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Pathogenicity and Bioefficacy of Two Most Promising WP Formulations of *Metarhizium anisopliae* (Metschnikoff) Sorokin 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 *Metarhizium anisopliae* (Metschnikoff) Sorokin. The results revealed that the LC_{50} values of formulation A1 ($M_{30}S_{1/1}C_{1/2}$) were 0.0174% and 0.0180% for II and III instar larvae of *S. litura*, respectively. The LC_{50} values of formulation B1 ($M_{30}S_{1/1}H_{1/1}$) were 0.0149% and 0.0163% against II and III instar larvae of *S.litura*, respectively. LC_{90} values of formulation A1($M_{30}S_{1/1}C_{1/2}$) were 0.0940% and 0.1088% for II and III instar larvae of *S. litura*, respectively. The LC_{90} values of formulation B1 ($M_{30}S_{1/1}H_{1/1}$) were 0.0928% and 0.1006% against II and III instar larvae of *S. litura*, respectively. It indicated that among two larval instar of *S. litura* tested, II instar of larvae was most susceptible to *M. anisopliae* WP formulation A1 ($M_{30}S_{1/1}C_{1/2}$) and B1 ($M_{30}S_{1/1}H_{1/1}$). The data indicated that LT_{50} values of formulation B1 ($M_{30}S_{1/1}H_{1/1}$) at concentration 0.02 per cent was 7.78 days and it was the lowest time registered for 50 per cent kill of II instar larvae compared to formulation A1 ($M_{30}S_{1/1}C_{1/2}$. The formulation A1 ($M_{30}S_{1/1}C_{1/2}$) recorded 8.12 days for 50 per cent kill of second instar larvae of *S. litura.* In case of III instar larvae of *S. litura* formulation B1 ($M_{30}S_{1/1}H_{1/1}$) registered 8.68 days while formulation A1 ($M_{30}S_{1/1}C_{1/2}$) recorded 9.22 days for 50 per cent mortality of larvae of *S. litura.* Thus, it was established from the results that formulation B1 ($M_{30}S_{1/1}H_{1/1}$) taken minimum time to kill 50 per cent population and was most virulent. The mortality at 10 DAT was higher in the formulation B1 ($M_{30}S_{1/1}H_{1/1}$) registered highest (85.0%) mortality of II instar larvae. However, it was at par with formulation B1 0.03% (82.50%), A1 ($M_{30}S_{1/1}C_{1/2}$) 0.04% (82.50%) and A1 ($M_{30}S_{1/1}C_{1/2}$) 0.03% (77.50%). The next promising treatments were formulation B1 ($M_{30}S_{1/1}H_{1/1}$) and A1 ($M_{30}S_{1/1}C_{1/2}$) both 0.025% (72.50 and 70.0%). The concentration 0.01% of both formulations showed up to 50.0 per cent mortality. In case of third instar larvae the highest (82.50%) per cent mortality in formulation B1 ($M_{30}S_{1/1}H_{1/1}$) 0.04% was recorded which was on par to formulation A1 ($M_{30}S_{1/1}C_{1/2}$) 0.04% (80.0%), B1 0.03% (77.50%), and A1 0.03% (77.50%).

Key words : *Metarhizium anisopliae, Spodoptera litura, LT_{50}, LC_{50} and LC_{90}*

Introduction

The use of entomopathogenic fungi due to their amenability to mass production has emerging future in insect pest management. The green muscardine fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin, Moniliales, Moniliaceae is potential entomopathogenic candidates for biological control. (1) was first to isolate fungus *M. anisopliae* from the larvae of grain weevil and also first to demonstrate entomopathogenic nature of the fungus against chrysomelid, curculonid and scarabaeid beetles. *M. anisopliae* capable of infecting more than 100 different insect pests belonging to a variety of insect orders *viz.*, Orthoptera (Grasshopper and Cockroaches), Homoptera (Spittle bug, *Nilaparvata lugens*) and Lepidoptera (*Helicoverpa armigera, S. litura*) (2). (3), reported 80-100% mortality of *H. armigera* by *M. anisopliae*. It also used for the control of *Earias insuana* (4), diamond back moth (5). Shelf life under ambient conditions with the developed formulation (6). This fungus is also used for control of sucking pests of important field crops. Virulence of *M. anisopliae* against mustard aphid (7). *M. anisopliae* is characterized as green muscardine fungus due to green colour of the sporulating colonies. It forms a mycelia mat on cuticle of insects. The infective unit is conidia or blastospores which germinate and forms short germ tube bearing appresoria with infective peg attach to cuticle. The infective peg penetrates in layer of integument by enzymatic dissolution of chitin and protein. It reaches the haemocoel and internal organs and insect is filled with fungus. The death of insects occurs due to obliteration of tissues, also production of toxins (destruxin A,B,C,D,E) and proteolytic enzymes secreted by the fungus. Infected insects show symptoms like loss of

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

Sr. No.	Formulations *M. anisopliae* 3% WP	Host tested (*S. litura* larvae)	Chi-square	Regression equation	LC50 on BAI (%)	Fiducial limit		LC90 on BAI (%)	Fiducial limit	
						Lower	Upper		Lower	Upper
1.	A1 ($M_{30}S_{1/1}C_{1/2}$)	II instar	0.80	Y = 2.8218 + 1.7530 X	0.0174	0.0138	0.0220	0.0940	0.0418	0.2117
2.	A1 ($M_{30}S_{1/1}C_{1/2}$)	III instar	0.51	Y = 2.9360 + 1.6227 X	0.0180	0.0141	0.0229	0.1088	0.0446	0.2649
3.	B1 ($M_{30}S_{1/1}H_{1/1}$)	II instar	1.21	Y = 3.1012 + 1.6162 X	0.0149	0.0112	0.0199	0.0928	0.0413	0.2086
4.	B1 ($M_{30}S_{1/1}H_{1/1}$)	III instar	0.80	Y = 3.0297 + 1.6238 X	0.0163	0.0125	0.0212	0.1006	0.0423	0.2388

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

Sr. No.	Formulations *M. anisopliae* 3% WP	Host tested (*S. litura* larvae)	Chi-square	Regression equation	LT_{50} (days)	Fiducial limit	
						Lower	Upper
1	A1 ($M_{30}S_{1/1}C_{1/2}$)	II instar	0.341	Y = 2.489 + 2.762X	8.117	6.859	10.856
2	A1 ($M_{30}S_{1/1}C_{1/2}$)	III instar	0.714	Y = 1.436 + 3.694X	9.221	8.006	11.896
3	B1 ($M_{30}S_{1/1}H_{1/1}$)	II instar	1.019	Y = 1.929 + 3.447 X	7.780	6.783	9.413
4	B1 ($M_{30}S_{1/1}H_{1/1}$)	III instar	0.364	Y = 1.311 + 3.931 X	8.679	7.641	10.628

appetite, decreased irritability, general or partial paralysis, loss of mobility, discolouration and mummification.

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 *M. anisopliae* 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 and formulation without adjuvants having three replications in completely randomized design were carried out in the biological control laboratory, Dept. of Entomology, MPKV, Rahuri during 2009 to 2012.

Pathogenicity and bioefficacy of formulations of *M. anisopliae* : The bioassay of the two developed WP formulation of *M. anisopliae* 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 *M. anisopliae* 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 (8). The data on cumulative per cent mortality obtained 10 days after inoculation (DAI) were subjected to Probit Analysis (9).

Results and Discussion

Pathogenicity of promising WP formulations of *M. anisopliae:*

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 LC_{50} values of *M. anisopliae* for II and III instar larvae of *S. litura* were determined through bioassay and probit analysis. The results are presented in Table 1. The results revealed that the LC_{50} values of formulation A1 ($M_{30}S_{1/1}C_{1/2}$) were 0.0174% and 0.0180% for II and III

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

Tr. No.	Treatment Formulation	BAI Conc. (%)	Dose g/l	Larval mortality (%)		
				5 DAT	7 DAT	10 DAT
T_1	*M. anisopliae* 5% WP-A1	0.01	2.0	22.50 (28.32)*	32.50 (34.76)	50.00 (45.00)
T_2	*M. anisopliae* 5% WP-A1	0.015	3.0	27.50 (31.63)	42.50 (40.69)	52.50 (46.43)
T_3	*M. anisopliae* 5% WP-A1	0.02	4.0	45.00 (42.13)	60.00 (50.77)	67.50 (55.24)
T_4	*M. anisopliae* 5% WP-A1	0.025	5.0	52.50 (46.43)	60.00 (50.77)	70.00 (56.79)
T_5	*M. anisopliae* 5% WP-A1	0.03	6.0	57.50 (49.31)	72.50 (58.37)	77.50 (61.68)
T_6	*M. anisopliae* 5% WP-A1	0.04	8.0	60.00 (50.77)	72.50 (58.37)	82.50 (65.27)
T_7	*M. anisopliae* 5% WP-B1	0.01	2.0	22.50 (28.32)	42.50 (40.69)	52.50 (46.43)
T_8	*M. anisopliae* 5% WP-B1	0.015	3.0	27.50 (31.63)	42.50 (40.69)	52.50 (46.43)
T_9	*M. anisopliae* 5% WP-B1	0.02	4.0	42.50 (40.69)	60.00 (50.77)	65.00 (53.73)
T_{10}	*M. anisopliae* 5% WP-B1	0.025	5.0	52.50 (46.43)	65.00 (53.73)	72.50 (58.37)
T_{11}	*M. anisopliae* 5% WP-B1	0.03	6.0	62.50 (52.24)	70.00 (56.79)	82.50 (65.27)
T_{12}	*M. anisopliae* 5% WP-B1	0.04	8.0	65.00 (53.73)	75.00 (60.00)	85.00 (67.21)
T_{13}	*M. anisopliae* alone 5%WP	0.02	4.0	22.50 (28.32)	37.50 (37.76)	46.67 (43.11)
T_{14}	Control (water spray)	-	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E+			1.73	2.12	2.56
	C.D.(P=0.05)			4.96	6.06	7.32

*Figures in parentheses are arcsin values, DAT = Days after treatment, M.a. = Metarhizium anisopliae, BAI = Bioactive ingredient, A1 =($M_{30}S_{1/1}C_{1/2}$), B1 = ($M_{30}S_{1/1}H_{1/1}$)

Table-4 : Bioefficacy of WP formulations of *M. anisopliae* 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	*M. anisopliae* 5% WP-A1	0.01	2.0	15.00 (22.79)*	32.50 (34.76)	42.50 (40.69)
T_2	*M. anisopliae* 5% WP-A1	0.015	3.0	25.00 (30.00)	40.00 (39.23)	47.50 (43.57)
T_3	*M. anisopliae* 5% WP-A1	0.02	4.0	40.00 (39.23)	57.50 (49.31)	65.00 (53.73)
T_4	*M. anisopliae* 5% WP-A1	0.025	5.0	47.50 (43.57)	60.00 (50.77)	67.50 (55.24)
T_5	*M. anisopliae* 5% WP-A1	0.03	6.0	52.50 (46.43)	65.00 (53.73)	77.50 (61.68)
T_6	*M. anisopliae* 5% WP-A1	0.04	8.0	55.00 (47.87)	67.50 (55.24)	80.00 (63.44)
T_7	*M. anisopliae* 5% WP-B1	0.01	2.0	17.50 (24.73)	37.50 (37.76)	47.50 (43.57)
T_8	*M. anisopliae* 5% WP-B1	0.015	3.0	25.00 (30.00)	42.50 (40.69)	52.50 (46.43)
T_9	*M. anisopliae* 5% WP-B1	0.02	4.0	40.00 (39.23)	57.50 (49.31)	65.00 (53.73)
T_{10}	*M. anisopliae* 5% WP-B1	0.025	5.0	47.50 (43.57)	62.50 (52.24)	70.00 (56.79)
T_{11}	*M. anisopliae* 5% WP-B1	0.03	6.0	52.50 (46.43)	67.50 (55.24)	77.50 (61.68)
T_{12}	*M. anisopliae* 5% WP-B1	0.04	8.0	55.00 (47.87)	70.00 (56.79)	82.50 (65.27)
T_{13}	*M. anisopliae* alone 5%WP	0.02	4.0	16.67 (24.12)	33.33 (35.24)	46.67 (43.11)
T_{14}	Control (water spray)	-	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E+			2.55	1.64	1.67
	C.D.(P=0.05)			7.30	4.69	4.77

*Figures in parentheses are arcsin values, DAT = Days after treatment, BAI = Bioactive ingredient, A1 = ($M_{30}S_{1/1}C_{1/2}$), B1 = ($M_{30}S_{1/1}H_{1/1}$)

instar larvae of *S. litura*, respectively. The LC_{50} values of formulation B1 ($M_{30}S_{1/1}H_{1/1}$) were 0.0149% and 0.0163% against II and III instar larvae of *S. litura*, respectively. LC_{90} values of formulation A1 ($M_{30}S_{1/1}C_{1/2}$) were 0.0940% and 0.1088% for II and III instar larvae of *S. litura*, respectively. The LC_{90} values of formulation B1 were 0.0928% and 0.1006% against II and III instar larvae of *S. litura*, respectively. It indicated that among two larval instar of *S. litura* tested, II instar of larvae was most susceptible to *M. anisopliae* WP formulation A1 ($M_{30}S_{1/1}C_{1/2}$) and B1 ($M_{30}S_{1/1}H_{1/1}$). The LC_{50} value of *M. anisopliae* formulation B1 (0.0149) showed the lesser value than A1 (0.0174) for II instar larvae. The formulation B1 ($M_{30}S_{1/1}H_{1/1}$) of *M. anisopliae* 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 *M. anisopliae* WP formulations to kill it. These results are in conformity with the results reported by (7,10,11,12,13, 14,15). The results of the bioassays indicated that susceptibility of the pest decreased with the age of the larvae interms 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 *M. anisopliae* 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 (10). (16) 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. (17) recorded LC_{50} values of $80.09x10^3$ conidia/ml of wettable powder formulation. (10) worked out LC_{50} values of *M. anisopliae* for II instar larvae of *S. litura* was $12.52x10^5$conidia/ml. (11) reported the LC_{50} values of *M. anisopliae* isolate MUCL8237 was $21.32x10^5$ conidia/ml.

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 *M. anisopliae* and results are given in Table-2. The data indicated that LT_{50} values of formulation B1 ($M_{30}S_{1/1}H_{1/1}$) at concentration 0.02 per cent was 7.78 days and it was the lowest time registered for 50 per cent kill of II instar larvae compared to formulation A1 ($M_{30}S_{1/1}C_{1/2}$. The formulation A1 recorded 8.12 days for 50 per cent kill of second instar larvae of *S. litura.* In case of III instar larvae of *S. litura* formulation B1 registered 8.68 days while formulation A1 recorded 9.22 days for 50 per cent mortality of larvae of *S. litura.* Thus, it was established from the results that formulation B1 taken minimum time to kill 50 per cent population and was most virulent. It was noticed from the comparative performance of two formulation *viz.,* formulation A1($M_{30}S_{1/1}C_{1/2}$) and formulation B1 ($M_{30}S_{1/1}H_{1/1}$) 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 to be proportionate to BAI concentrations. The formulation B1 ($M_{30}S_{1/1}H_{1/1}$) found superior in causing mortality which is evidenced from LC_{50} and LT_{50} value compared to other formulation.

The LT_{50} values that were calculated at highest concentrations of the *M. anisopliae* were 101.16, 116.51 and 149.75h for 4-5, 10-11 and 15-16 days old larvae of *S. litura* (11,15).

Bioefficacy of WP formulations of *M. anisopliae* against *S. litura*

II instar larvae : The formulation A1 ($M_{30}S_{1/1}C_{1/2}$) and formulation B1 ($M_{30}S_{1/1}H_{1/1}$) evaluated at concentrations (Table-3) of 0.01 to 0.04% against II instar larvae of *S. litura* to find out the suitable dose to be used in the field. The mortality among treatments was in the range of 22.50 to 65.0 per cent at 5 DAT. Formulation B1 0.03% and 0.04% registered highest (62.50 and 65.0%) mortality at 5 DAT. The concentrations at 0.04% and 0.03% of formulation B1 were on par to the concentration 0.04% and 0.03% of formulation A1 recording 60.0 and 57.50 per cent mortality, respectively. The minimum (22.50%) mortality was observed in treatment with formulation A1 and B1 0.01%. The treatment with control (*M.a.* alone) 0.02% recorded 22.50 per cent kill, when it was 45.0 and 42.50 per cent in treatment with 0.02% each of formulation A1 and B1. The trend of mortality at 7 DAT was more or less same. The mortality in formulation A1 ranged 32.50 to 72.50 per cent; while, it was 42.50 to 75.0 per cent in formulation B1. The mortality at 10 DAT was higher in the formulation B1 registered highest (85.0%) mortality of II instar larvae. However, it was at par with formulation B1 0.03% (82.50%), A1 0.04% (82.50%) and A1 0.03% (77.50%). The next promising treatments were formulation B1 and A1 both 0.025% (72.50 and 70.0%). The concentration 0.01% of both formulations showed up to 50.0 per cent mortality.

III instar larvae : The per cent mortality (Table-4) in the treatments was 15.0 to 55.0, 32.50 to 70.0 and 42.50 to 82.50 per cent at 5, 7 and 10 DAT, respectively. It was highest (82.50%) in formulation B1 ($M_{30}S_{1/1}H_{1/1}$) 0.04%; which was on par to formulation A1 ($M_{30}S_{1/1}C_{1/2}$) 0.04% (80.0%), B1 0.03% (77.50%), and A1 0.03% (77.50%). The next best treatments were formulation B1 0.025% (70.0%), A1 0.025% (67.50%), B1 and A1 0.02% (65.0%). It was established from the study on bioefficacy of final stage WP formulation of *N. rileyi* and *M. anisopliae* against II and III instar larvae of *S. litura* that there was increase in mortality with increase in dose and decrease in duration for lethal effect. The cumulative performance of formulations at test concentrations proved that the dose of 8, 6 and 5g/l of formulation both fungi were equality (at par) effective. The dose of 4g/l of the formulations was found next best. Hence, the dosages of 4, 5 and 6g/l of water were selected for evaluation of the bioefficacy of entomopathogenic fungi formulations under field conditions.

(14) reported that *M. anisopliae* gave the highest mortality of the II instar larvae and IV instar larvae of *S.litua* with lethal time 7 and 10 days, respectively. The LT_{50} values that were calculated at highest concentrations

of the *M. anisopliae* were 101.16, 116.51 and 149.75h for 4-5, 10-11 and 15-16 days old larvae of *S. litura* (11,15).

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