https://doi.org/10.17576/serangga-2024-2901-12

EFFECTIVE TREATMENT OF THE LEAF EATING CATERPILLAR, *Trilocha varians* (LEPIDOPTERA: BOMBYCIDAE) WITH *Beauveria bassiana* IN COMBINATION WITH AN INSECTICIDE UNDER LABORATORY CONDITIONS

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Received: 5 June 2023; Accepted: 1 February 2024

ABSTRACT

Ficus benjamina (Moraceae) commonly called fig, is an ornamental plant planted alongside the canal, roads and inside the parks and academic institutes to increase their beauty. The aesthetic value of *F. benjamina* is declining due to attack of leaf eating caterpillar, *Trilocha varians*. The current study was conducted under laboratory conditions to determine the effect of *Beauveria bassiana* with and without the combination of insecticide (emmamectin benzoate) on 2nd and 3rd larvae of *T. varians*. Complete larval mortality (100%) of *T. varians* was recorded at the highest (2×10^5) concentration of *Beauveria bassiana* without insecticide after seven days of post treatments but when the *B. bassiana* treatment was conducted together with insecticide, the result showed 100% mortality of the second larval instar just after six days. The second instar larvae were more susceptible to all concentrations of fungus with and without the combination with insecticides ($\chi^2_{1,40} = 952.99$, $P<0.001$). Even though both insecticide and fungus could become the best control for *T. varians*, it was proven that treatment with fungus together with insecticide showed faster effect. Hence, this finding should be tested under the field conditions in future.

Keywords: Entomopathogenic fungi, *Ficus benjamina*, insecticides, leaf eating caterpillar, Pakistan, *Trilocha varians,* weeping fig

ABSTRAK

Ficus benjamina (Moraceae) yang biasanya dipanggil ara, adalah tumbuhan hiasan yang ditanam di tepi terusan, jalan dan di dalam taman dan institusi akademik untuk meningkatkan keindahan permandangan. Nilai estetika *F. benjamina* semakin berkurangan akibat serangan ulat pemakan daun, *Trilocha varians*. Kajian dijalankan di dalam makmal untuk menentukan kesan *Beauveria bassiana* dengan dan tanpa kombinasi racun serangga (emmamectin benzoate) ke atas larva instar *T. varians* ke-2 dan ke-3. Kesemua larva *T. varians* merekodkan kematian (100%) pada kepekatan tertinggi (2×105) *Beauveria bassiana* tanpa racun serangga selepas tujuh hari rawatan tetapi apabila rawatan *B. bassiana* dijalankan bersama racun serangga, keputusan menunjukkan 100 % kematian larva instar kedua hanya selepas enam hari. Larva instar kedua lebih mudah terdedah kepada semua kepekatan kulat dengan dan tanpa kombinasi dengan racun serangga ($\chi^2_{1,40}$ = 952.99, *P<0.001*). Kedua-dua racun serangga dan kulat boleh menjadi kawalan terbaik untuk *T. varians* tetapi terbukti bahawa rawatan dengan kulat bersama racun serangga menunjukkan kesan yang lebih cepat. Oleh itu, penemuan ini harus diuji di dalam keadaan lapangan pada masa akan datang.

Kata kunci: Kulat entomopatogenik, *Ficus benjamina*, racun serangga, ulat pemakan daun, Pakistan, *Trilocha varians*, Beringin

INTRODUCTION

Genus *Ficus* is the large group of plant species with more than 800 species of vines, shrubs and trees. *Ficus benjamina*, *Ficus elastic, Ficus religiosa, Ficus microcarpa*, *Ficus benghalensis* and *Ficus infectoria* are the most important species grown in the world. Among these reported species, *F. benjamina* (Weeping fig), is planted alongside the park, road and academic institutes (school, colleges and universities) to increase their aesthetic value and adaptability against harsh environmental conditions. This is mostly planted in tropical and subtropical regions of the world including Pakistan, India and Malaysia (Basari et al. 2019; Kedar et al. 2014; Ramzan et al. 2020). This *Ficus* species not only planted as an ornamental plant but also have several chemicals which extracted and used for the treatment of several diseases especially ulcers occurred in many animals and humans (Chuenban et al. 2017; Lansky et al. 2008; Mousa et al. 1994; Ramzan et al. 2021).

Ficus benjamina is attacked by several sucking and chewing insect pests. Among chewing insect pests, leaf eating caterpillar, *Trilocha varians* (Lepidoptera: Bombycidae), is the most destructive and damaging pest of *F. benjamina,* spreading in the various countries like Pakistan (Ramzan et al. 2020) and Malaysia (Basari et al. 2019). During severe attack of pest, 100% defoliation of plant occur, decrease the beauty and aesthetic value of the areas (Basari et al. 2019; Ramzan et al. 2020).

There is the need to control this noxious pest especially in Pakistan because this pest is becoming a serious threat for the ornamental plants and may become primary pest of other horticultural and agricultural crops in the globe (Arya 2020; Lee et al. 2015; Mansoor et al. 2022; Naeem-Ullah et al. 2020; Ramzan et al. 2023). No management strategies have been adopted to control this emerging pests in the world except insecticidal studies conducted in Pakistan and Malaysia (Basari et al. 2019; Ramzan et al. 2020). To fill this need and gap, the recent study was conducted against larvae of *T. varians* using *Beauveria bassiana* fungus with and without the combination of insecticide (emmamectin benzoate) on 2^{nd} and 3^{rd} larvae of *T. varians*.

MATERIALS AND METHODS

Trilocha varians Rearing and Culture Maintenance

The rearing of *T. varians* was conducted at the Institute of Plant Protection, MNS-University of Agriculture, Multan, Pakistan under laboratory or favorable conditions *i.e.,* 26° C and 50–60% relative humidity (RH) up to five generations on *Ficus* species like *F. virens* and *F. benjamina.* The rearing of *T. varians* was done following the procedures put forward by Basari et al. (2019), Ramzan et al. (2021), Sajid et al. (2023), and Pervez et al. (2023). The larval instars were identified by referring to the physical characteristics as mentioned by Navasero & Navasero (2014) and Basari et al. (2019).

Fungal and Insecticide Sources

Beauveria bassiana fungus stock was obtained from University of Agriculture Faisalabad (UAF) and insecticide, Emmamectin benzoate; Runner (Ali Akbar Group) 1.9% EC was purchased from pesticide shop.

Spore Formation

Five concentrations of fungus (*B. bassiana*) mixed with distilled water were prepared (i.e., 2×10^1, 2×10^2, 2×10^3, 2×10^4 and 2×10^5 spores/ml). The spore concentrations were counted with the help of Neubauer Hemocytometer. The stock solution of *B. bassiana* was placed in the refrigerator until desired. In control experiment, only water was sprayed on the larvae.

Bioassay

To perform the bioassay study, second and third larval instars that reached near to molting stage were picked up with the help of fine forceps and placed into petri dishes containing moistened filter paper for the protection of larval damage. When the larvae were left in the petri dish until molted into second (L_2) and third (L_3) instar larvae. The larvae usually took around 24 hours to molt after they were transferred into the petri dish. Six treatments including control were conducted. Each treatment contains five replications and ten larvae were used in each replication. For the first experiment to determine the effect of fungus, *B. bassiana* without insecticide, larvae were dipped into a prepared solution of *B. bassiana* for 10s, kept in sterile Petri dishes (15cm diameter), and allowed to dry for 15 minutes. For the effect of fungus, *B. bassiana* with insecticide, the leaf dip method was used for insecticide bioassay. For the second treatment, the same procedures as the first treatment were followed but after 15 minutes of drying, the larvae were placed into petri plates containing leaves treated in different insecticide concentrations to check their combination effect as shown in Figure 1.

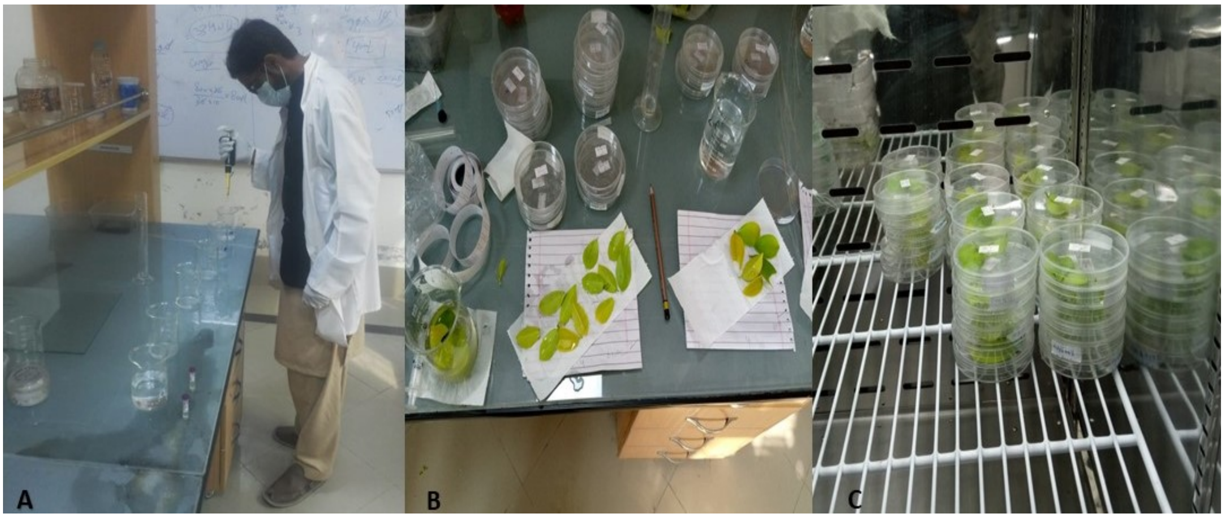

Figure 1. Preparation and application of insecticides (*B. bassiana* and insecticide) on *T. varians*. (A) preparation of stock solution, (B) indicates method of application (leaf dip) and C shows the treated larvae that placed in incubator for data recording

Data Collection and Data Analysis

Mortality data was recorded daily for 10 days of application. Dead larvae were removed from the treated culture and kept in other Petri dishes for developing mycosis to verify the death of larvae by fungus. The difference in the total mortality was analyzed using Chi-Square Test (SPSS, IBM v.25).

RESULTS

The study was conducted to determine the effect of *Beauveria bassiana* (BB) with and without the combination with emmamectin benzoate against the second and third larval instar of *T. varians* under laboratory conditions. The study revealed that the highest (2×10^5) concentration of conidial spores of *B. bassiana* without insecticide was proven to be more effective showing 100% mortality after 7 to 8 days of post treatments while 2×10^1 was proven to be least effective and showed only 49% mortality (Table 1).

Table 1. Percentage mortality (%) of second and third larvae of *T. varians* treated with *Beauveria bassiana* (BB)

Concentration	Larval Instars	Mortality (%) In Days Post Treatment									
		1	2	3	4	5	6	7	8	9	10
2×10^1 BB	L2	0	0	0	1	8	16	23	34	45	49
	L3	0	0	0	4	5	13	18	29	36	40
2×10^2 BB	L2	0	0	5	8	17	31	42	65	78	95
	L3	0	0	8	5	13	23	32	43	54	76
2×10^3 BB	L2	0	1	10	25	46	76	99	95	100	-
	L3	0	0	13	19	34	70	80	89	93	100
2×10^4 BB	L2	0	3	15	32	56	89	95	100	-	-
	L3	0	1	18	29	45	79	89	95	100	-
2×10^5 BB	L2	0	6	19	54	66	96	100	-	-	-
	L3	0	2	21	43	57	84	94	100	-	-
Control	L2	0	0	0	0	0	0	0	0	1	0
	L3	0	0	0	0	0	0	0	0	0	0

The current study also showed that *B. bassiana* caused less mortality against the third larval instar as compared to the second instars. The highest concentrations, 2×10^5, caused 100% and 94% mortality against the second and third larval instar after 7 days of post-treatment. The concentrations, 2×10^3 were also toxic and caused 100% and 95% mortality against second and third larval instar but only after 9 days of application. The mortality of larvae began after 24 hours of post-treatment.

The effect of the combination of emmamectin benzoate and *B. bassiana* is described in Table 2. The present study showed that the combination of insecticides and entomopathogenic fungi (EPF) was also effective against the 2nd and 3rd larval instars of *T. varians.* The 100% mortality of 2nd instar larvae was recorded in the combination of 2×10^5 and emmamectin benzoate after 6 days of application. It was observed that 100% mortality was recorded on the seventh, eighth, ninth, and tenth days of post-treatment for the three concentrations of EPF (2×10^5, $2\times10^{4,}$ and 2×10^3 spores/ml) applied together with insecticide. Moreover, 31% mortality for L_3 was also recorded in the lowest concentration of EPF with the insecticide (2×10^1+ emmamectin benzoate) on the eighth day of treatment (Table 2). In this study, results showed that the mortality percentage increased with the increase in concentration against L_2 and L_3. It was also recorded that the second instar larvae were more susceptible to all concentrations of fungus with and without the combination with insecticides ($\chi^2_{1,40} = 952.99$, $P<0.001$).

Table 2. Percentage mortality (%) of second and third larvae of *T. varians* with combine applications of *Beauveria bassiana* and emmamectin benzoate

Concentration	Larval Instars	Mortality (%) In Days Post Treatment									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
2×10^1 + emmamectin benzoate	L2	0	9	12	16	22	29	33	42	63	80
	L3	0	3	0	4	17	19	23	31	59	70
2×10^2 + emmamectin benzoate	L2	0	10	15	21	37	43	75	81	99	100
	L3	0	0	8	5	31	35	44	59	75	86
2×10^3 + emmamectin benzoate	L2	0	13	19	27	74	86	99	100	-	-
	L3	0	0	13	19	53	76	89	99	100	-
2×10^4 + emmamectin benzoate	L2	1	15	20	39	86	91	100	-	-	-
	L3	0	6	14	26	69	79	99	100	-	-
2×10^5 + emmamectin benzoate	L2	1	18	23	56	97	100	-	-	-	-
	L3	0	2	21	79	84	96	100	-	-	-
Control	L2	0	0	0	0	0	0	1	-	0	1
	L3	0	0	0	0	0	1	0	0	0	1

DISCUSSION

The microbial control is an effective and alternative method to manage the pest population that is applied all over the world (Alm et al. 1994; Bednarek et al. 2000; Benyakir et al. 1995; Sani et al. 2020). It can be considered an important tactic of Integrated Pest Management and improved the efficacy of other management approaches like insecticide. EPF are cheap method and ecofriendly that doesn't harm the natural enemies and risks of insect resistance (Roberts et al. 1991; Rosset & Moore 1997). It is also compatible with insecticides against insect pest populations (Inglis et al. 2001; Murtaza et al. 2020).

A study was conducted to determine the susceptibility of entomopathogenic fungus against different instars of silkworm, *B. mori* in Japan 2010. The study concluded that the second instar was found more susceptible at all conidial suspensions of fungus. The susceptibility difference was recorded between the strains (Wada et al. 2011) due to insect genes that showed susceptibility (Wang et al. 2005; Yamamoto et al. 2008). In various regions of the world, many studies have been conducted to investigate the entomopathogenic fungi such as *Beaveria bassiana* against *B. mori* larvae (Alves 1998; Kumari et al. 2011) and showed effective infestation on larvae but no such study reported on *T. varians*. Our laboratory experiments proved that treatment with fungus together with insecticide showed a faster effect and could become the best control for *T. varians*. Hence, this finding should be tested under field conditions in the future and check their effects on larval growth and development. Further studies are needed to check the fungi and insecticide effects on food consumption, relative consumption rate, efficiency of conversion of ingested food, efficiency of conversion of digested food, and relative growth rate values of *T. varians* under laboratory and field conditions. The EPF may be an alternative to synthetic pesticides to protect *F. benjamina* against *T. varians*. Entomopathogenic fungi are the eco-friendly and pollution-free biological approaches that have been tested and recommended by researchers and scientists throughout the globe (Murtaza et al. 2019, 2020).

CONCLUSION

The insecticides have been found very destructive in many aspects of life, causing pollution, and harmful to beneficial fauna like silkworms. The efficacy or toxicity of insecticides against silkworms has been checked by many early workers all over the world. They have recorded insect mortality, growth, development, food consumption rate, survival days, fertility, and fecundity of insects. Further studies are needed to control this pest on ornamental plants and protect the figs.

ACKNOWLEDGMENT

The authors are highly thankful to the concerned institutes.

AUTHOR DECLARATIONS

Funding Statement
This research received no specific grant from any funding agency.

Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Declarations
No ethical issue is required for this research.

Data Availability Statement
My manuscript has no associated data.

Authors' Contributions
MR and MH conceived this research and designed experiments; MY and SMFB participated in the design and interpretation of the data; MR performed experiments and analysis; MR and

NB wrote the paper and participated in the revisions. All authors read and approved the final manuscript after critical review.

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