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Research Note

Evaluation of *Metarhizium* (*Nomuraea*) *rileyi* (Farlow) Samson isolates against *Spodoptera litura* F. under *in vitro* conditions

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ABSTRACT: Fifteen isolates of *Metarhizium* (*Nomuraea*) *rileyi* collected from field populations of *Spodoptera litura* (F.) and *Spodoptera frugiperda* J. E. Smith in different crops across different agroclimatic zones of Andhra Pradesh were evaluated for their pathogenicity against third instar larvae of *S. litura* at the Biocontrol Laboratory, Regional Agricultural Research Station, Tirupati, India. Virulence was tested by applying conidial suspension @ $1x10^7$ spores ml^{-1} on the groundnut leaves on which the larvae were allowed to feed. The cumulative mean larval mortality to the extent of 75.00 to 85.00% was observed with isolates viz.Nr.Yj.KZ, Nr.Dp.KZ, Nr.Rt.KZ, Nr.Rd.HATZ, Nr.Pp.HATZ and Nr.Cp.HATZ whereas the isolates Nr.Ik.SRZ, Nr.Vg.SRZ, Nr.Pl.SZ, Nr.Ag.SZ, Nr.Np.SRZ, Nr.Rp.SZ, Nr.Rg.NCZ, Nr.Hv.SRZ and Nr.Cd.SZ recorded 65.00 to 73.33% mortality.

KEY WORDS: Agroclimatic zones, larval mortality, *Metarhizium rileyi,* pathogenicity, *Spodoptera litura*

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The *Spodoptera litura* (Fabricius) commonly known as tobacco caterpillar, is a serious insect pest which can infest over 290 plant species in different parts of the world including India, Japan, China and other countries of Southeast Asia. In India, *S. litura* causes 26-100% yield losses in groundnut at peak incidence, by infesting new tender shoots, new flushes in the crop plants during the cropping season (Dhir *et al*., 1992). Insecticides for control of *S. litura* have become ineffective because of the development of resistance and cause toxic residues in the crops. (Rajan and Muthukrishnan, 2009).

Concerns about the negative effects of chemical insecticides have led to emphasis on alternative strategies for pest control. Pest management involving biocontrol agents is assuming prominence and has been considered as an important and safe strategy in insect population reduction. Among the several micro-organisms viz., bacteria, fungi, virus, protozoans and nematodes, entomopathogenic fungi fills an extremely important niche for control of insect pests. They are often reported to cause high level of epizootics in nature and are the most versatile biological control agent and environmentally safe. They have great potential for integrated pest management programs due to their specificity, mode of action by infecting the insects through cuticle, amenability for easy mass production and ease of application. *Metarhizium* (*Nomuraea*) *rileyi* is an important entomopathogenic fungus which induces epizootics in several lepidopteran larvae throughout the world. The present study was taken up to evaluate the *M. rileyi* isolates collected from survey to study their pathogenicity levels against the third instar larvae of *S. litura.*

Culturing of *Spodoptera litura* in the laboratory

Studies were carried out at the Department of Entomology, Regional Agricultural Research Station, Tirupati. Egg masses of *S. litura* were collected from groundnut and castor fields at Regional Agricultural Research Station, Tirupati. The leaves with egg masses of *S. litura* after collection were placed in sterilized plastic boxes along with additional tender groundnut/castor leaves. Fresh leaves were fed to the larvae daily after cleaning the boxes by removing dried leaves and excreta of larvae. The pupae were placed in a broad Petriplate lid and they were placed in rearing cages. In the rearing cage, after adult emergence, conical flasks of 500 ml capacity containing groundnut twigs/castor leaves with water were placed. Cotton swab dipped in 10% honey solution was placed in a Petriplate and kept in the corner of

rearing cage. After egg laying on the leaves and twigs, the egg masses were collected into rearing containers every day and were reared on castor leaves under aseptic conditions to get disease free larvae. The hatched larvae were provided with fresh castor leaves every day. Freshly moulted third instar larvae were selected for laboratory studies.

Bioassay of *Metarhizium rileyi* isolates

Fifteen *M. rileyi* isolates used to test their pathogenicity against the third instar larvae of *S. litura* were 1) Nr.Rg.NCZ, 2) Nr.Cp.HATZ, 3) Nr.Rd.HATZ, 4) Nr.Pp.HATZ, 5) Nr.Dp. KZ, 6) Nr.Rt.KZ,7) Nr.Yj.KZ, 8) Nr.Hv.SRZ, 9) Nr.Ik.SRZ, 10) Nr.Np.SRZ, 11) Nr.Vg.SRZ,12) Nr.Cd.SZ, 13) Nr.Pl.SZ, 14) Nr.Rp.SZ and 15) Nr.Ag.SZ. The isolates were collected from North coastal zone, High altitude tribal zone, Krishna zone, scarce rainfall zone and Southern zone of Andhra Pradesh, India. The isolates were identified based on spore and culture morphology.

In a 100 ml capacity conical flask, 50 ml of distilled water was taken with 0.1% Tween-20. The spores of fifteen *M. rileyi* isolates from culture plates grown on Sabouraud Maltose Agar + Yeast medium were harvested with the help of fine brush by pouring little quantity of prepared water into the Petriplate. After thorough mixing with glass rod, the suspension was filtered through muslin cloth into a separate beaker or conical flask. The spore count was estimated by using the Improved Neubaeur Haemocytometer under Research microscope (OLYMPUS BX41). The concentration of was 1 x 10^7 spores ml^{-1}.

Groundnut leaves were cleaned with brush. In sterilized petriplates, the leaves were placed and the suspension of *M. rileyi* isolate was applied with fine hair brush/atomizer. The leaves were allowed to air dry. Ten third instar larvae of uniform size (newly moulted) were selected and released onto leaves in the petriplates to crawl and feed. Like this, for fifteen *M. rileyi* isolates, the procedure was done. An untreated control treatment was maintained. All the sixteen treatments were replicated thrice and conducted twice as test-I and test-II. From the next day of the treatment, fresh leaves were fed as food material. Daily observations on incubation period, post treatment changes in larvae, larval mortality were recorded. The experiment was conducted in the laboratory at 25 ±2 °C temperature. The pathogenicity tests were conducted as test-I and test-II with an interval of one month.

The larval mortality was converted to Per cent values and then transformed to arc-sine values before subjecting to statistical analysis through SPSS (V 20). Means were separated by DMRT. The larval mortality was expressed as per cent larval mortality by using the formula.

Changes observed in *Metarhizium rileyi* infected larvae before mortality

At two days after treatment the infected *S. litura* larvae observed to become sluggish and consumed less amount of food. On nearing to the death, the larvae stopped feeding and reduction in body weight with fragile shrivelled integument was observed and documented.

Changes observed after death of larvae

A few hours after death, the cadavers became stiff with bulged segmentation. The cadaver surface was covered with white mycelial threads in about 2-3 days after death. In next 2-4 days the cadaver surface was appeared in malachite green color due to development of conidiophores and conidia and found cadavers adhered to the leaves. The stiffness of the cadaver after the death may be due to the maximum growth stage of fungus inside the body. However, the toxins might have also been produced by the fungus inside the body.

Per cent mortality of third instar larvae (Test-I)

The results indicated that in the test-I mean percent larval mortality of 73.33-86.67 were observed in isolates Nr.Cp.HATZ, Nr.Yj.KZ, Nr.Dp.KZ, Nr.Rt.KZ, Nr.Rd.HATZ and Nr.Pp. HATZ. The isolates Nr.Ag.SZ, Nr.Rp.SZ, Nr.Pl.SZ, Nr.Vg. SRZ, Nr.Ik.SRZ, Nr.Cd.SZ, Nr.Np.SRZ, Nr.Hv.SRZ and Nr.Rg. NCZ showed 63.33-73.33% larval mortality. No mortality was recorded in untreated control treatment (Table 1).

Per cent mortality of third instar larvae (Test-II)

The results of the test-II revealed that the isolates Nr.Yj.KZ, Nr.Dp.KZ, Nr.Rt.KZ, Nr.Pp.HATZ, Nr.Rd.HATZ and Nr.Cp.HATZ had shown 76.67-83.33 per cent larval mortality. The mean per cent larval mortality of 66.67-73.33 was observed in the isolates Nr.Ik.SRZ, Nr.Rp.SZ, Nr.Vg. SRZ, Nr.Hv.SRZ, Nr.Rg.NCZ, Nr.Ag.SZ, Nr.Pl.SZ, Nr.Cd. SZ and Nr.Np.SRZ. The larval mortality was not noticed in untreated control (Table 1).

Mean per cent mortality of third instar larvae (Average of Test-I and Test-II)

Pathogenicity of *M. rileyi* isolates (mean of test-I and test-II) revealed that larval mortality of 75.00-85.00% was observed in isolates Nr.Yj.KZ, Nr.Dp.KZ, Nr.Rt.KZ, Nr.Rd. HATZ, Nr.Pp.HATZ and Nr.Cp.HATZ. Isolates Nr.Ik.SRZ, Nr.Vg.SRZ, Nr.Np.SRZ, Nr.Rp.SZ, Nr.Rg.NCZ, Nr.Hv.SRZ, Nr.Cd.SZ, Nr.Ag.SZ and Nr.Pl.SZ recorded 65.00-73.33% mortality (Table 1).

Table 1. Pathogenicity of *Metarhizium rileyi* isolates to third instar *Spodoptera litura*

S.No	Isolate	Host insect (Crop)	larval mortality (Test-I)	larval mortality (Test-II)	Overall Mean per cent larval mortality
1	*Nr.*Rg.NCZ	*S. litura* (blackgram)	70.00^{cde} (56.79)	70.00^{bc} (56.79)	70.00^{a} (56.79)
2	*Nr.*Cp.HATZ	*S. frugiperda* (maize)	73.33^{bcde} (59.00)	76.67^{abc} (61.22)	75.00^{cde} (60.11)
3	*Nr.*Rd. HATZ	*S. litura* (maize)	80.00^{abc} (63.43)	80.00^{ab} (63.43)	80.00^{abc} (63.43)
4	*Nr.*Pp. HATZ	*S. frugiperda* (maize)	76.67^{abcd} (61.22)	80.00^{ab} (63.43)	78.33^{bcd} (62.33)
5	*Nr.*Dp.KZ	*S. litura* (groundnut)	86.67^{a} (68.86)	83.33^{a} (66.14)	85.00^{a} (67.50)
6	*Nr.*Rt. KZ	*S. litura* (blackgram)	83.33^{ab} (66.14)	80.00^{ab} (63.43)	81.67^{ab} (64.79)
7	*Nr.*Yj.KZ	*S. litura* (blackgram)	86.67^{a} (68.86)	83.33^{a} (66.14)	85.00^{a} (67.50)
8	*Nr.*Hv.SRZ	*S. frugiperda* (maize)	70.00^{cde} (56.79)	70.00^{bc} (56.79)	70.00^{efg} (56.79)
9	*Nr.*Ik.SRZ	*S. frugiperda* (maize)	73.33^{bcde} (59.00)	73.33^{bc} (59.00)	73.33^{cdef} (59.00)
10	*Nr.*Np. SRZ	*S. frugiperda* (maize)	70.00^{cde} (56.79)	66.67^{c} (54.78)	68.33^{efg} (55.79)
11	*Nr.*Vg.SRZ	*S. frugiperda* (maize)	73.33^{bcde} (59.00)	70.00^{bc} (56.79)	71.67^{defg} (57.90)
12	*Nr.*Cd.SZ	*S. frugiperda* (maize)	70.00^{cde} (56.79)	66.67^{c} (54.78)	68.33^{efg} (55.79)
13	*Nr.*Pl.SZ	*S. litura* (groundnut)	63.33^{e} (52.78)	66.67^{c} (54.78)	65.00^{g} (53.78)
14	*Nr.*Rp.SZ	*S. frugiperda* (maize)	66.67^{de} (54.78)	70.00bc (56.79)	68.33^{efg} (55.79)
15	*Nr.*Ag.SZ	*S. litura* (chilli)	66.67^{de} (54.78)	66.67^{c} (54.78)	66.67^{fg} (54.78)
16	Untreated control		0.00^{f} (0.00)	0.00^{d} (0.00)	0.00^{h} (0.00)

*Per cent larval mortality is the mean of three replications
*Values in parentheses are arc sine transformed values
*Values followed by same letter are not significantly different as per DMRT

The incubation periods of *M. rileyi* on the larva varied from 5 to 7 days. In the test-I the isolates had recorded 63.33 to 86.67%, in the test-II the isolates had recorded 66.67 to 83.33% larval mortality where as in the cumulative test the isolates had recorded 65.00 to 85.00% larval mortality showing that all the isolates were active regarding pathogenicity. All the isolates have recorded greater than 63% larval mortality.

In the test-I, test-II and in the mean percent mortality, higher mean percent larval mortality were observed in isolates Nr.Yj.KZ and Nr.Rt.KZ isolated from *S. litura* on blackgram crop and Nr.Dp.KZ isolated from *S. litura* on groundnut in Krishna zone, Nr.Rd.HATZ isolated from *S. litura* on groundnut in High altitude tribal zone. The isolates were followed by Nr.Pp.HATZ and Nr.Cp.HATZ isolated from *S. frugiperda* on maize in High altitude tribal zone, Nr.Ik. SRZ and Nr.Vg.SRZ isolated from *S. frugiperda* on maize in Scarce rainfall zone, Nr.Rg.NCZ isolated from *S. litura* on blackgram crop in North coastal zone. They were followed by Nr.Hv.SRZ and Nr.Np.SRZ isolated from *S. frugiperda* in scarce rainfall zone, Nr.Rp.SZ and Nr.Cd.SZ isolated from *S. frugiperda* on maize in Southern zone, Nr.Pl.SZ isolated from *S. litura* on groundnut in southern zone and Nr.Ag.SZ isolated from *S. litura* on chillies. Variations observed in *M. rileyi* isolates regarding the mean larval per cent mortality may be due to difference in virulence and capacity of *M.*

rileyi isolates which were collected from host insects, crops and agroclimatic zones.

From the results, it was observed that comparatively higher mean percent larval mortality was observed in Nr.Yj. KZ, Nr.Dp.KZ and Nr.Rt.KZ *M. rileyi* isolates which were collected from Krishna Zone from host insect *S. litura* on blackgram and groundnut which would have more virulence of *M. rileyi* due to the crops grown, weather factors that causes regular infections to insects, every season. Considerably lower mortality was observed in Nr.Pl.SZ and Nr.Ag.SZ *M. rileyi* isolates collected from Southern Zone from host insect, *S. litura* on groundnut and chillies, respectively which would have comparatively less virulence of *M. rileyi*.

The present findings are in line with the results of Gopalakrishan (2000) who evaluated three isolates of *M. rileyi* (IHR-NR, AN-NR and PU-NR) and found all the isolates were equally effective against *S. litura*. Ramanujam *et al.* (2003) reported that *M (N). rileyi* isolates showed the maximum mean mortality of 54.44%, 76.66% in case of *Helicoverpa armigera* and *S. litura,* respectively. Among the three *M (N). rileyi* isolates *Nr-3* caused the highest mortality for both the insects.

Devi *et al*. (2003) studied efficacy of 11 geographical isolates of the *M. rileyi* against the two host insects *H. armigera* and *S. litura*. Laboratory bioassays concentration of 2×10^8 conidia ml^{-1} indicated that *M (N).rileyi* isolates of *S. litura* origin were better in terms of mortality in both the test larvae. In the case of *S. litura* 77–80% mortality in 7 days whereas mortality in case of *H. armigera* was 79–85% in 8 days. Among the isolates geographical isolates from Hyderabad and Karimnagar of *S. litura* origin were found superior in terms of high per cent kill as well as 100% germination of conidia within 48 hours. Indrayani *et al*. (2013) conducted a study at the Insect Pathology Laboratory of the Indonesian Sweetener and Fiber Crops Research Institute (ISFCRI), Malang, East Java, from January to December 2011, to determine pathogenicities of two local isolates of *M. rileyi* to *H. armigera*. The ranges of percentage of *H. armigera* larval mortality due to infections by isolates ML01and LG2 were 51.1-85.56 per cent and 43.36-78.90 per cent, respectively.

Krutmuang and Thungrabeab (2017) tested eight strains of *M. rileyi* for control of *S. litura* and recorded the larval mortality ranged from 2.5 to 92.5% at 7 days after inoculation and documented two strains, BCC 14653 and BCC 14671 as highly virulent. Padanad and Krishnaraj (2009) isolated entomopathogenic fungus *M. rileyi* from *S. litura* and *H. armigera* insect cadavers collected from different sampling sites and evaluated its pathogenicity against *S. litura* by topical application of 10^8 conidia/ml concentration suspension. All ten isolates of *M. rileyi* were active against third instar *S.litura*, resulting in 85 to 97% mortality.

CONCLUSION

Pathogenicity tests of *M (N). rileyi* isolates collected from surveys, indicated that the considerably higher mean per cent larval mortality was observed in Nr.Yj.KZ, Nr.Dp.KZ and Nr.Rt.KZ isolates. Comparatively lower larval mortality was observed in Nr.Cd.SZ and Nr.Pl.SZ.

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