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Evaluation of the entomopathogenic fungi as a non-traditional control of the rice leaf roller, *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae) under controlled conditions

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Abstract

The rice leaf roller or leaf folder, *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae), is a serious pest of rice crop in Pakistan. The present study was carried out to evaluate the virulence of the entomopathogenic fungi (EPF) *Beauveria bassiana*, *Verticillium lecanii*, and *Metarhizium anisopliae* against the third instar of *C. medinalis* larvae. Larvae were exposed to fungi under controlled conditions at the available commercial concentration (1×10^8 conidia ml^{-1}). The results showed 73.33, 57.78, and 74.44% mortality rates in the in vitro assay and 56.67, 41.11 and 52.78% in the greenhouse assay of *B. bassiana*, *V. lecanii*, and *M. anisopliae*, respectively. The maximum mycosis from cadavers of *C. medinalis* was observed at *B. bassiana* treatment in the in vitro assay (70%) and in the greenhouse assay (53.78%). The maximum sporulation from *C. medinalis* cadavers was observed at *B. bassiana* treatment in the in vitro assay (144.67 conidia ml^{-1}) and in greenhouse assay (96.67 conidia ml^{-1}). These results favor the alternative use of EPF in organic rice production for management of *C. medinalis*.

Keywords: *Cnaphalocrocis medinalis*, Entomopathogenic fungi, In vitro assay, Greenhouse assay, Virulence

Background

Rice, *Oryza sativa* L., is the second cash crop of Pakistan after cotton. It also ranks second after wheat in cereals in terms of area and plays a significant role in the economy of Pakistan (Sherawat et al. 2007). In Pakistan, the rice stem borers, rice plant hoppers, rice leaf rollers, and grasshoppers are key pests of the rice crop (Saleem et al. 2004). The rice leaf roller, *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae), gained the status of a major pest that may cause 30–40% leaf infestation and 20–30% yield losses to the rice crop (Haider et al. 2014 and Prakash et al. 2008).

There are effective insecticides available to cope with this pest, but this solution is not a long-term strategy because of apprehensions about health and environmental hazards, exposure risks, residual perseverance, and development of resistance (Natarajan and Ramaraju 1997). Therefore, in recent years, the focus has been shifted towards biological control. Earlier researches suggested the possibility of the successful use of entomopathogenic fungi (EPF) such as *Beauveria bassiana* (Sivasundaram et al. 2007 and Ullah et al. 2018). EPF such as *Metarhizium anisopliae* and *B. bassiana* have been effectively used for biological control of aphids, lepidopteran caterpillars, and other pests. These fungicides are valued tools for non-chemical pest management strategies. *M. anisopliae* and *B. bassiana* are active agents against different stages of insect pests (Sivasundaram et al. 2007).

This study was carried out to evaluate the efficacy of EPF against *C. medinalis* for possible use in an organic rice production.

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Materials and methods

Plant material and *C. medinalis* mass rearing

Fine rice Basmati-515 variety was used for the evaluation of EPF against *C. medinalis* at 28 ± 2 °C and 65 ± 5% RH, at the Rice Research Institute, Kala Shah Kaku, Punjab, Pakistan, during the year 2018. Larvae of *C. medinalis* were collected from grown rice plants in nursery trays, seedlings were transferred from the nursery after 40 days to pots (30 cm height and 9 cm diameter). Stock culture of the insect was maintained, following the method of Fujiyoshi et al. (1980), by releasing the newly emerged adults in ovipositional cages (120 × 80 × 50 cm). Moths were provided with a 10% honey solution as food and left for mating and egg laying. Ovipositional cages were observed daily till the hatched larvae reached the third instar (slightly dark green in color and a brownish patch on either side of pronotum) and then chosen for the bioassay tests. For the bioassay test, the third instar larvae were kept unfed for 3 h before each test, then transferred into Petri dishes, containing moistened filter paper at their bottom, to maintain the freshness and turgidity of clipped leaves.

Entomopathogenic fungi

Three fungi, *B. bassiana*, *V. lecanii*, and *M. anisopliae*, in talc forms, were obtained from AgriLife SOM Phytopharma (India) Limited (www.agrilife.in). All fungi were tested against *C. medinalis*, at a feasible conidial concentration (1×10^8 CFU/ml) (Dal Bello et al. 2018). The quality of the treatments was checked by a heamocytometer. Potato dextrose agar (PDA) was used to determine the conidial germination. The conidial germination was measured, based on the counts of 200 random conidia per plate, 18 h post-incubation at 25 ± 2 °C (Ayala--Zermeño et al. 2015).

Fungal pathogenicity against *C. medinalis* larvae

In vitro assay

Available concentration [1×10^8 colony-forming unit/gram (CFU/g)] of fungi was used in the laboratory in order to study the pathogenicity of the tested fungi against the third instar larvae of *C. medinalis*. Twenty larvae were used in each replication. The tested larvae were collected from the potted plants in vials, starved for 3 h, then dipped in each tested fungi solution at the chosen concentration for 10 s, as described by Negasi et al. (1998). Sterilized distilled water was used as a control treatment. Mortality counts of the larvae were recorded for 10 days (Riasat et al. 2011).

Greenhouse assay

A rice nursery was grown in plastic trays with 60 plugs in each. The nursery was transplanted in a greenhouse after 30 days. Four plants were grown at (22.5 cm row × row and plant × plant distance) and considered for each treatment replication. At the age of 60 days, the plants were sprayed by the help of a Pump Pressure Sprayer (Hommold, Lahore) with the fungal talc formulation. The third instar of *C. medinalis* larvae were collected, kept starved for 3 h, and then shifted on the treated rice plants. Three replications, of 12 larvae, were used in each case. The mortality rate of the larvae was recorded after 10 days. The cadavers of *C. medinalis* were used to record the mycosis percentage. The cadavers were collected and preserved in sterile Petri plates. The cadavers were washed three times in sterile distilled water, and then the surface was sterilized for 2–3 min by a 0.05% sodium hypochlorite solution. Then, these cadavers were placed on Sabouraud dextrose agar (SDA) plates and incubated at 25 ± 2 °C, 75 ± 5 RH for 7 days to observe the external white fungal growth under a stereomicroscope (Cole-Parmer 625 East Bunker Court Vernon Hills, IL, 60061, USA) (Riasat et al. 2011). Sporulation data were determined by mixing mycosed cadavers from each replicate in a beaker with a drop of Tween 80 with 20 ml of distilled water (Tefera and Pringle 2003). Treatments were replicated three times independently. The solution was carefully stirred and the number of conidia was counted by using a hemocytometer under the microscope (Riasat et al. 2011).

Statistical analysis

All statistical analyses were conducted using Statistix software (version 8.1) (Tallahassee, FL). One-way ANOVA was applied in CRD to understand the mortality of *C. medinalis* and mycosis and sporulation from cadavers of tested EPF in in vitro and greenhouse assays. The means were separated, using the Tukey's HSD test at $P = 0.05$.

Results and discussion

The pathogenicity of EPF, as a percent mortality ($F = 94.3$, DF = 3/11), percent mycosis ($F = 633$, DF = 3/11), and sporulation ($F = 426$, DF = 3/11), was highly significant ($P < 0.01$) in the in vitro assay, as well as in the greenhouse assay, where the percent mortality ($F = 312$, DF = 3/11), percent mycosis ($F = 469$, DF = 3/11), and sporulation ($F = 148$, DF = 3/11) were recorded.

In the in vitro assay, the maximum percent mortality of *C. medinalis* (74.44 ± 4.44%) was observed by *M. anisopliae*, while the minimum (57.78 ± 5.30%) was recorded in *V. lecanii*. The highest percent of mycosis from *C. medinalis* cadavers (70.00 ± 1.53%) was observed at *B. bassiana* treatment, while the lowest (61.33 ± 1.76%) was recorded in *M. anisopliae*. Also, the maximum sporulation from *C. medinalis* cadavers (144.67 ± 4.06 conidia ml^{-1}) was observed at *B. bassiana* treatment, while the minimum (133.33 ± 3.28 conidia ml^{-1}) was recorded in *M. anisopliae* (Table 1).

Table 1 Percentage mortality of the third instar of *Cnaphalocrosis medinalis* larvae and percentage of mycosis and sporulation from their cadavers assayed with the entomopathogenic fungi, *Beauvaria bessiana*, *Verticelium lecanii*, and *Metarhizium anisopliae* in in vitro

Entomopathogenic fungi	Percent mortality	Percent mycosis	Sporulation (conidia ml^{-1})
Beauveria bassiana	73.33 ± 0.96ab	70.00 ± 1.53a	144.67 ± 4.06a
Verticillium lecanii	57.78 ± 5.30b	65.67 ± 1.20ab	122.33 ± 3.48b
Metarhizium anisopliae	74.44 ± 4.44a	61.33 ± 1.76b	113.33 ± 3.28b
Control	2.22 ± 0.56c	0.00 ± 0.00c	0.00 ± 0.00c

Means with different lowercase letters are significantly different (Tukey HSD at $P = 0.05$)

In the greenhouse assay, the maximum percent mortality of *C. medinalis* (56.67 ± 1.67%) was observed at *B. bassiana* treatment, while the minimum (41.11 ± 1.47%) was recorded in *V. lecanii*. the maximum percent mycosis from cadavers of *C. medinalis* (53.78 ± 0.87%) was showed at *B. bassiana* treatment, while the minimum (39.78 ± 1.56%) was recorded in *V. lecanii*. The highest sporulation from *C. medinalis* cadavers (96.67 ± 4.26 conidia ml^{-1}) was observed at *B. bassiana* treatment, while the lowest (68.33 ± 2.85 conidia ml^{-1}) was recorded by *V. lecanii* (Table 2).

There was a clear percent mean mortality difference between in vitro and greenhouse assays. It could be due to continuous favorable and controlled conditions for fungal activity and pathogenicity in the case of the in vitro assay than in the greenhouse. Moreover, the larvae of *C. medinalis* were directly dipped in an EPF solution in the in vitro assay, while they were just released on sprayed plants in the case of the greenhouse assay.

Obtained data showed that microbial insecticides have shown promising results against *C. medinalis* larvae even the less percent mortality under greenhouse conditions is still satisfactory. Present data is in accordance with the findings of Alice et al. (2003) and Ambethgar et al. (2007) who reported that the *B. bassiana* was the most efficient for biological control of *C. naphalocrocis* under controlled conditions. Padmaja and Kaur (2001) and Ambethgar (2003) also recorded successful trials using *B. bassiana* against *C. medinalis* larvae.

Table 2 Percentage mortality of the third instar of *Cnaphalocrosis medinalis* larvae and percentage of mycosis and sporulation from their cadavers assayed with the entomopathogenic fungi, *Beauvaria bessiana*, *Verticelium lecanii*, and *Metarhizium anisopliae* in a greenhouse

Entomopathogenic fungi	Percent mortality	Percent mycosis	Sporulation (conidia ml^{-1})
Beauveria bassiana	56.67 ± 1.67a	53.78 ± 0.87a	96.67 ± 4.26a
Verticillium lecanii	41.11 ± 1.47b	39.78 ± 1.56b	84.67 ± 2.85a
Metarhizium anisopliae	52.78 ± 1.11a	43.56 ± 1.25b	68.33 ± 4.91b
Control	7.22 ± 0.56c	0.00 ± 0.00c	0.00 ± 0.00c

Means with different lowercase letters are significantly different (Tukey HSD at $P = 0.05$)

EPF are one of the bio-control agents that are recommended in IPM strategies (Feng et al. 2004). The talc-based formulation of beneficial microbes was found to be effective and cheap for pest and disease management in different crops (Saravanakumar et al. 2007). The fungal species against *C. medinalis* were documented earlier (Padmaja and Kaur 2001; Ambethgar et al. 2007; and Sivasundaram et al. 2007).

The difference of efficacy among the tested three fungi under controlled and greenhouse conditions may be due to environmental abiotic factors of temperature (°C) and relative humidity (RH), which influence the pathogenicity of fungi. High temperature reduces the germination of conidia and subsequently the efficacy of EPF (Sun et al. 2003). Ouedraogo et al. (1997) reported that 25 °C was an optimum temperature for *M. anisopliae*. As well, the relative humidity affects the fungal growth as reported by Michalaki et al. (2006) that fungal growth is maximum at 51–74% RH.

Conclusion

EPF are among the alternative tools beside the chemicals for controlling *C. medinalis*. Recently, the studies, regarding EPF, have gained special considerations against insect pests. However, further studies are required to evaluate their efficacy and compatibility against *C. medinalis* under field conditions in various ecological zones of Pakistan.

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Authors' contributions
MR and BA designed the study and wrote the manuscript with input from all authors. AMS analyzed the data. MY and AQ read and approved the final manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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