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Evaluation of various aqua suspension formulations of *Metarhizium anisopliae* (Metschnikoff) Sorokin

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ABSTRACT

Studies on evaluation of eleven aqua suspension formulations of entomopathogenic fungus, *Metarhizium aniosopliae* comprising 1) *M.a.*+ TW(0.5%) + CMC (0.5%) 2) *M.a.*+SFO(1.0%) + CMC(0.5%),3) *M.a.*+ SFO(1.0%)+HO(1.0%) 4) *M.a.*+ GNO(0.5%) + BA (2.0%),5) *M.a.*+ GNO(0.5%) + CMC(0.5%),6) *M.a.*+ GNO (0.5 %) + GH (0.5%), 7) *M.a.*+ GH (0.5%)+HO (1.0%), 8) Control (*M.a.*alone) were carried out. At 3 DAI, AS formulation with *M.a.*+GNO(0.5%)+CMC (0.5%) registered significantly highest (88.33%) surface coverage. The next promising formulations were *M.a.*+ SFO(1.0%) + CMC (0.5%) (78.33%), *M.a.*+ GNO (0.5%)+GH(0.5%) (56.67%) and *M.a.* + GH (0.5%) + HO (1.0%) (51.67%). Least (8.33%) growth of the fungus was recorded in *M.a.*+TW (0.5%)+CMC(0.5%); when, control recorded 30.0 per cent surface coverage. On 10 DAI, the differences (6.67 to 11.43g) for biomass production were significant. *M.a.* + SFO (1.0%)+CMC (0.5%) maintained its superiority over rest of the formulations by producing 11.43g biomass. However, it was at par with *M.a.*+SFO(1.0%)+HO(1.0) (11.10g). Maximum cfu count was observed in *M.a.*+SFO (1.0%)+ HO (1.0%) ($2.33x10^9$). However, it was at par with *M.a.*+SFO (1.0%) + CMC (0.5%) ($2.27x10^9$). The least (30.0%, 6.67g and $17.672x10^8$ cfu/ml) surface coverage biomass and cfu count were recorded in control *i.e.* the formulation (*M.a.*alone), respectively. In case of bioefficacy of various formulations against larvae of *Spodoptera litura*, the formulation with *M.a.*+ SFO (1.0%) + HO(1.0%) registered significantly highest mortality of 80.00 and 73.33 per cent of II and III instar larvae of *S.litura.*

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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. Metschnikoff (1879)

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*) (McCoy *et al*.,1988). Gopalkrishnan and Narayan (1987), reported 80-100 per cent mortality of *H.armigera* by *M.anisopliae*. It also used for the control of *Earias insuana* (Aly and Rashad, 1997), diamond back moth (Silva *et al*., 2003). This fungus is also used for control of sucking pests of important field crops. Virulence of *M. anisopliae* against mustard aphid (Pandey and Kanaujia, 2004). *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 appetite, decreased irritability, general or partial paralysis, loss of mobility, discolouration and mummification.

The formulation of the fungi still awaits a serious efforts in formulation technology. Exploring formulation of *M. aniosopliae* as a tool in the pest management of Lepidopteran pests is one of requisite mandate. The foregoing problem can largely be overcome by developing suitable formulations. The performance and shelf life can be improved by adding suitable adjuvants subsequently leading to growth, development and viability of the fungus that may act as nutrient, adhesive, UV protectants, wetting agents etc. For developing wettable powder formulation, basic research on standardization of bioactive ingredient and suitable adjuvants is necessary before the formulation. Studies on aqua suspension formulations were limited. Hence, the present study was undertaken with an object to determine the effectiveness of aqua suspension formulations of *M.aniosopliae* for growth, development, viability and bioefficacy against *Spodoptera litura*.

MATERIAL AND METHODS

Fungus culture :

The pure fungus culture of *M.aniosopliae* was made, available from isolates in Biological control laboratory, Dept. of Entomology, MPKV, Rahuri

Laboratory studies with eight aqua suspension formulations having three replications in completely randomized design were carried out in the biological control laboratory, Dept. of Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri during 2009 to 2012.

Aqua suspension formulations of *M.aniosopliae* :

The highly promising eight formulations with tween-80 + carboxymethyl cellulose, sunflower oil + carboxymethyl cellulose, sunflower oil+honey,groundnut oil + boric acid, groundnut oil + carboxymethyl cellulose, groundnut oil + *Ghee, Ghee*+ honey and control without adjuvants (*M.anisopliae* alone) of *M.anisopliae* were tested for their growth, development, viability and bioeffficacy against II and III instar larvae of *S.litura.*

Effect on growth and development of *M. aniosopliae*:

Various formulations were made and tested for growth and development of *M. aniosopliae.* The bottles were plugged with cotton wool and incubated at ambient temperature. One ml of the formulated liquid was added to 40 ml Sabouraud's dextrose broth with yeast extract medium in glass bottle and closed with cotton wool. The whole process was carried out in laminar flow cabinet. The observations on per cent surface coverage by fungus on 3rd, 7th and 10th days and fungal biomass on 10th day after inoculation were noted. The experimental data were subjected to statistical analysis. These experiments were carried out in CRD with three replications.

The promising 8 formulations of *M. aniosopliae* were tested for their growth, development and viability.

The viability index was worked out by designing following formula :

$$\text{Viobility index} = \frac{T\,(E \times F) - C\,(E \times F)}{C\,(E \times F)} \times 100$$

where,

T (E × F) = Biomass in treatment × cfu in treatment

C (E × F) = Biomass in control × cfu in control.

Testing cfu count of formulation of *M.aniosopliae*:

The method suggested by Ming *et al.* (1990) was used. The autoclaved Sabouraud's dextrose agar with

yeast extract (SDA) medium in petridishes, (100 mm diameter) was inoculated with the help of micropipette by releasing 1 ml *M.aniosopliae* suspension prepared in the distilled water in laminar flow cabinet. Other petridishes with the medium were prepared in similar manner and inoculated with various dilutions in the series (10^1 to 10^{10} cfu/ ml) at 27 $\pm$ 1^0C. After 48 hrs from the 10 samples in each petridishes the numbers of colonies/ petrideshes were counted and cfu/ml was calculated.

Bioefficacy of aqua suspension formulations of *M.aniosopliae* :

The promising 8 formulations of *M.aniosopliae* as mentioned above were tested against II and III instar larvae of *S.litura*. One ml of each preparation was mixed in 99 ml of water and sprayed on II and III instar larvae of *S.litura*.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 upto 10 days. Per cent mortality was calculated and corrected by formula given by Abbott (1925).

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Potential for growth, development and viability:

Effect on growth :

The data on the growth, development and viability of *M. anisopliae* (AS) and the results are presented in Table 1 and depicted in Fig.1. At 3 DAI, T_5- *M.a.* +

Table 1: Influence of advanced test formulations of *M.anisopliae* (AS) on growth, development and viability

Tr. No	Treatments	Conc. adj. (%)	Surface coverage (%)			Biomass g/40ml medium	cfu (x10^8 /ml	Viability Index	pH at 10 DAI
			3 DAI	7 DAI	10 DAI				
1.	*M.a.*+ TW+CMC	0.5+0.5	8.33 (16.78)*	91.67 (73.26)	100.00 (90.00)	10.23	18.33 (4.33)**	59.10	7.10
2.	*M.a.*+ SFO+CMC	1.0+0.5	78.33 (62.24)	100.00 (90.00)	100.00 (90.00)	11.43	22.67 (4.81)	119.85	8.01
3.	*M.a.*+ SFO+HO	1.0+1.0	36.67 (37.29)	100.00 (90.00)	100.00 (90.00)	11.10	23.33 (4.88)	119.72	8.03
4.	*M.a.*+ GNO+BA	0.5+2.0	31.36 (34.27)	100.00 (90.00)	100.00 (90.00)	10.70	19.33 (4.45)	75.49	8.04
5.	*M.a.*+ GNO+CMC	0.5+0.5	88.33 (70.00)	100.00 (90.00)	100.00 (90.00)	10.67	19.00 (4.42)	72.01	8.08
6.	*M.a.*+ GNO+GH	0.5+0.5	56.67 (48.85)	100.00 (90.00)	100.00 (90.00)	10.70	18.67 (4.38)	69.50	8.17
7.	*M.a.*+ GH+HO	0.5+1.0	51.67 (45.97)	100.00 (90.00)	100.00 (90.00)	10.77	19.33 (4.45)	76.63	8.50
8.	Control (*M.a.*alone)	-	30.00 (33.21)	100.00 (90.00)	100.00 (90.00)	6.67	17.67 (4.26)	-	8.10
	S.E.+		1.42	0.66	-	0.04	0.05	-	-
	C.D. (P=0.05)		4.29	1.99	-	0.11	0.14	-	-

*Figures in parentheses are arc sin values. **Figures in parentheses for cfu are values $\sqrt{n+0.5}$

DAI = Days after inoculation *M.a.* = *Metarhizium anisopliae*
TW = Tween-80 CMC = Carboxymethyl Cellulose
SFO = Sunflower oil HO = Honey
GNO = Groundnut oil BA = Boric acid GH = *Ghee*

GNO + CMC registered significantly highest (88.33%) surface coverage. The next promising formulations were T_2- *M.a.*+ SFO+CMC (78.33%), T_6- *M.a.*+ GNO+GH (56.67%) and T_7- *M.a.*+GH+HO (51.67%). Least (8.33%) growth of the fungus was recorded in T_1- TW+CMC; when, control recorded 30.0 per cent surface coverage. At 7 DAI, all the formulations, except T_1- *M.a.*+TW+CMC (91.67%) recorded significantly cent per cent surface area coverage by fungus. At 10 DAI, all treatments including control covered cent per cent surface of the medium.

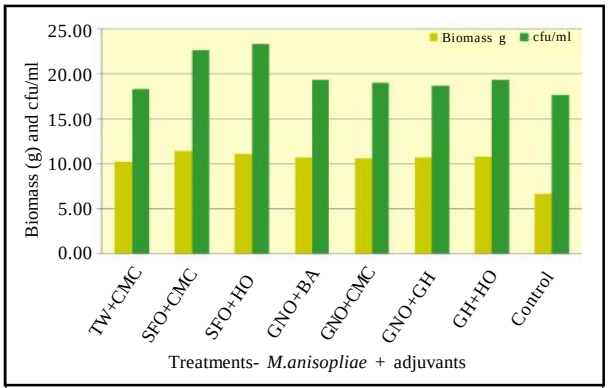

Fig. 1 : Influence of advanced test formulations (AS) of M. anisopliae+ on biomass and viability

Effect on biomass and pH :

On 10 DAI, the differences (6.67 to 11.43g) for biomass production were significant. T_2- *M.a.* + SFO + CMC maintained its superiority over rest of the formulations by producing 11.43 g biomass. However, it was at par with T_3- *M.a.*+SFO+HO (11.10g). The most pH of *M.anisopliae* cultures developed from the inoculums in highly promising formulations was 7.10 to 8.17 except pH of 8.50 in T_7- *M.a.*+GH+HO and 8.10 in T_8-Control. Although the adjuvants in the formulations are variable but ultimately at 10 DAI the fungus maintained the pH between 7.10 to 8.50 and produced the higher biomass of 10.23 to 11.43g than the 6.67g/40 ml medium in control. On the basis of results of pH, it has been established that requirement of neutral pH for all the entomopathogenic fungi is not necessary parameter as part of quality control or insisted in Insecticide Act. There is no published information in relation of pH, growth and development of *M.anisopliae* or any other mycoagent.

Effect on viability (cfu/ml) :

The viability in terms of cfu/ml was significantly different in test formulations. Maximum cfu count was observed in T_3-*M.a.*+SFO+HO ($2.33x10^9$). However, it was at par with T_2- *M.a.*+SFO+CMC ($2.27x10^9$). Maximum (119.85) viability index was registered in T_2- SFO+CMC. On the basis of the index next best formulation was followed by T_3- *M.a.*+ SFO+HO (119.72) and T_7-*M.a.*+GH+HO (76.63).

It was evident from the study that the adjuvants substantially contributed to cfu. T_2- *M.a.*+SFO+CMC and T_3- *M.a.* + SFO+HO of *M.anisopliae* were the best formulations resulted in higher cfu than control with higher viability index than rest ones. These were considered as highly potent formulations for highest growth and biomass production of entomopathogenic fungi.

Prior *et al.* (1988) reported that mixtures of oil and powder formulations stimulated the mycelial growth of *M. anisopliae*. Alves *et al*. (2001) observed that oil formulation did not cause any negative effect on conidial germination of *M.anisopliae*. Rodriguez Colorado *et al*. (2002) reported that viability was greater than 90 per cent where maize oil adjuvant; while it was less than 85 per cent in glycerine and neem oil. Wiwat (2004) found that carbohydrates (glucose, lactose and sucrose) were essential source for germination and conidial production of *N.rileyi*. The highest biomass production (21.88 mg/ml) of *M.anisopliae* for sunflower oil was reported by Silva *et al*. (2005). The utility of oils and carbohydrates in present findings are in corroboration with the reports as above investigators.

Bioefficacy of *M.anisopliae* against larvae of *S.litura*:

The II instar larvae :

Data presented in Table 2 and depicted in Fig.2 revealed that the larval mortality among the treatments ranged from 16.67 to 46.67, 37.50 to 66.67 and 50.00 to 80.00 per cent at 5,7 and 10 DAT, respectively. There was significant variation in mortality among the treatments; all the treatments were superior over untreated control which reported zero per cent mortality upto last 10 DAT. T_3- *M.a.*+SFO+HO registered significantly highest mortality of 50.0 per cent at 5 DAT. However, it was at par with T_2- *M.a.*+SFO +CMC and T_6- *M.a.*+ GNO + GH which recorded 43.33 to 46.67 per cent mortality. The pattern of mortality at 7 DAT was more or less same.

At 10 DAT, the kill was again significantly highest

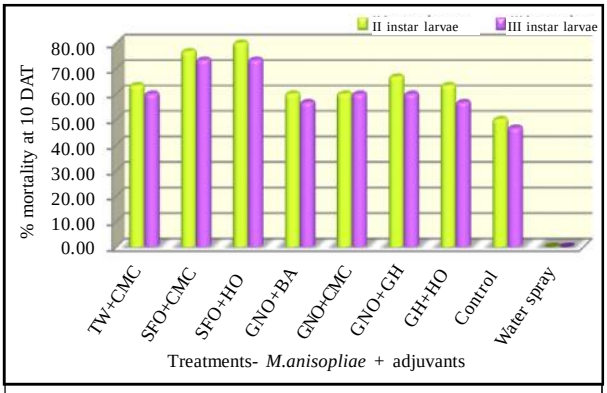

Fig. 2 : Influence of advanced test formulations (AS) of *M. anisopliae* on mortality of II and III instar larvae of *S. litura*

(80.0%) in formulation with adjuvants *M.a.*+SFO+HO (T_3). However, it was at par with T_2- *M.a.*+SFO+CMC (76.67%). The next promising formulations for larval morality were T_6- *M.a.*+GNO+GH (66.67%), T_1- *M.a.*+TW+CMC (63.33), T_7- *M.a.*+GH+HO (63.33%), T_4- *M.a.*+GNO+BA (60.0) and T_5- *M.a.*+GNO+CMC (60.0%). The lowest mortality (50.0%) was recorded in T_{11}- control (*M.a.*alone).

The III instar larvae:

The per cent larval mortality was in the range of 13.33 to 40.0, 26.67 to 56.67 and 46.67 to 73.33 at 5, 7 and 10 DAT, respectively. At 5 DAT, T_3- *M.a.*+SFO+HO recorded significantly highest larval mortality of 40.0 per cent. The rest of the formulations were significantly superior to control (13.33%). However, T_3- *M.a.* + SFO + HO was at par with T_2- *M.a.*+SFO+CMC (36.67%), T_6- *M.a.*+GNO+GH (33.33%), T_5- *M.a.* + GNO + CMC (30.00%) and T_7- *M.a.*+GH+HO (30.0%). The pattern of larval mortality was more or less same at 7 DAT. At 10 DAT, all the formulations were significantly superior (56.67 to 73.33%) than control (*M.a.*alone) (46.67%) for the larval mortality. T_2- *M.a.*+SFO+CMC and T_3- *M.a.*+SFO+HO (73.33% each) were proved to be significantly superior to the rest of the treatments. The next promising formulations were T_1- *M.a.*+TW+CMC, T_5- *M.a.*+GNO+CMC and T_6- *M.a.*+GNO+GH, (60.0% each). Lowest larval mortality of 46.67 per cent was recorded in control (*M.a.*alone).

The formulations, T_2- *M.a.*+SFO+CMC and T_3- *M.a.*+SFO+HO were most promising for *M.anisopliae* growth and development among 8 formulations. These

Table 2 : Effect of advanced test formulations of *M.anisopliae* (AS) on mortality of II and III instar larvae of *S. litura*

Tr. No.	Treatment	Conc. of adjuvant(%)	Larval mortality (%) II instar			Larval mortality (%)III instar		
			5 DAT	7 DAT	10 DAT	5 DAT	7 DAT	10 DAT
1.	*M.a.*+ TW+CMC	0.5+0.5	33.33	53.33	63.33	23.33	43.33	60.00
			(35.24)*	(46.89)	(52.71)	(28.86)*	(41.15)	(50.77)
2.	*M.a.*+ SFO+CMC	1.0+0.5	46.67	63.33	76.67	36.67	53.33	73.33
			(43.11)	(52.71)	(61.14)	(37.29)	(46.89)	(58.89)
3.	*M.a.*+ SFO+HO	1.0+1.0	50.00	66.67	80.00	40.00	56.67	73.33
			(45.00)	(54.76)	(63.44)	(39.23)	(48.85)	(58.89)
4.	*M.a.*+ GNO+BA	0.5+2.0	26.67	46.67	60.00	16.67	36.67	56.67
			(31.11)	(43.11)	(50.77)	(24.12)	(37.29)	(48.85)
5.	*M.a.*+ GNO+CMC	0.5+0.5	36.67	50.00	60.00	30.00	43.33	60.00
			(37.29)	(45.00)	(50.77)	(33.21)	(41.15)	(50.77)
6.	*M.a.*+ GNO+GH	0.5+0.5	43.33	56.67	66.67	33.33	43.33	60.00
			(41.15)	(48.85)	(54.76)	(35.24)	(37.29)	(50.77)
7.	*M.a.*+ GH+HO	0.5+1.0	36.67	53.33	63.33	30.00	43.33	56.67
			(37.29)	(46.89)	(52.71)	(33.21)	(41.15)	(48.85)
8.	Control (*M.a.* alone)	-	16.67	37.50	50.00	13.33	26.67	46.67
			(24.12)	(37.76)	(45.00)	(21.39)	(31.11)	(43.11)
	S.E. +		2.18	2.40	2.64	2.09	1.87	2.01
	C.D.(P=0.05)		6.48	7.13	7.84	6.20	5.55	5.98

*Figures in parentheses indicate arcsin values. DAI = Days after inoculation
M.a. = *Metarhizium anisopliae* TW = Tween-80 CMC = Carboxymethyl Cellulose SFO =Sunflower oil
HO = Honey GNO = Groundnut oil BA = Boric acid GH = *Ghee*

included combination of sunflower oil, honey and carboxymethyl cellulose with mycoagent, *M. anisopliae.* Many scientist experienced effectiveness of *M.anisopliae*.

There is no literature on synergistic effect of *M. anisopliae* formulation containing sunflower oil, CMC and honey as best adjuvants in most promising formulations against *S.litura* in present study. So few relevant references in support of the results on bioefficacy have been accordance with the findings of scientists. Ramoska (1984) noted that *M.anisopliae* conidia in oil caused much infection at low humidity and penetration at intersegmental membrane of chinch bug. Prior *et al*. (1988) pointed out that the oil formulation of aerial conidia of *M.anisopliae* are more efficacious. Kaaya and Hassan (2000) observed that *M.anisopliae* oil formulation (10^9conidia/ml) inducted 100 per cent mortality in larvae of *Rhipicephalus appendiarlates.* Alves *et al*. (2001) reported that the fungal activity was enhanced by 2.25 per cent adjuvant oil and effectivity against *T.molitor*. Malsam *et al*. (2002) reported that the increase in efficacy of *M. anisopliae* for the control of white fly by addition of sublethal concentrations of oil. The sunflower oil gave higher synergistic effect reaching nearly 100 per cent control and increasing and speed of action. The fungus at concentration of $5x10^6$ conidia/ml of *M.anisopliae* recorded 66.7 to 100 per cent mortality of white fly and also killed the nymphs and adults of red spider mite within 3 to 4 days.

Liu Bing Lan *et al*. (2000) reported that maltose and peptone were the best carbon and nitrogen sources for the production of destruxins. Sharma *et al*. (1998) reported that the addition of carboxy methyl cellulose to the *M.anisopliae* reduced time to 100 per cent mortality by more than a week in first instar larvae of *Holotrichia consanguinea.* The present investigation is in conformity with the results reported by Dayakar and Kanaujia (2001 and 2003); Pandey and Kanaujia (2004); Purvar and Sachan (2005); Amer *et al*. (2008) and Dayakar and Subbarao (2011) who reported the considerable mortality of *S.litura*.

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