JKL	16.0 (4) HIJ	24.0 (6) GH	52.0 (13) E	64.0 (16) CD	80.0 (20)	92.0 (23)A	100.0 (25) A	54.50 (109) A
T_2	4.0 (1) KL	4.0 (1) KL	20.0 (5) GHI	28.0 (7) G	60.0 (15) D	72.0 (18) C	56.0 (14)DE	68.0 (17) C	33.50 (67) B
T_3	0.0 (0) L	0.0 (0) L	12.0 (3) IJK	16.0 (4) HIJ	36.0 (9) F	48.0 (12) E	40.0 (10)F	56.9 (14) DE	22.0 (44)C
T_4	0.0 (0) L	0.0 (0) L	4.0 (1) KL	4.0 (1) KL	120.0 (3) JK	16.0 (4) IJ	20.0 (5)GHI	20.9 (5) GHI	7.0 (14)D
Mean	3.0 (3) G	5.0 (5) G	15.0 (15) F	25.0 (25) E	46.0 (46) C	55.0 (55) B	61.0 (61)A	61.0 (61) A	-

Note:
1 = LSD for treatments at alpha 0.05 = 9.813
2 = LSD for timings at alpha 0.05 = 5.405
3 = LSD treatment x timings interaction at alpha 0.05 = 10.93
4 = Figures followed by the same letters are not significantly different from each other
T_1 = 0.8×10^4 spores/ml
T = 0.6×10^4 spores/ml
T_3 = 0.4×10^4 spores/ml; T_4 = Check

The figures in paranthesis indicate the original data.

The above table shows that like larvae and pupae the average effect of different doses differed significantly from each other. Mean percent mortality recorded after 192 hours of treatment was 54.50, 33.50, 22.0 and 7.0 in T_1, T_2, T_3 and T_4 respectively. In T_1 mortality of adults was very low even after 72 hours i.e. 24% which increased to 92% mortality occurred after 192 hours. In T_3 no mortality occurred upto 48 hours and only 12% mortality was recorded after 72 hours which very slowly increased and reached to 56% after 192 hours. In T_4 no mortality occurred till 48 hours but 20% occurred after 192 hours.

Conclusion

These studies showed that Hadda bettle *Epilachna duodecastigma* is suscieptible to Entomopathogenic fungus, *Beauveria bassiana* and the fungus could be utilized against the pest as the control agent.

References

Aguda, R. M. and M. C. Rombach. 1987. Bioassay of *Beauveria bassiana* and *Nomuraea rileyi* (Deutermycotina; Hypghomycetes) against the rice leaf folder (LF), International Rice Research Newsletter, 12(3): 36.

Badilla, F. F. and S. B. Alves. 1991. Control of Sugar cane Weevil Sphenophorus levis Vaurie, 1978 (Coleoptera; Curculionidae) with *Beauveria* spp. and the laboratory and field conditions, Directoria de Pesquisa Extensao, Apartado, San Jose, Costa Rica. Rev. Agr. Ent. 20 (2): 251-263.

Gopalakrishnan, C. and K. Narayanan. 1990. Studies on the dose-morality relationship between the entomofungal pathogen *Beauveria bassiana* (Bals), Vuillemin and Heliothis armigera Hub, Indian Institute of Horticultural Research (ICAR), Bangalore, India, Journal of Biological Control. 4(2): 112-115.

Guerin, A. 1986. A study of the efficacy of a preparation of *Beauveria bassiana* against the maize pyralid in the vienna Department. Colloques de LIRNA. 34: 141-147.

Mietkiewski, R. 1985. The mycoflora of dead larvae of the brown tail moth (*Euproctis chrysorrhoea* L.) during winter diapause, Roczniki Nak Roliczych, E. *(Orchrona Roslin)*; 15(1-2): 139-150.

Prestidge, R. A. and B. Willoughby. 1990. Control of the garden weevil (*Phyctinus callosus*) larvae and pupae with a parasitic nematode and the fungal pathogen, Rúakura Agricultural Centre, New Zealand. Rev. Agr. Ent. 63-66.

Rombach, M. C., R. M. Aguda, B. M. Shepard and D. W. Roberts. 1986. Entomopathogenic fungi (Deutermycotina) in the control of the back bug of rice, *Scotinophara coarctata* (Hemiptera; Pentatomidae), Jour. Invert. Path. 48(2): 174-179.