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Evaluation of *Metarhizium rileyi* Farlow (Samson) impregnated with azadirachtin and indoxacarb against *Helicoverpa armigera* (Hubner)

Bhisham Dev, Subhash Chander Verma, Prem Lal Sharma, Rajeshwar Singh Chandel, Mahesh Balaso Gaikwad, Tanuja Banshtu and Priyanka Sharma[*]

Abstract

Background: Entomopathogenic fungi are the most versatile having a wide host range, capable of infecting insects at different developmental stages. In the present study, *Metarhizium rileyi,* at the concentrations of 10^2, 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 conidia/ml and sub-lethal concentrations of azadirachtin (1.02 and 1.53 ppm) and indoxacarb (0.72 ppm) were evaluated against the 1st, 2nd, 3rd, 4th and 5th larval instars of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) *under* laboratory conditions.

Results: *M. rileyi* applied at 10^6 conidia/ml caused a maximum mortality of 83.33 and 80.00% of 1st and 2nd larval instars of *H. armigera,* respectively. The maximum mortality of 3rd, 4th and 5th larval instars of *H. armigera* with 10^8 conidia/ml of *M. rileyi* was 83.33, 76.67 and 53.33%, respectively. When *M. rileyi* blended with azadirachtin at 1.02 ppm, the highest mortality rate of 86.21% at 10^6 conidia/ml against 2nd instar larvae was resulted. Similarly, *M. rileyi* applied at 10^8 conidia /ml mixed with azadirachtin (1.53 ppm) showed 89.66% mortality of 3rd instar larvae. The 2nd instar larvae treated with *M. rileyi* at 10^6 conidia/ml, mixed with indoxacarb (0.72 ppm), the corrected mortality rate was 82.14%. Concentration mortality response of 3rd instar larvae to *M. rileyi* blended with indoxacarb (0.72 ppm) was 85.71% at 10^8 conidia/ml. The median lethal concentration (LC_{50}) values were 5.51×10^3, 1.86×10^4, 2.81×10^5 and 5.55×10^5 conidia/ml for 1st, 2nd, 3rd and 4th larval instars, respectively, after 7 days of treatment. *M. rileyi* when mixed with sub-lethal concentrations of azadirachtin (1.02 ppm) and indoxacarb (0.72 ppm) resulted LC_{50} values of 1.09×10^4 conidia/ml and 1.37×10^4 conidia/ml against 2nd instar larvae, respectively, after 24 hours. Similarly, *M. rileyi* mixed with sub-lethal concentrations of azadirachtin (1.53 ppm) and indoxacarb (0.72 ppm) resulted LC_{50} values of 3.12×10^8 and 3.06×10^5 conidia/ml against 3rd instar larvae, respectively, after 24 hours. The study revealed that the susceptibility of larvae decreased in case of large larval instars.

Conclusions: *M. rileyi* can be utilized as one of the component of Integrated Pest Management Program for the eco-friendly management of *H. armigera*. As the application of *M. rileyi* @ 10^7 conidia/ml alone or in combination with azadirachtin (1.02 and 1.53 ppm) or indoxacarb (0.72 ppm) resulted to the highest mortality.

Keywords: *Helicoverpa armigera*, Entomopathogenic fungus, *Metarhizium rileyi*, Azadirachtin, Indoxacarb, Evaluation

Background

The noctuid moth, *Helicoverpa armigera* (Hubner) is a cosmopolitan widely distributed crop pest and damage more than 200 plant species belonging to greater

*Correspondence: ps366502@gmail.com
Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan, HP 173230, India

Dev *et al. Egyptian Journal of Biological Pest Control*   (2021) 31:142

Page 2 of 7

than 47 families (Bird 2017). *H. armigera* has a high fecundity, high rate of fertility, high dispersal rate, long distance movement, overlapping generations per year under tropical and subtropical conditions, respectively, and resistance development against insecticides (Jones et al. 2019). For the management of this noctuid pest farmers mainly used synthetic insecticides, Excessive use of chemical insecticides to control the pest has led to development of pest resistance, pest resurgence, killing of natural enemies, environmental pollution besides being costly. Therefore, there is a need of development of alternative tools. Entomopathogenic fungi (EPF) are an alternative to chemical pesticides which is ecofriendly, safe to non-target organisms and prevent pesticides resistance (Leahy et al. 2014). EPF are the most versatile due to their wide host range, capable of infecting insects at different developmental stages and ability to penetrate through the host cuticle (Vega et al. 2012). *Metarhizium rileyi* is a dimorphic hypomycete fungus and initially named as *Botrytis rileyi* (Farlow) and later on described as *Spicaria rileyi* (Farlow) Charles. Kish et al. (1974) re-described the fungus and kept in the genus, *Nomuraea.* According to Boucias et al. (2000), *N. rileyi* isolates were more closely related to *Metarhizium anisopliae* and *M. flavoviride* than to *N. atypicola* and *N. anemonoides. Metarhizium* spp. have been extensively exploited because it is ecofriendly and easy to mass produce (Greenfield et al. 2015). *Metarhizium* genus was originally comprised of four species, which were *M. anisopliae, M. taii, M. pingshaense* and *M. guizhouense. N. rileyi* isolates were closely related to *M. anisopliae* and *M. flavoviride* than to *N. atypicola* and *N. anemonoides.* Based on morphological and molecular characterization, *N. rileyi* has been changed to *M. rileyi* (Kepler et al. 2014). It is observed that sometimes farmers spray the crop with insecticides alone or in combination with EPF for the management of the pests. Therefore, it is necessitated to know the action of synthetic chemical insecticides in combination with the *M. rileyi* and determine their compatibility. Many authors have conducted the studies on the combination of pesticides with EPF (Kachhadiya et al. 2014). On the other hand, Ignoffo et al. (1975) reported that several chemical products applied in soybean crop inhibited growth as well as virulence of *N. rileyi.* The information on combined action of sub-lethal concentrations of synthetic chemical pesticides and *M. rileyi* is scanty. Therefore, the aim of present study was to evaluate the susceptibility of *H. armigera* larvae to *M. rileyi* incorporated with sub-lethal concentrations of azadirachtin and indoxacarb under laboratory conditions.

Methods
Rearing of insect culture
The culture of *H. armigera* was raised in in vitro (25 ± 0.5 °C, $70 \pm 5\%$ RH and 14L:10D photoperiod) conditions from caterpillars collected from the field on chickpea crop. The larvae were reared individually in rearing trays on chickpea sprouts. Larval food was changed daily or as per requirement until pupation. Pupae obtained were transferred to glass jars for adult emergence. Adults on emergence were shifted to rearing cages for mating and egg laying. Adults were provided with 30% honey solution (in cotton swabs) as food and strips of filter paper as substrate for egg lying. The insect was reared for 2 generations before using in experimentations.

Rearing of culture of *M. rileyi* and treatment of *H. armigera*
The nucleus culture of *M. rileyi* was obtained from National Bureau of Agricultural Insect Resources (NBAIR) Bangaluru and further multiplied on SDAY (Sabouraud dextrose agar + yeast extract medium). Newly inoculated slants were incubated at 25 ± 0.5 °C and $70 \pm 5\%$ RH. *M. rileyi* was evaluated against 1st, 2nd, 3rd, 4th and 5th larval instars of *H. armigera.* Harvesting of conidia was carried out from 15-days-old well sporulated culture in tubes by pouring 10 ml sterilized emulsified (0.5% Tween 80) distilled water in each tube. The concentration of conidia in the suspension was determined by a Neubauer Hemocytometer and further adjusted the conidial suspension of 10^8 or 10^7 conidia/ml depending upon the harvested. Conidial suspension thus obtained was serially diluted in 1:9 ratio with sterilized emulsified distilled water to get test concentrations of 10^6, 10^5, 10^4, 10^3 and 10^2 conidia/ml. For the combinations, different concentrations of azadirachtin and indoxacarb were fortified with the conidial suspension and larvae of *H. armigera* where treated by larval dip method for 10 s, and data were recorded after 24 h of treatment upto 7 days.

Data analysis
Mortality data were subjected to probit analysis as per Finney (1952). The mortality data falls in the range of 20–80% were subjected to probit analysis, and LC_{50}/LC_{90} values were calculated by IBM SPSS Statistics 20.

Results
Concentration mortality response of different larval instars
M. rileyi tested at the concentrations of 10^2, 10^3, 10^4, 10^5, 10^6, 10^7 conidia/ml against 1st instar larvae of *H. armigera* showed that the corrected mortality was maximum (96.67%) at 10^7 conidia/ml and minimum

Dev *et al. Egyptian Journal of Biological Pest Control*   (2021) 31:142

Page 3 of 7

(20%) at 10^2 conidia/ml. Similar trend was observed with 2nd instar larvae of *H. armigera* where 93.33% mortality was recorded at 10^7 conidia/ml and 16.67% at 10^2 conidia/ml. However, *M. rileyi* at the concentration of 10^2 conidia/ml no mortality of 3rd instar larvae of *H. armigera* was observed. The maximum (83.33%) mortality rate of 3rd instar larvae was recorded at 10^8 conidia/ml whereas the minimum (20%) was recorded at 10^3 conidia/ml. Similarly, the applied concentration of *M. rileyi* at 10^3 conidia/ml resulted 16.76% mortality rate of 4th instar larvae of *H. armigera,* whereas at 10^8 conidia/ml the mortality was 76.67%. The results also showed that with the advancement of larval instars mortality decreased even at high concentration. Conidial concentration of 10^8 and 10^2 conidia/ml resulted 53.33 and 13.33% mortality rate on the 5th instar *H. armigera* larvae (Table 1).

In case of 1st instar larvae of *H. armigera,* the concentration and % mortality were directly proportional with LC$_{50}$ of 5.51×10^3 conidia/ml (95% fiducial limits: 1.65×10^3 and 1.62×10^4 conidia/ml) and LC$_{90}$ of 2.86×10^6 conidia/ml (95% fiducial limits: 4.93×10^5 and 8.04×10^7 conidia/ml) on 7 DAT (Day After Treatment). Probit kill had linear relationship at log concentration ($Y = 0.49X - 1.76$), χ^2 showed that the data were homogenous at $p = 0.05$. For 2nd instar larvae, the LC$_{50}$ value of 1.86×10^4 conidia/ml with fiducial limits (95%) of 5.85×10^3 and 6.87×10^4 conidia/ml was calculated whereas, LC$_{90}$ was 1.56×10^7 conidia/ml (95% fiducial limits: 1.79×10^6 and 1.27×10^9 conidia/ml). The χ^2 showed that the data were homogenous as the χ^2_{cal} (0.25)

was less as compared to χ^2_{tab} (7.81) at 5% level of significance and 4 degrees of freedom. The regression equation of probit kill (Y) was linear dependent on log concentrations (X) i.e., $Y = 0.43X - 1.87$. Similarly, for 3rd instar larvae, Probit kill had linear relationship with log concentration as $Y = 0.35X - 1.97$. χ^2—test showed homogeneity of data (χ^2_{cal}: 0.17, $\chi^2_{(tab)}$: 9.48 at 5 df). The median lethal concentration (LC$_{50}$) was 2.81×10^5 conidia/ml with fiducial limits of 6.77×10^4 and 1.72×10^9 conidia/ml after 7 days of treatment. The concentration of *M. rileyi* to kill 90% of larvae (LC$_{90}$) was 1.72×10^4 conidia/ml with fiducial limits of 1.37×10^8 and 2.53×10^{11} conidia/ml. In 4th instar larvae, the mortality due to fungus and concentration were directly proportional with LC$_{50}$ of 5.55×10^5 conidia/ml (fiducial limits: 1.44×10^5 and 2.35×10^6 conidia/ml) and LC$_{90}$ of 2.87×10^9 (fiducial limits: 2.19×10^8 and 4.41×10^{11} conidia/ml), whereas, regression equation of Probit kill (Y) on log concentration (X) was $Y = 0.35X - 1.97$, χ^2 test showed that the data were homogeneous as χ^2_{cal} (0.17) was quite less than χ^2_{tab} (9.48) at 5% level of significance and 4 degree of freedom (Table 2).

Concentration mortality response of 2nd and 3rd larval instars when *M. rileyi* fortified with azadirachtin (1.02 and 1.53 ppm)

When *M. rileyi* blended with azadirachtin at 1.02 ppm applied at concentrations of 10^2, 10^3, 10^4, 10^5 and 10^6 conidia/ml against 2nd instar larvae of *H. armigera* resulted corrected mortality of 10.34, 27.59, 44.83, 68.97 and 86.21%, respectively, after 7 days of treatment

Table 1 Concentration mortality response of *Metarhizium rileyi* to different larval instars of *Helicoverpa armigera* after 7 days of treatment

Larval instars	Conidial suspension/ml							Control
	10^8	10^7	10^6	10^5	10^4	10^3	10^2	
1st	−	−	83.33	76.67	53.33	36.67	20.00	0
2nd	−	−	80.00	60.00	43.33	30.00	16.67	0
3rd	83.33	66.67	56.67	43.33	33.33	20.00	−	0
4th	76.67	66.67	56.67	40.00	26.67	16.67	−	0
5th	53.33	46.66	36.66	23.22	20.00	13.33	−	0

Table 2 Pathogenicity parameters of *Metarhizium rileyi* to larval instars of *Helicoverpa armigera* after 7 days of treatment

Larval instars	LC$_{50}$	95% fiducial limits (LC$_{50}$)	LC$_{90}$	95% fiducial limits (LC$_{90}$)	Regression equation	χ^2 calculated	χ^2 tabulated
1st	5.51×10^3	1.65×10^3 and 1.62×10^4	2.86×10^6	4.93×10^5 and 8.04×10^7	$Y = 0.49X - 1.76$	0.44	7.81
2nd	1.86×10^4	5.85×10^3 and 6.87×10^4	1.56×10^7	1.79×10^6 and 1.27×10^9	$Y = 0.43X - 1.87$	0.25	7.81
3rd	2.81×10^5	6.77×10^4 and 1.11×10^6	1.72×10^9	1.37×10^8 and 2.53×10^{11}	$Y = 0.33X - 1.84$	0.38	9.48
4th	5.55×10^5	1.44×10^5 and 2.35×10^6	2.87×10^9	2.19×10^8 and 4.41×10^{11}	$Y = 0.35X - 1.97$	0.17	9.48

Table 3 Concentration mortality response of 2nd and 3rd larval instars of *Helicoverpa armigera* to *Metarhizium rileyi* incorporated with azadirachtin (1.02 and 1.53 ppm)

Azadirachtin conc (ppm)	Larval instars	Conidial suspension/ml							Control (water)
		10^8	10^7	10^6	10^5	10^4	10^3	10^2	
		Larval mortality (%)							
1.02	2nd	–	–	86.21	68.97	44.83	27.59	10.34	0
1.53	3rd	89.66	72.41	51.72	37.93	2759	13.79	–	0

Table 4 Pathogenicity parameters of *Metarhizium rileyi* incorporated with azadirachtin to 2nd and 3rd larval instars of *Helicoverpa armigera* at 7 days of treatments

Parameters	Azadirachtin (1.02 ppm)	Azadirachtin (1.53 ppm)
	2nd instar	3rd-instar
Reg. equation	$Y = 0.54X - 2.21$	$Y = 0.42X - 2.29$
χ^2 calculated	0.12	1.12
χ^2 tabulated	7.81	9.48
LC_{50} (conidia/ml)	1.09×10^4	2.79×10^3
Fiducial limits (conidia/ml)	4.10×10^3 and 2.93×10^4	8.83×10^4 and 8.80×10^5
LC_{90} (conidia/ml)	2.39×10^6	3.12×10^8
Fiducial limits (Conidia/ml)	5.13×10^5 and 3.62×10^7	4.73×10^7 and 8.27×10^9

(Table 3). The median concentration of fungus to kill % population (LC_{50}) was 1.09×10^4 conidia/ml with 95% fiducial limits of 4.10×10^3 and 2.93×10^4 conidia/ml, and concentration to kill 90% population (LC_{90}) was 2.39×10^6 conidia/ml with 95% fiducial limits of 5.13×10^5 and 3.62×10^7 conidia/ml. χ^2 test proved that data were homogenous as χ^2_{cal} (0.12) was less than χ^2_{tab} (7.81) at 5% level of significance and 3 degree of freedom. The Probit kill was linearly related with log concentration; $Y = 0.54X - 2.21$ (Table 4).

Similarly, *M. rileyi* at the concentrations of 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 conidia/ml mixed with azadirachtin (1.53 ppm) showed 13.79, 27.59, 37.93, 51.72, 72.41 and 89.66% corrected mortality rates, after 7 days of treatments (Table 3). Concentration to kill 50 and 90% of the treated larvae were 2.79×10^3 conidia/ml (fiducial limits: 8.83×10^4 and 8.80×10^5 conidia/ml) and 3.12×10^8 conidia/ml (fiducial limits: 4.73×10^7 and 8.27×10^9 conidia/ml). Probit kill followed a straight line curve with a log concentration $Y = 0.42X - 2.29$, and the data were homogenous as χ^2_{cal} (1.12) was quite less than χ^2_{tab} (9.48) at 5% level of significance and 4 degree of freedom (Table 4).

Effect of azadirachtin and indoxacarb on growth of *M. rileyi*

M. rileyi was tested against both azadirachtin and indoxacarb at tested concentrations and founded

Table 5 Effect of azadirachtin and indoxacarb on the growth of *Metarhizium rileyi*

Treatment	Conc. (ppm)	Mean growth (cm²) and inhibition (%) over control	
		Growth	Inhibition
Azadirachtin	1.02	1.54	47.31
	1.53	1.09	62.47
Indoxacarb	0.72	0.80	72.39
Control	–	2.92	–
CD ($p = 0.05$)	–	0.29	–

that they inhibited the growth of fungus over control (Table 5). Maximum growth (1.54 cm²) was obtained on media mixed with azadirachtin (1.02 ppm), whereas mean radial growth of *M. rileyi* recorded on culture mixed with azadirachtin (1.53 ppm) + indoxacarb (0.72 ppm) was 1.09 and 0.80 cm², respectively, as compared to 2.92 cm² in control. Indoxacarb at 0.72 ppm resulted in the maximum inhibition (72.39%) of the fungus, followed by azadirachtin 1.53 ppm (62.47%) and azadirachtin 1.02 ppm (47.31%).

Dev *et al. Egyptian Journal of Biological Pest Control*   (2021) 31:142

Page 5 of 7

Table 6 Concentration mortality response of 2nd and 3rd larval instars of *Helicoverpa armigera* to *Metarhizium rileyi* incorporated with indoxacarb (0.72 ppm)

Larval instar	Conidial suspension/ml							Control (water)
	10^8	10^7	10^6	10^5	10^4	10^3	10^2	
	Larval mortality (%)							
2nd	–	–	82.14	64.29	46.43	32.14	14.29	0
3rd	85.71	67.86	50.00	32.14	25.00	17.86	–	0

Concentration mortality response of 2nd and 3rd larval instars when *M. rileyi* fortified with indoxacarb (0.72 ppm)

Data contained in Table 6 revealed that when 2nd instar larvae of *H. armigera* was treated by *M. rileyi* at 10^2, 10^3, 10^4, 10^5 and 10^6 conidia/ml mixed with indoxacarb (0.72 ppm), the corrected mortality was calculated as 14.29, 32.14, 46.43, 64.29 and 82.14, respectively, after 7 days of treatments. After subjecting the data to probit analysis, LC_{50} was 1.37×10^4 conidia/ml (fiducial limits: 4.62×10^3 and 4.35×10^4 conidia/ml), and LC_{90} was 6.73×10^6 conidia/ml (fiducial limits: 1.02×10^6 and 2.46×10^8 conidia/ml). The χ^2 showed that the data were homogenous at 5% level of significance and 3 degrees of freedom, since the χ^2_{cal} (0.20) was less than χ^2_{tab} (7.81), and probit kill had the linear relationship with log concentration; $Y = 0.47X - 1.97$ (Table 7).

Concentration mortality response of 3rd instar larvae of *H. armigera* to *M. rileyi* blended with indoxacarb (0.72 ppm) revealed that at the concentrations of 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 conidia /ml resulted corrected mortality of 17.86, 25, 32.14, 50, 67.86 and 85.71%, respectively, after 7 days of treatments (Table 7). The concentrations to kill 50 and 90% of the treated larvae were 3.06×10^5 conidia/ml (fiducial limts: 8.14×10^4 and 1.16×10^6 conidia/ml) and 1.11×10^9 conidia/ml (fiducial limits: 1.07×10^8 and 9.35×10^{10} conidia/ml, respectively. χ^2 test proved that data were homogeneous (the $\chi^2_{cal} = 1.65$; $\chi^2_{tab} = 9.48$) at 5% level of significance and 4

degree of freedom. Linear regression equation of probit mortality on log concentration was $Y = 0.36X - 1.97$ (Table 7).

Discussion

In the present study, high mortality rate of the early instars of *H. armigera* may be due to fragile and thin cuticle of the larvae which was easy for the germ tube of conidia to penetrate, germinate and caused mycelium growth. The present findings were in agreement with the findings of Manjula and Krishnamurthy (2005) who found mortality of 80–95% at 1st and 2nd larval instars of *H. armigera* at the concentration of 10^7 conidia/ml of *M. rileyi*. Similar to the present findings Gundannavar et al. (2008) recorded 100 and 97.50% mortality of the 1st instar larvae due to *M. rileyi,* at the concentration of 10^8 conidia/ml and 10^7 conidia/ml, respectively, whereas, a mortality of 95% at the concentration of 10^8 conidia/ml of *M. rileyi* was recorded with the 2nd instar larvae. In the present study, *M. rileyi* killed 83.33% of 3rd instar larvae of *H. armigera* at concentration of 10^8 conidia/ml. These findings were in line with findings of Gundannavar et al. (2008) who reported 82.50% mortality at 10^8 conidia/ml. Similar, to present findings, Padanad and Krishnaraj (2009) reported that *M. rileyi* isolates tested against 3rd instar larvae of *S. litura* caused mortality in the range of 85–97%. *M. rileyi* @ 10^8 conidia/ml caused 76.67% mortality to the 4th instar larvae of *H. armigera*.

Table 7 Pathogenicity parameters of *Metarhizium rileyi* incorporated with indoxacarb (0.72 ppm) to 2nd and 3rd larval instars of *Helicoverpa armigera* at 7 days of treatment

Parameters	2nd instar	3rd instar
Reg. equation	$Y = 0.47X - 1.97$	$Y = 0.36X - 1.97$
χ^2 calculated	0.20	1.65
χ^2 tabulated	7.81	9.48
LC_{50} (conidia/ml)	1.37×10^4	3.06×10^5
Fiducial limits (conidia/ml)	4.62×10^3 and 4.35×10^4	8.14×10^4 and 1.16×10^6
LC_{90} (conidia/ml)	6.73×10^6	1.11×10^9
Fiducial limits (conidia/ml)	1.02×10^6 and 2.46×10^8	1.07×10^8 and 9.35×10^{10}

Dev *et al. Egyptian Journal of Biological Pest Control*   (2021) 31:142

Page 6 of 7

These findings were in accordance with the findings of Gundannavar et al. (2008) who recorded 75% mortality at same concentration. *M. rileyi* caused 53.33% mortality on the 5th instar *H. armigera* larvae, at concentration of 10^8 conidia/ml. The lowest mortality to the 5th instar larvae than early instar may be due to thick cuticle of the oldest instar larvae, which makes it difficult for the fungus to penetrate, germinate, and form mycelial growth and kill the larvae. Similar to present findings, Namasivayam and Arvind (2015) reported that the LC_{50} values increased as the larvae grew older. As the instars advanced, a decrease in mortality was recorded. The present study was in agreement with the study of Patil et al. (2014) who noticed that early instars were highly susceptible with a mortality of 70.17% and mortality decreased significantly with the increase in age of the larvae. The present findings also corroborate the findings of Gundannavar et al. (2008) who reported 47.50% mortality at 10^8 conidia/ml. Whereas, Mohamed et al. (1978) observed a high mortality (63%) at a concentration of 10^9 conidia/ml *M. rileyi*. In the present investigations, *M. rileyi* mixed with azadirachtin and indoxacarb separately enhanced the lethal effect of *M. rileyi*. The increase in the efficacy of the *M. rileyi* in the presence of azadirachtin and indoxacarb may be due to increased susceptibility of larvae. *M. rileyi* with indoxacarb (0.72 ppm) showed better performance than *M. rileyi* with azadiracthin (1.02 ppm) against 2nd instar larvae of *H. armigera*. The superiority of indoxacarb over azadirachtin may be due to more stress exhibited to the larvae. *M. rileyi* with azadirachtin (1.53 ppm) resulted to a slightly high mortality than *M. rileyi* mixed with indoxacarb (0.72 ppm) to the 3rd instar larvae of *H. armigera* might be due to interference of neem (azadirachtin) with insect development and formation of cuticle or the molting process (Rembold 1989). According to Zimmermann (1994), if new cuticle formation was affected in term of deposition, hardening and tanning it will reduce the barricading ability to fungus, thus the chance of mycosis might increase.

Conclusions

Susceptibility of larvae decreased with the increase in larval instars of *H. armigera*. *M. rileyi* impregnated with azadirachtin (1.02 and 1.53 ppm) and indoxacarb (0.72 ppm) inhibited the growth of *M. rileyi* but increased the lethal effect against *H. armigera*. Thus, it can be concluded that either *M. rileyi* at 10^7 conidia/ml alone or impregnated with azadirachtin (1.02 and 1.53 ppm) or indoxacarb (0.72 ppm) resulted almost equal mortality to the larvae of *H. armigera*. Hence, *M. rileyi* can be utilized as one of the components of IPM program for the eco-friendly management of *H. armigera*.

References
Bird LJ (2017) Genetics, cross-resistance and synergism of indoxacarb resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae). Pest Manag Sci 73:575–581

Boucias DG, Tigano MS, Sosa-Gomez DR, Glare TR, Inglis PW (2000) Genotypic properties of the entomopathogenic fungus *Nomuraea rileyi*. Biol Control 19:124–138

Finney DJ (1952) Probit analysis: a statistical treatment of the sigmoid response curve. Cambridge University Press, Cambridge, p 331

Greenfield BP, Peace A, Evans H, Dudley E, Ansari MA, Butt TM (2015) Identification of *Metarhizium* strains highly efficacious against *Aedes, Anopheles* and *Culex* larvae. Biocontrol Sci Technol 25:487–502

Gundannavar KP, Lingappa S, Giraddi RS, Kulkarni KA (2008) Susceptibility of *Helicoverpa armigera* (Hübner) to *Nomuraea rileyi* (Farlow) Samson. J Entomol Res 32:11–13

Ignoffo CM, Hostetter DL, Garcia C, Pinnel RE (1975) Sensitivity of the entomopathogenic fungus *Nomuraea rileyi* to chemicals pesticides used on soybeans. Environ Entomol 4:765–768

Jones CM, Parry H, Tay WT, Reynolds DR, Chapman JW (2019) Movement ecology of pest *Helicoverpa*: implications for ongoing spread. Annu Rev Entomol 64:277–295

Kachhadiya NM, Kapadia MN, Jethva D (2014) Bio-efficacy of *Nomuraea rileyi* (Farlow) samson alone and in combination with insecticides against *Spodoptera litura* (Fabricius) on groundnut under laboratory condition. Pestology 38:19–21

Kepler RM, Humber RA, Bischoff JF, Rehner SA (2014) Clarification of generic "ans" species boundaries for *Metarhizium* and related fungi through multigene phylogenetics. Mycologia 106:824

Kish LP, Samson RA, Allen GE (1974) The genus *Nomuraea* Maublanc. J Invertebr Pathol 24:154–158

Leahy J, Mendelsohn M, Kough J, Jones R, Berckes N (2014) Biopesticide oversight and registration at the US Environmental Protection Agency. In: Gross AD, Coats JR, Duke SO, Seiber JN (eds) Biopesticides: state of the art and future opportunities. ACS Publications, Washington, pp 3–18

Manjula K, Krishnamurthy KVM (2005) Efficacy of Nomuraea rileyi against different instars of Spodoptera litura and Helicoverpa armigera. Ann Plant Prot Sci 13:347–350

Mohamed AKA, Bell JV, Sikorowski PP (1978) Field cage tests with *Nomuraea rileyi* against corn earworm larvae on sweet corn. J Econ Entomol 71:102–104

Namasivayam SKR, Arvind BRS (2015) Biocontrol potential of entomopathogenic fungi *Nomuraea rileyi* (f.) Samson against major groundnut defoliator *Spodoptera litura* (F.) (Lepidoptera; Noctuidae). Adv Plants Agric Res 2:221–225

Padanad MS, Krishnaraj PU (2009) Pathogenicity of native entomopathogenic fungus *Nomuraea rileyi* against *Spodoptera litura*. Plant Health Prog 10:1–9

Patil RK, Bhagat YS, Halappal B, Bhat RS (2014) Evaluation of entomopathogenic fungus, *Nomuraea rileyi* (Farlow) samson for the control of

groundnut *Spodoptera litura* (F.) and its compatibility with synthetic and botanical pesticides. J Biopest 7:106–115

Rembold H (1989) Azadirachtins: their structure and mode of action. In: Arnason J (eds) Insecticides of plant origin, symposium series of American Chemical Society, Washington, DC, vol 380, pp 150–167

Vega FE, Meyling NV, Luangsa-ard JJ, Blackwell M (2012) Fungal entomopathogens. Insect Pathol 2:171–220

Zimmermann G (1994) Strategies for the utilization of entomopathogenic fungi. In: Proceedings, VIth international colloquium on invertebrate pathology and microbial control. Montpellier, France. Society for Invertebrate Pathology, pp 67–73

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