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BIO–EFFICACY OF ENTOMOGENOUS FUNGUS, *NOMURAEA RILEYI* (FARLOW) SAMSON AGAINST *HELICOVERPA ARMIGERA* (HÜBNER)

B.L. TANDI

Department of Entomology, Agricultural Research Station, Durgapura, Jaipur 302018
Swami Keshwanand Rajasthan Agriculture University, Bikaner, Rajasthan, India
Email: banwarilaltandi@gmail.com

ABSTRACT

Effectiveness of entomopathogenic fungus, *Nomuraea rileyi* was tested against II and IV instar larvae of *Helicoverpa armigera* on chickpea by two methods of application *i.e.* leaf dip and leaf spray under laboratory conditions. It caused 85 per cent mortality of II instar larvae within 5 days and 75 per cent mortality of IV instar larvae within 10 days, when applied at 5×10^8 conidia/ml to chickpea leaves by dipping method of application. The LD_{50} values of fungus computed for II instar larvae were 8.06×10^6 conidia/ml with dip method and 2.42×10^7 conidia/ml with spray method, 4 days after the treatment. However, 3 to 4 times more conidial suspension (2.68×10^7 and 1.09×10^8) of the fungus is required to kill 50 per cent population of IV instar larvae, after 7 days of treatment. The LT_{50} of II instar larvae with the dose of 5×10^6 conidia/ml were 4.38 and 5.42 days with dip and spray methods, respectively. However, LT_{50} of IV instar larvae with dip and spray methods with the dose of 5×10^7 conidia/ml were 7.18 and 8.84 days, respectively.

Key words: *Nomuraea rileyi*, *Helicoverpa armigera,* chickpea, mortality, suspension

INTRODUCTION

American bollworm, *Helicoverpa armigera* (Hübner) is a serious pest of cotton, legumes and vegetables in the Indian sub–continent and South–east Asia (King, 1994). Continuous use of chemical insecticides in insect pest management resulted in adverse effects like development of resistance, pest resurgence and pollution to environment. Thus, there is a need for alternative methods of pest control like microbial control.

Microorganisms like viruses, bacteria, fungi, nematodes and protozoa, infecting insect pests and causing epizootic, are of great importance for the management of pests. *Nomuraea rileyi* is an important entomopathogenic fungus (Vimla Devi 1994 and Tang and Roger 1998). In view of this, a study was carried out to evaluate the effectiveness of *N. rileyi* on *H. armigera*.

MATERIALS AND METHODS

A study was carried out to evaluate the effectiveness of *Nomuraea rileyi* against II and IV instar larvae of *Helicoverpa armigera* by two methods (leaf dipping and leaf spraying) in the laboratory of Department of Entomology, Agriculture Research Station, Durgapura, Jaipur.

The culture of *N. rileyi* was procured from Senior Pathologist, Directorate of Oilseeds Research, Rajender Nagar, Hyderabad. The conidial mass was harvested from the culture tubes and suspended in sterilized distilled water containing 0.05 per cent emulsifier (Tween 80). The strength of the stock conidial suspension was adjusted to 5×10^8 conidia/ml. From this suspension, further dilutions were made with serial dilution. Five concentrations *viz.,* 5×10^8, 5×10^7, 5×10^6, 5×10^5 and 5×10^4 conidia/ml were prepared to find their efficacy. The fungal concentration 5×10^4 conidia/ml was not evaluated against IV instar larvae.

PVC containers of size 65×55 mm were disinfected with 1% formalin solution and left over night for drying. Dried containers were used for maintaining individually II and IV instar larvae along with the treated food.

For the experimentation the tender twigs of chickpea plants from the field were brought to laboratory in polythene bags. They were washed under ordinary tap water. The washed twigs halved into two equal portions. Half portion divided into five lots were dipped into different concentrations and offered as food to each II instar larvae after fan drying for the period of half an hour. Similarly, the remaining half was divided in five lots for spraying with different doses of *N. rileyi* suspensions and offered to II instar larvae individually. Forty larvae were tested for each dose. Simultaneously, a control was also run with each method of application, spraying the leaves only with 0.05% emulsifier (Tween 80) solution.

The same procedure was adopted for treating the food of IV instar larvae.

The larvae tested were incubated with treated food at 25±1°C for 48 hours. They were then transferred to untreated food for rearing.

The larval mortality recorded on 4th day for II instar and 7th day for IV instar were utilized to determine medium lethal dose (LD_{50}) value by Probit Analysis Method (Finney 1971). Similarly, the mortality recorded in the dose of 5×10^6 conidia/ml against II instar larvae and in the dose of 5×10^7 conidia/ml against IV instar larvae were subjected to Probit Analysis to determine median lethal time (LT_{50}) value.

RESULTS AND DISCUSSION

Bio–efficacy of *N. rileyi* on *H. armigera* larvae fed upon dipped chickpea leaves: It is evident from the results Table 1A that *N. rileyi* at 5×10^4 conidia/ml dose caused 5 to 25 per cent mortality of II instar *H. armigera* larvae within 2 to 5 days. Mortality of the larvae with suspension strength of 5×10^5, 5×10^6 and 5×10^7 conidia/ml was uniformly seen to raise mortality levels from 10 to 60, 15 to 75 and 17.5 to 100 per cent, respectively, within a week's time. The larval mortality recorded on 4th day of treatment was subjected to probit analysis to compute the LD_{50} as 8.6×10^6 conidia/ml (Table 1B). The data of mortality of 5×10^6 conidia/ml of *N. rileyi* was utilized in computing LT_{50} as 4.38 days (Table 1C).

It is clear from the data of Table 2A that the mortality of 7.50 and 17.50 per cent on 3rd day and 17.50 and 30.00 per cent on 4th day was recorded in doses 5×10^7 and 5×10^8 conidia/ml against IV instar larvae of *H. armigera*, respectively, whereas, in lower doses of 5×10^5 and 5×10^6 conidia/ml, the respective larval mortality 15.00 and 22.50 per cent was recorded on 5th day of treatment. With higher dosages 5×10^7 and 5×10^8, the per cent larval mortality of 35.00 and 47.50, 55.00 and 67.50, and 62.50 and 75.00 was recorded on 5, 7 and 10th day of treatment, respectively. However, in the lower dosages of 5×10^5 and 5×10^6 conidia/ml, the per cent mortality against IV instar larvae on 7th and 10th day were 25.00 and 40.00, 30.00 and 47.50 per cent, respectively. The larval mortality caused 7 days after application of different doses of *N. rileyi* on dipped chickpea was utilized for calculating the LD_{50} of IV instar *H. armigera* larvae and it was 2.68×10^7 conidia/ml (Table 2B). Simultaneously, the mortality counts recorded with the dose of 5×10^7 conidia/ml from 3–10 days after treatment was analysed to determine the LT_{50} as 7.18 days (Table 2C).

Table 1A. Bio efficacy of fungus, *N. rileyi* to II instar *H. armigera* larvae on dipped chickpea leaves

Dose (conidia/ml)	No. of larvae tested	Per cent mortality after days					
		1	2	3	4	5	7
5×10^8	40	0.00	22.50	47.50	77.50	85.00	100.00
5×10^7	40	0.00	17.50	35.00	65.00	77.50	100.00
5×10^6	40	0.00	15.00	27.50	45.00	57.50	75.00
5×10^5	40	0.00	10.00	20.00	30.00	40.00	60.00
5×10^4	40	0.00	5.00	10.00	17.50	25.00	40.00

Table 1B. Dose mortality relationship of II instar *H. armigera* larvae to *N. rileyi* dipped chickpea leaves after 4 days

Regression equation	LD_{50} (conidia/ml)
Y = 2.0291 + 0.4301 x	8.06×10^6

x = Log dose, Y = Probit kill, LD_{50} = Median lethal dose

Table 1C: Time mortality relationship of II instar *H. armigera* larvae to *N. rileyi* dipped chickpea leaves 5×10^6 conidia/ml

Regression equation	LD_{50} (conidia/ml)
Y = 2.9344 + 3.2200 x	4.38

x = Log dose, Y = Probit kill, LD_{50} = Median lethal time

Table 2A. Bio efficacy of fungus, *N. rileyi* to IV instar *H. armigera* larvae on dipped chickpea leaves

Dose (conidia/ml)	No. of larvae tested	Per cent mortality after days				
		3	4	5	7	10
5×10^8	40	17.50	30.00	47.50	67.50	75.00
5×10^7	40	7.50	17.50	35.00	55.00	62.50
5×10^6	40	0.00	0.00	22.50	40.00	47.50
5×10^5	40	0.00	0.00	15.00	25.00	30.00

Table 2B. Dose mortality relationship of IV instar *H. armigera* larvae to *N. rileyi* dipped chickpea leaves after 7 days

Regression equation	LD_{50} (conidia/ml)
Y = 2.2085 + 0.3758 x	2.68×10^7

x = Log dose, Y = Probit kill, LD_{50} = Median lethal dose

Table 2C. Time mortality relationship of IV instar *H. armigera* larvae to *N. rileyi* dipped chickpea leaves 5×10^7 conidia/ml

Regression equation	LD_{50} (conidia/ml)
Y = 2.1214 + 3.3623 x	7.18

x = Log dose, Y = Probit kill, LD_{50} = Median lethal time

Bio–efficacy of *N. rileyi* on *H. armigera* larvae fed upon sprayed chickpea leaves: The data presented in Table 3A reveal that the fungal concentrations 5×10^6, 5×10^6, 5×10^7 and 5×10^8 conidia/ml caused 7.50, 10.00 and 15.00 per cent mortality of the II instar larvae, respectively 2 days after the treatment. Further, it was seen that larval mortality increased in each concentration with lapse of time and reached to 60.00, 82.50 and 97.50 per cent at the end of 7 days. However, in dosages 5×10^4 and 5×10^5 conidia/ml, the per cent mortality of the insect ranged between 7.50 to 35.00 and 12.50 to 52.50 in 3 to 7 days period, respectively. The mortality counts recorded in different conidial doses of *N. rileyi* on 4th day of treatment were further subjected to probit analysis and the value was 2.42×10^7 conidia/ml (Table 3B). Similarly, the mortality recorded in 5×10^6 conidia/ml dose at 2, 3, 4, 5 and 7 days was utilized to calculate LT_{50} value of the test insect as 5.42 days (Table 3C).

It clear from the data of Table 4A that in dosages 5×10^7 and 5×10^8 conidia/ml did not cause mortality against IV instar larvae upto 3 days of treatment. After 4 days of treatment mortality was 15.00 and 25.00 per cent, respectively. Whereas, in the dosages 5×10^5 and 5×10^6 conidia/ml it took 5 days to cause 10.00 and 20.00 per cent mortality. After 10 days the mortality caused by this green muscardine fungus against IV instar larvae of *H. armigera* was 25.00, 40.00, 50.00 and 67.50 per cent with their respective dosages of 5×10^5, 5×10^6, 5×10^7 and 5×10^8 conidia/ml. The cumulative larval mortality recorded 7 days after the treatment was computed to find the LD_{50} value as 1.09×10^8 conidia/ml (Table 4B). Likewise, the cumulative mortality recorded in the dose 5×10^7 conidia/ml at 4, 5, 7 and 10 days after treatment was treated to determine the LD_{50} of this dose against IV instar larvae of *H. armigera* and the lethal time was calculated as 8.84 days (Table 4C).

Table 3A. Bio efficacy of fungus, *N. rileyi* to II instar *H. armigera* larvae on sprayed chickpea leaves

Dose (conidia/ml)	No. of larvae tested	Per cent mortality after days					
		1	2	3	4	5	7
5×10^8	40	0.00	15.00	42.50	70.00	80.00	97.50
5×10^7	40	0.00	10.00	30.00	57.50	70.00	82.50
5×10^6	40	0.00	7.50	22.50	37.50	47.50	60.00
5×10^5	40	0.00	0.00	12.50	22.50	32.50	52.50
5×10^4	40	0.00	0.00	7.50	15.00	17.50	35.00

Table 3B. Dose mortality relationship of II instar *H. armigera* larvae to *N. rileyi* sprayed chickpea leaves after 4 days

Regression equation	LD_{50} (conidia/ml)
Y = 1.9713 + 0.4101 x	2.42×10^7

x = Log dose, Y = Probit kill, LD_{50} = Median lethal dose

Table 3C. Time mortality relationship of II instar *H. armigera* larvae to *N. rileyi* sprayed chickpea leaves 5×10^6 conidia/ml

Regression equation	LD_{50} (conidia/ml)
Y = 2.772 + 3.0351 x	5.42

x = Log dose, Y = Probit kill, LD_{50} = Median lethal time

Table 4A. Bio efficacy of fungus, *N. rileyi* to IV instar *H. armigera* larvae on sprayed chickpea leaves

Dose (conidia/ml)	No. of larvae tested	Per cent mortality after days				
		3	4	5	7	10
5×10^8	40	0.00	25.00	42.50	57.50	67.50
5×10^7	40	0.00	15.00	30.00	47.50	50.00
5×10^6	40	0.00	0.00	20.00	32.50	40.00
5×10^5	40	0.00	0.00	10.00	20.00	25.00

Table 4B. Dose mortality relationship of IV instar *H. armigera* larvae to *N. rileyi* sprayed chickpea leaves after 7 days

Regression equation	LD_{50} (conidia/ml)
Y = 2.21554 + 0.3464 x	1.09×10^8

x = Log dose, Y = Probit kill, LD_{50} = Median lethal dose

Table 4C. Time mortality relationship of IV instar *H. armigera* larvae to *N. rileyi* sprayed chickpea leaves 5×10^7 conidia/ml

Regression equation	LD_{50} (conidia/ml)
Y = 2.6619 + 2.4709 x	8.84

x = Log dose, Y = Probit kill, LD_{50} = Median lethal time

The results of the study show that *N. rileyi* is pathogenic to the larvae of *H. armigera*, where the dosage 5×10^8 conidia/ml is able to cause over 80 per cent mortality of II instar larvae of *H. armigera* within 5 days of feeding on treated chickpea leaves either by dip method or by spray method. Moreover, the same conidial dose of the fungus causes around 70 per cent mortality of IV instar larvae in 10 days in a pathogenicity test Gopalakrishnan and Narayanan (1988) reported 100 per cent mortality of III instar larvae of *H. armigera* with a dose of 1.8×10^9 conidia/ml of *N. rileyi*. In a recent study Tang and Roger (1998) recorded more than 90 per cent mortality of IV instar larvae of *H. armigera* with aqueous suspension of *N. releyi* at the dose 1×10^7 conidia/ml on corn silk and leaves of soybean, tomato and chrysanthemum.

The present studies indicate that younger stage larvae of *H. armigera* can be killed by comparatively lesser conidial dosage of *N. rileyi* than the older ones. For achieving 50 per cent mortality of population of the larvae, the LD_{50} value of fungus computed for II instar larvae were 8.06×10^6 conidia/ml with dip method and 2.42×10^7 conidia/ml with spray method, 4 days after the treatment. However, 3 to 4 times more conidial suspensions (2.68×10^7 and 1.09×10^8) of the fungus is required to kill 50 per cent population of IV instar *H. armigera* larvae after 7 days of treatment. These findings are in agreement with the observations made by Vimla Devi (1994) who determined the LD_{50} values of *N. rileyi* as 2.23×10^7 conidia/ml for II instar and 2.89×10^7 conidia/ml for III instar larvae of *Spodoptera litura* 8 days after its application. Although time taken by *Spodoptera* is longer than the *H. armigera* in the present studies.

The present findings further show that II instar larvae of *H. armigera* take nearly 5 days for its 50 per cent kill with *N. rileyi* dosage of 5×10^6 conidia/ml, while IV instar larvae take nearly 8 days for achieving same level of mortality with a dosage of 5×10^7 conidia/ml. The results of IV instar are almost similar to results of Tang and Roger (1998) who established 5.9 to 6.7 days as LT_{50} value for IV instar larvae of *H. armigera* at a dose of 1.0×10^7 conidia/ml of *N. rileyi* on corn silk, leaves of soybean, tomato and chrysanthemum.

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