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Pathogenicity and Bioefficacy of Two Most Promising WP Formulations of *Nomuraea rileyi* (Farlow) Samson against *S. litura*

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Abstract

Laboratory studies with two promising WP formulations by spraying the dilution series (2.0, 3.0, 4.0, 5.0, 6.0 and 8.0 g/l of water or at concentrations 0.01, 0.015, 0.02, 0.025, 0.03 and 0.04%, respectively) of each of the formulations and formulation without adjuvants. having three replications in completely randomized design were carried out in the biological control laboratory, Dept. of Entomology, MPKV, Rahuri with an object to evaluate the pathogenicity and bio-efficacy of promising WP formulations *N. rileyi.* The results revealed that the LC_{50} values of formulation A ($N_{30}S_{1/1}$) on the basis of product (BAI) were 0.0116% and 0.0157% for II and III instar larvae of *S. litura*, respectively. The LC_{50} values of formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) were 0.0120% and 0.0176% against II and III instar larvae of *S. litura.* LC_{90} values of formulation A ($N_{30}S_{1/1}$) on the basis of product (BAI) were 0.0710% and 0.0820% for II and III instar larvae of *S. litura*, respectively. The LC_{90} values of formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) were 0.0748% and 0.0920% against II and III instar larvae of *S. litura.* It indicated that among two larval instars of *S. litura* tested, II instar larvae found to be most susceptible to the *N. rileyi* WP formulation A ($N_{30}S_{1/1}$) and B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$). The LC_{50} value of *N. rileyi* WP formulations was lower in formulation A ($N_{30}S_{1/1}$) (0.0116) than B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) (0.0120) for II instar larvae. The formulation A ($N_{30}S_{1/1}$) of *N. rileyi* and was the most virulent formulations as evidenced from lowest LC_{50} values. In case of III instar larvae of *S. litura* formulation A ($N_{30}S_{1/1}$) registered 7.12 days while formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) recorded 7.29 days for 50 per cent mortality of larvae of *S. litura.* Thus, it was established from the results that formulation A ($N_{30}S_{1/1}$) taken minimum time to kill 50 per cent population and was most virulent. The effectiveness of formulations at 10 DAT against II instar larvae of *S. litura* was highest (92.50%) in formulation A ($N_{30}S_{1/1}$) @ 0.04% and 0.03% and B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.04%. However, it was at par with formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.03% (90.0%), A ($N_{30}S_{1/1}$) @ 0.025% (87.50%) and B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.025% (85.0%). These were followed by formulation A ($N_{30}S_{1/1}$) @ 0.02% (77.50%) and B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.02% (75.0%). The concentrations at 0.01% and 0.015% of both formulations showed at par 52.50 and 60.0 to 65.0% mortality of the pest. At 10 DAT, the mortality of III instar larvae was highest (82.50%) in the treatment of formulation A ($N_{30}S_{1/1}$) @ 0.04%, which was on par to formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.04% (80.0%), A ($N_{30}S_{1/1}$) @ 0.03% (77.50%), B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.03% (77.50%) and formulation A ($N_{30}S_{1/1}$) @ 0.025% (72.50%) and B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.025% (70.0%).

Key words : *Nomuraea rileyi, Spodoptera litura, LT_{50}, LC_{50} and LC_{90}*

Introduction

The use of entomopathogenic fungi has potential in future strategies in insect pest management due to ability of their mass production. Most of the orders of the insects are more or less susceptible to fungal diseases. *Nomuraea rileyi* (Farlow) Samson Moniliales, Moniliaceae is an entomofungus of cosmopolitan nature (1). It is being regularly observed in epizootic form on *Spodoptera litura* (Fab.) in crops like cotton, soybean and groundnut in cooler months (2). *N.rileyi* infects mainly Lepidoptera, particularly economical important and polyphagous noctuid insect pests. *N. rileyi* is an entomopathogen causing natural mortality in as many as 51 Lepidopteran insects throughout the world (3). Progress of research on *N. rileyi* in India is slow though the results of the few studies have revealed that *N. rileyi* as a potential mycoinsecticide (4). It was considered as possible biological control agent in many years, because of its effectiveness in reducing or suppressing the population of lepidopteron caterpillars. Hence, the present study was taken up to evaluate the pathogenicity and bio-efficacy of promising WP formulations *N. rileyi.*

Materials and Methods

Fungus culture : The pure fungus culture of *N. rileyi* was made, available from isolates in Biocontrol Lab of Entomological centre, College of Agriculture, Pune. Laboratory studies with two promising WP formulations by spraying the dilution series (2.0, 3.0, 4.0, 5.0, 6.0 and 8.0 g/l of water or at concentrations 0.01, 0.015, 0.02, 0.025, 0.03 and 0.04%, respectively) of each of the formulations

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

Sr. No	Formulations *N. rileyi* 5% WP	Host tested (*S. litura* larvae)	Chi-square	Regression equation	LC50 on BAI (%)	Fiducial limit		LC_{90} on BAI (%)	Fiducial limit	
						Lower	Upper		Lower	Upper
1.	A (N_{30} $S_{1/1}$)	II instar	2.30	Y = 3.2547 + 1.6339 X	0.0116	0.0079	0.0172	0.0710	0.0301	0.1671
2.	A (N_{30} $S_{1/1}$)	III instar	1.38	Y = 1.8647 + 1.7850 X	0.0157	0.0122	0.0201	0.0820	0.0400	0.1684
3.	B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$)	II instar	1.43	Y = 3.2532 + 1.6960 X	0.0120	0.0083	0.0174	0.0748	0.0325	0.1722
4.	B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$)	III instar	0.39	Y = 2.7725 + 1.7867X	0.0176	0.0140	0.0220	0.0920	0.0438	0.1933

Table-2 : LT_{50} values of WP formulations of *N. rileyi* of II and III instar larvae of *S. litura.*

Sr. No.	Formulations *N. rileyi* 5% WP	Host tested (larvae of *S. litura*)	Chi-square	Regression equation	LT_{50} (days)	Fiducial limit	
						Lower	Upper
1.	A ($N_{30}S_{1/1}$)	II instar	0.114	Y = 1.147 + 4.805 X	6.337	5.654	7.027
2.	A ($N_{30}S_{1/1}$)	III instar	1.415	Y = 1.384 + 4.243 X	7.115	6.333	8.082
3.	B ($N_{30}T_{1/2}$ $G_{2/1}H_{1/1}$)	II instar	1.764	Y = 1.379 + 4.499 X	6.381	5.657	7.123
4.	B ($N_{30}T_{1/2}$ $G_{2/1}H_{1/1}$)	III instar	2.119	Y = 1.631 + 3.905 X	7.289	6.438	8.425

and formulation without adjuvants. having three replications in completely randomized design were carried out in the biological control laboratory, Dept. of Entomology, MPKV, Rahuri.

Pathogenicity and bioefficacy of formulations of *N. rileyi* : The bioassay of the two developed WP formulation of *N. rileyi* was carried out by spraying the dilution series (2.0, 3.0, 4.0, 5.0, 6.0 and 8.0 g/l of water or at concentrations 0.01, 0.015, 0.02, 0.025, 0.03 and 0.04%, respectively) of each of the formulations and formulation without adjuvants. The two promising WP formulations of *N. rileyi* were tested against II and III instar larvae of *S. litura* and calculated the LC_{50}, LC_{90} and LT_{50} of respective WP formulations. Laboratory experiment was carried out in Complete Randomized Design and three replications. Ten larvae were taken in a glass container along with castor leaves as food which were directly sprayed with 10 ml desired concentration of conidials suspension using hand atomizer and allowed to dry for about 15 minutes. Each larvae was transferred to a separate plastic vial (6 x 4cm) treated with antibiotics to avoid growth of other micro-organisms. Each vial containing moist filter paper at bottom with treated food. Fresh untreated castor leaves were provided to the larvae at every 24 hrs. Each treatment consisted of 10 larvae and replicated thrice. The treated larvae were incubated at room temperature at 25 $\pm$ 10 ^{o}C and RH of 70 $\pm$ 10%. The larval mortality was recorded at an interval of 24 hours up to 10 days. Percent mortality was calculated and corrected by formula given by (5). The data on cumulative per cent mortality obtained 10 days after inoculation (DAI) were subjected to Probit Analysis (6).

Results and Discussion

Pathogenicity of promising WP formulations of *N. rileyi :*

LC_{50} and LC_{90} values of *S. litura* : The LC_{50} values for *S. litura* of II and III instar larvae were determined through bioassay and probit analysis. The results are presented in Table-1. The results revealed that the LC_{50} values of formulation A ($N_{30}S_{1/1}$) on the basis of product (BAI) were 0.0116% and 0.0157% for II and III instar larvae of *S. litura*, respectively. The LC_{50} values of formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) were 0.0120% and 0.0176% against II and III instar larvae of *S. litura.* LC_{90} values of formulation A on the basis of product (BAI) were 0.0710% and 0.0820% for II and III instar larvae of *S. litura*, respectively. The LC_{90} values of formulation B were 0.0748% and 0.0920% against II and III instar larvae of *S. litura.* It indicated that among two larval instars of *S. litura* tested, II instar larvae found to be most susceptible to the *N. rileyi* WP formulation A ($N_{30}S_{1/1}$) and B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$).

The LC_{50} value of *N. rileyi* WP formulations was lower in formulation A (0.0116) than B (0.0120) for II instar larvae. The formulation A ($N_{30}S_{1/1}$) of *N. rileyi* and was the most virulent formulations as evidenced from lowest LC_{50} values. The chi-square test showed homogeneity of test population in all bioassays which indicated the good fit of the observed and expected responses.

It is established from the results that as the larval instar of *S. litura* advanced, it required higher doses of *N. rileyi* WP formulations to kill it. These results are in conformity with the results reported by (7,8,9,10,11) for *N.*

Table-3 : Bioefficacy of WP formulations of *N. rileyi* against II instar larvae of *S. litura*.

Tr. No.	Treatment Formulation	BAI Conc. (%)	Dose g/l	Larval mortalitly (%)		
				5 DAT	7 DAT	10 DAT
T_1	*N. rileyi* 5% WP-A	0.01	2.0	27.50 (31.63)*	42.50 (40.69)	52.50 (46.43)
T_2	*N. rileyi* 5% WP-A	0.015	3.0	37.50 (37.76)	57.50 (49.31)	65.00 (53.73)
T_3	*N. rileyi* 5% WP-A	0.02	4.0	55.00 (47.87)	72.50 (58.37)	77.50 (61.68)
T_4	*N. rileyi* 5% WP-A	0.025	5.0	57.50 (49.31)	77.50 (61.68)	87.50 (69.30)
T_5	N. rileyi 5% WP-A	0.03	6.0	65.00 (53.73)	80.00 (63.44)	92.50 (74.11)
T_6	*N. rileyi* 5% WP-A	0.04	8.0	65.00 (53.73)	82.50 (65.27)	92.50 (74.11)
T_7	*N. rileyi* 5% WP-B	0.01	2.0	22.50 (28.32)	42.50 (40.69)	52.50 (46.43)
T_8	*N. rileyi* 5% WP-B	0.015	3.0	35.00 (36.27)	55.00 (47.87)	60.00 (50.77)
T_9	*N. rileyi* 5% WP-B	0.02	4.0	52.50 (46.43)	70.00 (56.79)	75.00 (60.00)
T_{10}	*N. rileyi* 5% WP-B	0.025	5.0	55.00 (47.87)	75.00 (60.00)	85.00 (67.21)
T_{11}	*N. rileyi* 5% WP-B	0.03	6.0	60.00 (50.77)	77.50 (61.68)	90.00 (71.56)
T_{12}	*N. rileyi* 5% WP-B	0.04	8.0	62.50 (52.24)	80.00 (63.44)	92.50 (74.11)
T_{13}	*N. rileyi* alone 5% WP	0.02	4.0	27.50 (31.63)	42.50 (40.69)	57.50 (49.31)
T_{14}	Control (water spray)	-	-	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
	S.E +			1.49	1.39	2.59
	C.D.(P=0.05)			4.26	3.98	7.40

*Figures in parentheses are arcsin values DAT = Days after treatment BAI = Bioactive ingredient
A = ($N_{30}S_{1/1}$) B = ($N_{30}T_{1/2}G_{2/1}H_{1/1}$)

Table-4 : Bioefficacy of WP formulations of *N. rileyi* against III instar larvae of *S. litura*.

Tr. No.	Treatment Formulation	BAI Conc. (%)	Dose g/l	Larval mortalitly (%)		
				5 DAT	7 DAT	10 DAT
T_1	*N. rileyi* 5% WP-A	0.01	2	15.00 (22.79)*	37.50 (37.76)	47.50 (43.57)
T_2	*N. rileyi* 5% WP-A	0.015	3	25.00 (30.00)	42.50 (40.69)	52.50 (46.43)
T_3	*N. rileyi* 5% WP-A	0.02	4	45.00 (42.13)	62.50 (52.24)	67.50 (55.24)
T_4	*N. rileyi* 5% WP-A	0.025	5	47.50 (43.57)	65.00 (53.73)	72.50 (58.37)
T_5	*N. rileyi* 5% WP-A	0.03	6	52.50 (46.43)	72.50 (58.37)	77.50 (61.68)
T_6	*N. rileyi* 5% WP-A	0.04	8	55.00 (47.87)	75.00 (60.00)	82.50 (65.27)
T_7	*N. rileyi* 5% WP-B	0.01	2	17.50 (24.73)	32.50 (34.76)	42.50 (40.69)
T_8	*N. rileyi* 5% WP-B	0.015	3	30.00 (33.21)	42.50 (40.69)	47.50 (43.57)
T_9	*N. rileyi* 5% WP-B	0.02	4	47.50 (43.57)	57.50 (49.31)	65.00 (53.73)
T_{10}	*N. rileyi* 5% WP-B	0.025	5	47.50 (43.57)	60.00 (50.77)	70.00 (58.79)
T_{11}	*N. rileyi* 5% WP-B	0.03	6	52.50 (46.43)	67.50 (55.24)	77.50 (61.68)
T_{12}	*N. rileyi* 5% WP-B	0.04	8	55.00 (47.87)	72.50 (58.37)	80.00 (63.44)
T_{13}	*N. rileyi* alone 5%WP	0.02	4	16.67 (24.12)	36.67 (37.29)	55.00 (47.87)
T_{14}	Control (water spray)	-	-	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
	S.E +			1.57	1.86	2.71
	C.D.(P=0.05)			4.49	5.32	7.74

*Figures in parentheses are arcsin values, DAT = Days after treatment, BAI = Bioactive ingredient, A = (N30S1/1), B = ($N_{30}T_{1/2}G_{2/1}H_{1/1}$)

rileyi. The results of the bioassays indicated that susceptibility of the pest decreased with the age of the larvae in terms of both LC_{50} and LT_{50}. The present investigation on relative virulence demarcated that II instar larvae of *S. litura* were more susceptible to *N. rileyi* as compared to III instar larvae. However, all the researchers determined the LC_{50} values for *S. litura*. These were $16.11x10^5$ conidia/ml for II instar larvae (8). (9) reported that *N. rileyi* conidia along with bentonite and sucrose powder (1:7:7) and aluminium silicate (1:1:8), bentonite soil (1:7:7) and bentonite (1:1:8) recorded lower LC_{50} values of 168, 311, 416 and 586 conidia/larvae whereas that for fresh conidia was 797 conidia/larvae. (10) recorded LC_{50} values of $80.09x10^3$ conidia/ml of wettable powder formulation.

LT_{50} values of WP formulations of *N. rileyi* against II and III instar larvae of *S. litura* : The LT_{50} values were estimated from the data of bioassays of two formulation of

N. rileyi and results are given in Table-2. The data indicated that LT_{50} values of formulation A ($N_{30}S_{1/1}$) at concentration 0.02 per cent was 6.34 days and it was the lowest time registered for 50 per cent kill of II instar larvae compared to formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$). The formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) recorded in 6.38 days for 50 per cent kill of II instar larvae of *S. litura.*

In case of III instar larvae of *S. litura* formulation A registered 7.12 days while formulation B recorded 7.29 days for 50 per cent mortality of larvae of *S. litura.* Thus, it was established from the results that formulation A ($N_{30}S_{1/1}$) taken minimum time to kill 50 per cent population and was most virulent. It was noticed from the comparative performance of two formulations A ($N_{30}S_{1/1}$) and B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) that all caused mortality to II and III instar larvae of *S. litura* but there was significant variation on mortality at all intervals of observation. Their efficiency was found proportionate to bioactive ingredient concentrations. The formulation A ($N_{30}S_{1/1}$) was superior against *S. litura* which was evidenced from LC_{50} and LT_{50} value compared to other formulation.

(11) reported LT_{50} values for first to fifth instar larvae of *S. litura* of *N. rileyi* were 130.71, 137.77, 148.04, 235.55 and 263.10h respectively. Paulina Vega Aquino *et al*. (2010) observed the highest activity of *N. rileyi* in oil against *Spodoptera* spp. with LT_{50} values of 2.5 days.

Bioefficacy of WP formulations of *N. rileyi* against *S. litura* :

II instar larvae : The promising WP formulation A ($N_{30}S_{1/1}$) and formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) having outstanding performance for bioefficacy were evaluated at various doses (Table-3) ranged from 0.01 to 0.04% against II instar larvae of *S. litura* to decide the optimum dose to be used to suppress the pest in the field. The mortality was in the range of 27.50 to 65.0 per cent at 5 DAT. Formulation A ($N_{30}S_{1/1}$) 0.03% and 0.04% caused highest mortality of 65.0 per cent at 5 DAT. The concentrations 0.04% and 0.03% of formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) were on par to the concentration at 0.03% and 0.04% of formulation A ($N_{30}S_{1/1}$) for the effect.

The minimum (22.50%) mortality was noticed in treatment with formulation A and B (27.50%) @ 0.01% at 5 DAT. The treatment with formulation (*N.r.* alone*)* @ 0.02% recorded 27.50 per cent mortality. However, it was 55.00 and 52.50 per cent in treatment with formulation A ($N_{30}S_{1/1}$) and B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.02%. The pattern of the lethal effect at 7 DAT was more or less same. The kill in formulation A ($N_{30}S_{1/1}$) ranged from 42.50 to 82.50 per cent while it was 42.50 to 80.0 per cent in formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$). The formulation A ($N_{30}S_{1/1}$) @ 0.04% showed highest (82.50%) mortality which was on par to formulation A ($N_{30}S_{1/1}$) @ 0.03% (80.0%), B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.04% (80.0%), A($N_{30}S_{1/1}$) @ 0.03% (77.50%) and A ($N_{30}S_{1/1}$) @ 0.025% (77.50%). The effectiveness at 10 DAT was again highest (92.50%) in formulation A ($N_{30}S_{1/1}$) @ 0.04% and @ 0.03% and B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.04%. However, it was at par with formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.03% (90.0%), A ($N_{30}S_{1/1}$) @ 0.025% (87.50%) and B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.025% (85.0%). These were followed by formulation A ($N_{30}S_{1/1}$) @ 0.02% (77.50%) and B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.02% (75.0%). The concentrations at @ 0.01% and @ 0.015% of both formulations showed at par 52.50 and 60.0 to 65.0% mortality of the pest.

III instar larvae : The per cent mortality (Table-3) in the formulation treatments was 15.0 to 55.0 and 42.50 to 82.50 per cent at 5 and 10 DAT, respectively. At 10 DAT, the mortality was highest (82.50%) in the treatment of formulation A ($N_{30}S_{1/1}$) @ 0.04%, which was on par to formulation B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.04% (80.0%), A ($N_{30}S_{1/1}$) @ 0.03% (77.50%), B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.03% (77.50%) and formulation A ($N_{30}S_{1/1}$) @ 0.025% (72.50%) and B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.025% (70.0%). The next best treatments were formulation A ($N_{30}S_{1/1}$) @ 0.02% (65.0%), A ($N_{30}S_{1/1}$) @ 0.015% (52.50%) and B ($N_{30}T_{1/2}G_{2/1}H_{1/1}$) @ 0.015% (47.50%).

The present investigation is in accordance with the findings of (13) who reported higher (95%) mortality of *S. litura* with sunflower oil 2%+tween-80 0.02% formulations of *N. rileyi* than unformulated crude formulation (77.0%). The formulations with sunflower oil registered highest mortality in present investigation as it was also reported by (14) who opinionated that higher efficacy of oil based formulation might be due preventing the desiccation of the conidia, which helps in last survival period and better penetration of peg into the integument. The mortality of larvae increased with increase in days after treatment in present study. It is in conformity with that reported by (4,7,15,16) reported that *N. rileyi* with sunflower oil + triton-x-100 showed 83.90% mortality of *S. litura*. This supported the findings of present investigation. These results are also in conformity with those reported by (10) who opinionated where in groundnut oil registered 96.0% mortality of *S. litura* was used as adjuvants followed by sunflower oil and safflower oil. (16) observed that the mortality of *S. litura* was initiated at 3 days after treatment.

(17) reported that the I instar larvae of *S. litura* were found most susceptible to *N. rileyi* compared to IV instar larvae. They recorded 91.2% mortality of I instar larvae with $1x10^9$ spore/ml. The considerable mortality of *S. litura* by *N. rileyi* was reported by (8,11,15)

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