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Pathogenicity of Oil Formulations of Entomopathogenic Fungus, *Nomuraea rileyi* (Farlow) Samson Against *Spodoptera litura* (Fabricius)

T Sharmila, K Manjula and T Murali Krishna
Department of Entomology, SV Agricultural College, Tirupati 517 502

ABSTRACT

Three oil formulations of entomopathogenic fungus *Nomuraea rileyi* were evaluated for their efficacy in terms of LC_{50}, LC_{90} and LT_{50} against *Spodoptera litura* during 2011-2012 at the Department of Entomology, S.V. Agricultural College, Tirupati. The lowest LC_{50} (0.3×10^4 spores ml^{-1}) and LC_{90} (1.2×10^7 spores ml^{-1}) were obtained with groundnut oil formulation. LC_{50} and LC_{90} values of *N. rileyi* were lower with oil mixed application than applying the fungus as crude suspension. Lower LT_{50} of 3.93 days was obtained with groundnut oil formulation at 1×10^8 spores ml^{-1} concentration. The LT_{50} values recorded with sunflower oil, coconut oil and crude formulations are 4.81, 5.28, and 5.93 days respectively at higher concentration (1×10^8 spores ml^{-1}). LT_{50} values were higher at lower concentrations.

Key words : LC_{50}, LT_{50}, *Nomuraea rileyi*, Oil formulations, Pathogenicity.

Nomuraea rileyi (Farlow) Samson is an important natural control agent of many lepidopteran caterpillars throughout the world. It occurs in epizootic form in the humid regions and is easily multiplied at a low cost, is obviously a best candidate for exploitation at the field level. Therefore, it has great potential for development into myco-insecticide. The present investigation was carried out for evaluating pathogenicity of oil formulations of entomopathogenic fungus *N. rileyi* Samson against larvae of *S. litura*.

MATERIAL AND METHODS

An experiment was conducted during 2011-2012 in the Department of Entomology, S.V. Agricultural college, Tirupati to evaluate efficacy of *N. rileyi* when applied along with vegetable oils i.e., groundnut, sunflower and coconut oils. Mass production of *N. rileyi* was done in rice grain media. To 30 g of broken rice in 250 ml conical flask, 28 ml of 1 % yeast extract solution was added and soaked overnight. Next day the rice yeast mixture was autoclaved at 20^0C(15 Psi) for 30 minutes and cooled. The cooled conical flasks were inoculated with 2ml of *N. rileyi* spore suspension (10^8 spores ml^{-1}) under aseptic conditions in laminar air flow chamber. After inoculation they were incubated at 25 ± 2^0C temperature and around 85% RH for 20 days. After noticing sufficient sporulation, the spores are harvested, dried and powdered. The three oil formulations of *N. rileyi* were prepared separately by mixing @ one gram of conidial powder of *N. rileyi* obtained from broken rice culture (1×10^{10} spores g^{-1}) with ten ml of autoclaved and cooled oils containing Tween–20 (0.05%). Ten ml of each oil (i.e., sunflower oil or groundnut oil or coconut oil) formulation was mixed separately with 100 ml of distilled water along with Tween-20 (0.05%) and thoroughly shaked and filtered through muslin cloth and the spore count was adjusted to 1×10^8 spores ml^{-1}. Crude formulation was prepared by mixing one gram of conidial powder of *N. rileyi* obtained from broken rice culture (1×10^{10} spores g^{-1}) with 100 ml of distilled water along with Tween -20 (0.05%) and thoroughly shaked and filtered through muslin cloth and the spore count was adjusted to 1×10^8 spores ml^{-1}.

From this stock suspension, serial dilutions of 1×10^7 to 1×10^2 spores ml^{-1} were prepared. All the seven concentrations of each of these three (sunflower, groundnut and coconut) oil based suspensions of *N. rileyi* and *N. rileyi* without oil (crude formulation) were used for infecting laboratory reared second generation third instar *S. litura* larvae by leaf application method with hand automizer. All the treatments were replicated thrice

Table 1. LC_{50} and LC_{90} values of formulations of *N. rileyi* against III instar larvae of *S. litura.*

Formulation	Regression equation	LC_{50} values spores ml^{-1}	Fiducial limits spores ml^{-1}	LC_{90} values spores ml^{-1}	Fiducial limits spores ml^{-1}	Slope (b)
Sunfloweroil	Y= -1.67561 + 0.38878x	0.2 X 10^5	0.6 X 10^4 0.5 X 10^5	0.4 X 10^8	0.7 X 10^7 0.5 X 10^9	0.38
Groundnutoil	Y= -1.26618 + 0.35880x	0.3 X 10^4	0.6 X 10^3 0.1 X 10^5	1.2 X 10^7	0.2 X 10^7 2.2 X 10^8	0.35
Coconut oil	Y= -1.67561 + 0.38878x	0.5 X 10^5	0.1 X 10^5 0.2 X 10^6	1.1 X 10^9	0.8 X 10^8 1.5 X 10^{11}	0.29
Without oil	Y= -1.48804 + 0.27523x	0.2 X 10^6	0.5 X 10^5 0.14 X 10^7	1.1 X 10^{10}	0.4 X 10^9 5.7 X 10^{12}	0.27

with 10 larvae per replication. An untreated control was also maintained. Larval mortalities were recorded daily by providing fresh leaves of castor. The LC_{50} and LC_{90} values were calculated with the mortalities obtained through probit analysis. Similarly LT_{50} values were calculated with the larval mortalities obtained with different period of time.

RESULTS AND DISCUSSION

Corresponding to the higher mortalities of larvae, the lowest LC_{50} (0.3 x 10^4 spores ml^{-1}) and LC_{90} (1.2 x 10^7 spores ml^{-1}) were obtained with groundnut oil formulation. The LC_{50} and LC_{90} values recorded with sunflower oil, coconut oil and crude formulations are 0.2 x10^5 and 0.4 x 10^8; 0.5 x 10^5 and 1.1 x 10^9 ; 0.2 x 10^6 and 1.1 x 10^{10} spores ml^{-1} respectively. The data on regression equation, fiducial limits and slope were presented in table 1.

The results reveal that groundnut oil used as additive to *N. rileyi* shows the efficiency with enhanced larval mortalities of *S. litura* in terms of LC_{50} and LC_{90}. Other two oils tested i.e., sunflower and coconut oil also gave increased susceptibility of larvae when mixed with *N. rileyi*. Groundnut oil with its relative high viscous nature may have more contact with conidia and hence prevented from drying off. LC_{50} and LC_{90} values of *N. rileyi* were lower with oil mixed application than applying the fungus as crude suspension. Oils may protect the conidia from drying off before they germinate on larval integument. As the leaf surface is lipophilic in nature, the oil layer that surrounds the conidia has more affinity to the leaf surface, and may continue to be in contact with the leaf on which larvae crawl.

The above results are supported with the similar findings of earlier workers. Oil formulation prevents the desiccation of the conidia and helps in longer survival period and better penetration of peg into the integument (Burges,1998). Oil based formulations allow the fungal biopesticides to be applied in a non desicated manner, which reduced effects of thermal stress (McClatchie et al., 1994 and Hedgecock, et al., 1995). Ramegowda (2005) stated that among oil formulations of *N. rileyi*, against *S. litura,* safflower oil (1.42 × 10^4 conidia ml^{-1}) recorded lowest LC_{50} values followed by groundnut and sunflower oils.

Though oil formulations recorded lower LC_{50} and LC_{90} values, the crude formulation also gave considerable larval mortalities giving the LC_{50} of 0.2 x 10^6 spores ml^{-1}. Several workers reported the efficacy of *N. rileyi* applied as water suspension against lepidopteran larvae. According to Vimala Devi (1994) LC_{50} value of *N. rileyi* for III instar *S.litura* was 2.9 x 10^7 spores ml^{-1}. Zhang *et al*. (2002) evaluated two strains F_{889} and F_{815} of *N. rileyi* against III instar *Spodoptera litura* larvae and the LC_{50} values were 5.38 x 10^6 and 5.71 x 10^6 spores ml^{-1}, respectively.

Lower LT_{50} of 3.93 days was obtained with groundnut oil formulation at 1 x 10^8 spores ml^{-1} concentration (Table 2). The LT_{50} values recorded with sunflower oil, coconut oil and crude formulations are 4.81, 5.28, and 5.93 days (Table 2) respectively at higher concentration (1 x 10^8 spores

Table 2. Lethal Time 50 (LT_{50}) values of groundnut oil, sunflower oil, coconut oil and (without oil) crude formulations of *N. rileyi* against III instar larvae of *S. litura*

Concentration of *N. rileyi* (spores ml^{-1})	Formulation	Regression equation	LT_{50} values (days)	Fiducial limits (days)	Slope (b)
	Groundnut oil	Y = - 3.39644 + 5.70621x	3.93	3.48 – 4.31	5.70621
$1x10^8$	Sunflower oil	Y = - 4.05504 + 5.94045x	4.81	4.39 - 5.20	5.94045
	Coconut oil	Y = - 3.49252 + 4.83213x	5.28	4.77 – 5.75	4.83213
	Without oil	Y = - 3.56103 + 4.60568x	5.93	5.39 – 6.47	4.60568
	Groundnut oil	Y = - 2.76582 + 4.28266x	4.42	3.83 – 4.91	4.28266
$1x10^7$	Sunflower oil	Y = - 3.02095 + 4.12783x	5.39	4.81 – 5.94	4.12783
	Coconut oil	Y = - 3.28750 + 4.19606x	6.07	5.48 – 6.68	4.19606
	Without oil	Y = - 3.22147 + 3.84268x	6.89	6.21 – 7.73	3.84268
	Groundnut oil	Y = - 4.09541 + 5.50935x	5.53	5.06 – 5.98	5.50935
$1x10^6$	Sunflower oil	Y = - 3.71434 + 4.62442x	6.35	5.80 – 6.95	4.62442
	Coconut oil	Y = - 3.35688 + 3.91239x	7.21	6.51 – 8.13	3.91239
	Without oil	Y = - 3.33199 + 3.78887x	7.57	6.81 – 8.66	3.78887
	Groundnut oil	Y = - 3.65872 + 4.45222x	6.63	6.04 – 7.29	4.45222
$1x10^5$	Sunflower oil	Y = - 3.76483 + 4.42211x	7.10	6.48 – 7.86	4.42211
	Coconut oil	Y = - 2.01808 + 0.24447x	8.25	7.06 – 10.61	0.24447
	Without oil	-	-	-	-
	Groundnut oil	Y = - 4.80040 + 5.28249x	8.10	7.46 – 8.99	5.28249
$1x10^4$	Sunflower oil	Y = - 4.75843+ 5.07350x	8.66	7.92 – 9.84	5.07350
	Coconut oil	-	-	-	-
	Without oil	-	-	-	-
	Groundnut oil	-	-	-	-
$1x10^3$	Sunflower oil	-	-	-	-
	Coconut oil	-	-	-	-
	Without oil	-	-	-	-
	Groundnut oil	-	-	-	-
$1x10^2$	Sunflower oil	-	-	-	-
	Coconut oil	-	-	-	-
	Without oil	-	-	-	-

ml^{-1}). LT_{50} of 4.42, 5.53, 6.63 and 8.10 days was obtained with groundnut oil formulation at 1 x 10^7, 1 x 10^6, 1 x 10^5, 1 x 10^4 spores ml^{-1}concentrations respectively. LT_{50} of 5.39, 6.35, 7.10 and 8.66 days was obtained with sunflower oil formulation at 1 x 10^7, 1 x 10^6, 1 x 10^5, 1 x 10^4 spores ml^{-1} concentrations respectively. LT_{50} of 6.07, 7.21 and 8.25 days was obtained with coconut oil formulation at 1 x 10^7, 1 x 10^6, 1 x 10^5 spores ml^{-1} concentrations respectively. LT_{50} of 6.89 and 7.57 days was obtained with crude formulation at 1 x 10^7, 1 x 10^6, spores ml^{-1} concentrations respectively. The data on regression equation, fiducial limits and slope are presented in table 2.

With respect to each formulation, LT_{50} values were lower with higher concentration of fungal spores. Patil (2000) reported that LT_{50} values for 1st to 5th instar larvae of *S. litura* were 5.44, 5.74, 6.16, 9.81 and 10.9 days, respectively when treated with *N. rileyi*. Tadele Tefera and Pringle (2003) observed that, with increase in conidial concentration of *N. rileyi*, there was decrease in LT_{50}-.

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