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First record of the entomopathogenic fungus, *Nomuraea rileyi* (Farlow) Samson on *Diaphania caesalis* (Walker) (Lepidoptera: Crambidae) from India

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ABSTRACT

The entomopathogenic fungus, *Nomuraea rileyi* (Farlow) Samson was isolated from the cadavers of *Diaphania caesalis* (Walker) collected from the jackfruit field of ICAR_IIHR, Bengaluru. This detection represents the first report of *N. rileyi*on *D. caesalis*. Pathogenicity of *N. rileyi* isolate against different larval instars of *D. caesalis*was studied by topical application of spores at concentrations of $10^8$and 10^9conidia per ml. Early larval instars were more susceptible, which decreased significantly as the larval age advanced.

Key words: Natural enemies, Biological control, Bioassay, Pathogenicity

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INTRODUCTION

Diaphania caesalis Walker (Crambidae: Lepidoptera) is one of the most destructive jackfruit pests (Karim, 1995; Tandon, 1998; Soumya *et al.,* 2020). It has been reported from various Indian states, causing a fruit infestation of 27.44% (Khan and Islam, 2004). *Soumya et al.* (2015) reported an outbreak of *D. caesalis* in two border districts of Kerala, i.e., Kasaragod and Palakkad, indicated its invasiveness in other parts of India. The pests of jackfruit should be effectively contained to enhance jackfruit production (Sidhu, 2012). An increase in pest problems also leads to the indiscriminate use of pesticides, causing resurgence of many insect pests.

Biological control of insect pests is one of the most important components of Integrated Pest Management (IPM), wherein entomopathogens such as bacteria, viruses and fungi are exploited against insect pests. Several entomopathogens have been employed against different crop pests throughout the world. Microbe's role including fungi, virus and bacteria in removing the pest's population has received worldwide attention (Basavaraj *et al.,* 2007). Entomopathogens can provide an alternative and more environment-friendly approach for controlling pests due to their specificity, mode of action and ease of application.

Ignoffo (1981) studied the insecticidal activity of *Nomuraea rileyi* (Farlow) Samson on lepidopteran pests and found that the fungus was a candidate for further development as a microbial insecticide. Unlike bacteria or viruses, fungi infect insects by breaching the host cuticle. Entomopathogenic fungi also possess added advantage over other microbial control agents as they are capable of attacking all developmental stages of insects, including pupal stages (Anand *et al.,* 2009; Ferron, 1978).

Knowing the primary biocontrol agents (parasitoids, predators and pathogens) of a pest is the first step in any biocontrol programme and the most important component of IPM. Although parasitoids of *D. caesalis* was recorded earlier (Soumya *et al.,* 2019), no entomopathogen has been recorded so far on *D. caesalis*. The present study was conducted to bridge that knowledge gap and identify the entomopathogens of *D. caesalis* and evaluate their relative importance. The findings will also helpto control *D. caesalis* in other parts of the world.

MATERIALS AND METHODS

Surveys were conducted for the entomopathogens of *D. caesalis* in jackfruit orchards of ICAR-IIHR (Indian Council of Agricultural Research – Indian Institute of Horticultural Research), Bengaluru (12° 8′N; 77° 35′E), India during 2013-16. *Diaphania caesalis* larvae were collected based on the appearance of external symptoms like inactive without feeding or dead compared to the typical appearance of the pest. Such larvae were collected in clean screw cap tubes (to avoid contamination) and brought to the laboratory.

Isolation and Identification of *N. rileyi*

The dead larvae collected from the field were surface sterilized by immersing in 0.5 % $HgCl_2$ for two minutes, followed by rinsing in three changes of sterile water. The insects showing fungal infection symptoms (which had mycelial growth) were placed on a clear grease free glass slide in a sterile Petri plate. A moist tissue paper was placed within the lid of the Petri plate and the entire set up was kept undisturbed for about a week at room temperature to check for dehiscence. Meanwhile, Potato Dextrose Agar (PDA) media was prepared in conical flasks, plugged with cotton and sterilized at 121°C 15LBS for 15 minutes. On cooling, the media was poured into sterile Petri plates. The dehiscent fungal spores were collected in an inoculation loop and streaked on the PDA media inside the laminar airflow. The inoculated plates were incubated for 4-7 days for the mycelial mat and spore formation. The colonies were subcultured again to get a single colony for further identification, characterization and bioassay.

The isolates that came up on the PDA medium were identified as *N. rileyi* by microscopic examination according to the outlines given by Samson *et al.* (1988) and maintained as a pure culture. The microscopic examination revealed that the vegetative hyphae of *N. rileyi* appeared smooth septae, hyaline and conidiophores were erect. Branches formed near a septum were developed in whorls, each giving rise to 2 to 4 phialides. Conidia were single, smooth ellipsoidal.

Preparation of Fungal Culture Isolate

100 g of rice (substrate) was washed well and soaked for 2 - 3 hrs before starting the experiments. Excess water was drained by decanting and shade drying it for half an hour to remove the excess moisture further. The substrate was autoclaved at 121°C 15LBS for 15 minutes in individual 250 ml conical flasks plugged with cotton wool. After cooling, 1 ml of the spore suspension of the fungal pathogen was inoculated separately into each flask. All these procedures were done under a laminar airflow chamber. They were incubated in BOD incubator at 28°C for 15-days. After 7 days of inoculation to avoid clumping the flasks were shaken vigorously to separate the substrate and to break the mycelial mat. After 15-days of incubation, a 10 g homogenous substrate sample drawn from each replicates uniformly sporulating bottle/flasks was transferred to 100 ml sterilized distilled water containing Tween 80 (0.05%) solutions in 250 mL conical flasks. The flasks were shaken in a mechanical shaker for 10 min. The suspension was filtered through a double layered muslin cloth.

Bioassays of *N. riley*

Insecticidal activity of *N. rileyi* isolates on *D. Caesalis* was studied through larval bioassays. Spore suspensions of *N. rileyi* at 10^8 and 10^9 conidia/mL was topically applied on all five instars of *D. caesalis*. Controls consisted of larvae applied with 500 µL of distilled water. The *D.caesalis* larvae were released on the jackfruit leaves in anylon mesh cage under *invitro* condition. The *N. rileyi* spore suspension was sprayed using a hand sprayer. Five replications of each concentration were maintained. The first spray was carried out in the morning at 09.00AM. The second and third sprays were performed at five days intervals. Observations were made on the number dead larvae at 24 hurs interval till 72 hurs after each spray.

Statistical analysis

The mortality of *D. caesalis*in different doses (10^8 and 10^9) was analyzed using the Student t-test. The data on *D. caesalis* mortality in the laboratory during the screening of different biopesticides was subjected to One-way

Analysis of Variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) test for multiple comparisons at $P < 0.05$ (SPSS Inc. 21).

RESULTS AND DISCUSSION

Pathogenicity level

N. rileyi was found to be pathogenic against all the larval instars of *D. caesalis*. The percentage mortality of different larval instars of *D. caesalis* topically applied with the spore suspension of *N. rileyi* @10^8, and 10^9 Conidia/mL are presented in table 1 and table 2 respectively. The results of both bioassays revealed a gradual decrease in the percent mortality of *D. caesalis* as the larval age progressed.

The percentage mortality of first instar *D. caesalis* larva @ 10^8 Conidia/mL after 96 hours of application was significantly higher than that of 72 and 48 hours after application. Whereas in case of second, third, fourth and fifth larval instars the results after 72 and 48 hours were at par and significantly higher than 48 hours after application. The lowest mortality of 25.85 per cent was recorded in 5th instar after 48 hours of application.

The percentage mortality of first instar larval stages of *D. caesalis* @ 10^9 Conidia/mL after 96 and 72 hours was significantly higher than 48 hours after application. Whereas in case of second, third, fourth and fifth larval instars the results after 96 hours of application were significantly higher than 72 hours and 48 hours after application. The highest mortality of 100 per cent was recorded in 1st instar after 96 hours of application

Comparison of pathogenicity at 10^9 and 10^8 Conidia/mL against *D. caesalis*

The effect of different concentrations of *N. rileyi* 10^9 and 10^8 Conidia/ml screened against *D. caesalis* in the laboratory is given in figure 1. The average percent mortality of the pest is significantly higher in *N. rileyi* at 10^9 in all the larval instars than 10^8. The average mortality of *D. caesalis* was significantly lesser in control when compared to all other treatments.

Biological control by entomopathogenic fungi is important for reducing the population density of pests in IPM programs. Therefore, the preservation of entomopathogens that occur naturally or introduced for insect control should be adopted (Oliveira *et al.,* 2003). Besides, we must understand the compatibility of entomopathogenic fungi with other crop production techniques such as insecticides which may inhibit to a smaller or larger extent the development and reproduction of pathogen (Malo, 1993; Rogerio *et al.,* 2005). In this context, this study provides useful information about a fungal biological control agent against *D. caesalis*.

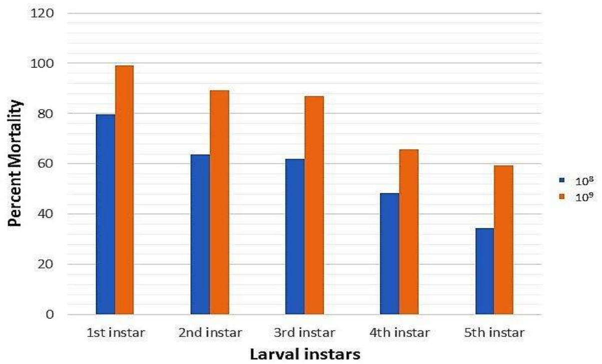

Fig. 1. Pathogenicity of *Nomuraea rileyi* (at 10^9 and 10^8 Conidia/ml) on*Diaphania caesalis*

In the present study, *N. rileyi* was highly infective to early instars of *D. caesalis* than later instars. The present finding conforms with Manjula and Krishna Murthy (2005), who reported that the highest larval mortality of 91.2 per cent was observed in the first instar of *S. litura* and 95 per cent in the second instar of *H. armigera* at the concentration of 1 x 10^9 of *N. rileyi* spores/mL. Vimala Devi and Prasad (2001) conducted field studies and found that *N. rileyi* was effective against *S. litura*as a foliar spray and soil application. Likewise, Sridhar and Prasad (1996) recorded up to 36.9 per cent infection of *N. rileyi* on *S. litura* in groundnut fields in Andhra Pradesh. *N. rileyi* was a key natural mortality factor of *S. litura* populations in coastal Andhra Pradesh (Sridhar and Prasad,1996). Vimala Devi *et al.* (2002) reported *S. Litura* larval mortality at 2 x 10^8*N. rileyi* spores mL/ l on castor. Navi *et al.* (2006) reported that *N. rileyi* caused more *D. caesalis* mortality under field conditions.

The mortality of different *D. caesalis* larval instars due to *N. rileyi* infection indicate that the level of infection is dependent on the age of the larvae. The larval mortality was highest in the first instar and least in the ultimate instar after 96 hours of infection. Our results conform with the findings of many workers (Ignoffo *et al.,* 1975; Garcia and Ignoffo, 1979; Ross and Brady, 1985; Silva *et al.,* 1993;Gopalkrishnan and Mohan, 1991; Tang and Hou,1998 and Kulkarni, 1999). As *N. rileyi* is found to be effective against *D. caesalis*, it can be used in IPM of the pest.

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