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Research article

LIQUID BIOFORMULATION OF *METARHIZIUM ANISOPLIAE* IS EFFECTIVE FOR THE MANAGEMENT OF COW PEA MOSAIC DISEASE

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ABSTRACT: Liquid bioformulation of *Metarhizium anisopliae* amended with oils and adjuvants was prepared. Six oils *viz*., sunflower, safflower, soybean, mustard, arachnid and coconut oils at three different concentrations, 0.1, 0.5 and 1.0 per cent and three adjuvants *viz*., glycerol @ 5, 8 and 10 per cent; tween-80 @ 0.02, 0.04, 0.06 and 1.0 per cent and arachnid oil @ 1, 5 and 10 per cent were tested to see their effect on growth and development of *M. anisopliae* in the bioformulation. Liquid bioformulation of *M. anisopliae* amended with Glycerol (10.0%) + Sunflower oil (0.5%) was found significantly effective which showed 84.33 per cent and 94.93 per cent higher surface area covered and biomass production respectively than the control. Efficacy of the bioformulation was tested against cow pea aphid, *Aphis craccivora* in pot as well as field condition. Liquid formulation of *M. anisopliae* supplemented with Glycerol (10.0%) + Sunflower oil (0.5%) was found to be significantly effective causing aphid mortality of 80 per cent at 30 days after spraying with protection of secondary spread of cow pea mosaic disease up to 100 per cent and 96.03 per cent in pot and field condition respectively. Spraying liquid formulation of *M. anisopliae* amended with Glycerol (10.0%) + Sunflower oil (0.5%) at 15 days interval for twice proved to be the best treatment with highest yield of 24.03 t/ha with a cost benefit ratio of 1:7.95 as compared to control (11.20 t/ha).

Key Words: Adjuvant, bioformulation, cow pea aphid, cow pea mosaic disease, *Metarhizium anisopliae*, oil

INTRODUCTION

Cow pea, *Vigna unguiculata* L. Walp an economiclly important legume crop is highly prone to cow pea aphid (*Aphis craccivora* Koch) with 80-100 per cent damage, by transmitting cow pea mosaic disease. With the growing interest towards biological approaches for management of pests and diseases. Entomopathogenic fungi like *Metarhizium anisopliae* (Metschnikoff)

Sorokin is one of the potential biological control agents against the cow pea aphid and as one component of integrated pest management (IPM) systems (Dutta. P, et al., 2013). In biological control, epizootics usually results from insects being infected directly by aerial conidia from sporulating cadavars (Hall. R.A, 1980; Hall. R. A and Burges. H. D, 1979) or mycelial conidia of foliage (Hall. R. A, 1982). Most of the biocontrol agents are formulated in solid form (contain only conidia) with shelf life of 6 months at 5ºC and 3 months at 25-35ºC. In the present study we hypothesized to standardize a liquid formulation protocol of *M. anisopliae* supplemented with oils, therefore the present study was conducted to see the effect of adjuvants and oils in bioformulation of *M. anisopliae* with higher shelf life and virulence.

MATERIALS AND METHODS

The experiment was carried out at Mycology Research Section, Department of Plant Pathology, Assam Agricultural University (AAU), Jorhat, Assam during 2012-13.

Culture of *M. anisopliae*

Pure fungus culture of *M. anisopliae* (ITCC No. 8882.12) isolated from naturally infested cow pea aphid, *Aphis craccivora* from Majuli island of Assam was collected from cultural bank of Mycology Research Section, Department of Plant Pathology, AAU, Jorhat. The fungus culture was maintained on Potato Dextrose Agar (PDA) medium throughout the experimentation period.

Preparation of formulation

Liquid based bioformulation was prepared in Potato Dextrose Broth (PDB) with conidial concentration of 1×10^6 cfu ml^{-1}. Effects of a range of adjuvants and vegetable oils at different concentration were amended to standardize the liquid formulation protocol. Observation on the growth and development of *M. anisopliae.*

Effect of adjuvants and oils in liquid formulation

Adjuvants like glycerol @ 5, 8 and 10 per cent; tween-80 @ 0.02, 0.04, 0.06 and 1.0 per cent and arachnid oil @ 1, 5 and 10 per cent and vegetable oils *viz.*, sunflower, safflower, soybean, mustard, arachnid and coconut oils at 0.1, 0.5 and 1.0 per cent were tested by adding in the optimum concentration ($1X10^6$ cfu/ml) of liquid formulation for satisfactory growth and development of *M. anisopliae*. One ml of each formulated medium was added individually to 40 ml of PDB medium as inoculants in 250 ml capacity erlenmeyer flask and incubated in BOD incubator at 21±1°C for 15 days. The experiment was replicated thrice and arranged in Completely Randomized Design (CRD). Observations on surface area covered (%) and biomass produced (g) were taken by visual observation at 3, 5, 7, 10 and 15 days after treatment.

Efficacy of liquid bioformulation of *M. anisopliae* against *A. craccivora* in pot condition

The experiment was conducted in plastic pots of 5 Kg capacity against the aphid, *A. craccivora*, vector of cow pea mosaic disease. Liquid formulation of *M. anisopliae* ($1X10^6$ spores/ml) mixed with best concentration of adjuvants and vegetable oils *viz*., Glycerol 10 per cent +Sunflower oil @ 0.5 per cent were sprayed thoroughly so that it yield 600 lit/ha. Two sprayings were done by the atomizer No 600 (Holm spray, Make: T.J. Holmes. Co. Inc. Charly, Mass) at an interval of 15 days starting from 10 days of sowing. Control treatment was sprayed with sterile water. Observations on estimation of aphid infestation, mortality of aphid and disease incidence were recorded. Estimation of aphid infestation was done by counting per cent leaf and shoot infestation per plant per pot. Aphid mortality was recorded at 15 and 30 days of spraying. Per cent disease incidence was calculated by using following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plant units}}{\text{Total number (healthy and infected) of units assessed}} \times 100$$

Test for efficacy of liquid bioformulation of *M. anisopliae* in field condition

The experiment was conducted during summer season, 2013-2014 at Experimental Farm, Department of Horticulture, AAU, Jorhat, Assam. Liquid formulation of *M. anisopliae* amended with best concentration of adjuvants and vegetable oils *viz*., Glycerol @ 10 per cent + Sunflower oil @ 0.5 per cent @ $1X10^6$ spores/ ml was sprayed thoroughly with the help of Knapsack sprayer (Make: Aspee). Per cent incidence of aphid was calculated by counting the healthy and infected plants per plot. Mortality of aphids and disease incidence were recorded as per the method mentioned in pot experiment. Pod yield for different treatment were recorded at the time of harvest and cost benefit ratio was worked out.

RESULTS AND DISCUSSION

Effect of adjuvants and vegetable oils on the bioformulation

In the present study liquid bioformulation amended with adjuvants and oils showed better performance in terms of surface area covered (%) and biomass production (g) than the untreated control. Amongst the adjuvants, glycerol @ 10.0 per cent was found significantly effective covering highest surface area of 98.33 per cent followed by glycerol @8.0 per cent (96.00%) on 15th day after treatment (Table 1).

Table 1. Effect of adjuvants on liquid bioformulation of *M. anisopliae*

Treatments	Concentration (%)	Surface area covered (%) by *M. anisopliae* after					Biomass on 15th day/40 ml (g)
		3 days	5 days	7 days	10days	15days	
Control (Only *M. anisopliae*)	-	5.43	10.00	10.22	15.00	15.17	0.20
Glycerol	5.0	19.63	31.50	39.83	39.83	50.33	2.52
	8.0	21.33	30.33	66.33	85.00	96.00	3.53
	10.0	23.33	33.33	80.00	86.67	98.33	3.62
Tween-80	0.02	5.17	15.00	15.00	15.00	15.25	0.22
	0.04	9.52	15.17	16.83	17.17	20.00	0.24
	0.06	10.23	16.92	18.33	20.00	20.83	0.26
	1.0	19.00	20.07	24.33	24.17	25.00	0.41
Arachnid oil	1.0	10.00	10.17	14.33	15.00	15.00	0.21
	5.0	5.51	10.42	10.47	18.50	18.50	0.23
	10.0	10.03	20.20	20.08	21.83	22.00	0.28
SEd(±)		0.17	0.06	0.25	0.89	0.11	0.03
$CD_{0.05}$		0.32	0.15	0.51	1.52	0.24	0.08

Data are mean of three replications

In case of biomass production at 15 days of treatment all the treatments were significantly superior to control. Glycerol @ 10.0 per cent was found significantly effective producing highest biomass of 3.62 g followed by Glycerol @ 8.0 per cent (3.53 g). Both these two treatments showed significantly higher biomass production than Glycerol @ 5.0 per cent (2.52 g). Liquid formulation amended with Tween-80 @ 1.0 per cent was found significantly effective with biomass of 0.41 g than 0.02, 0.04 and 0.06 per cent (Table 1). This might be due to addition of adjuvants which provides moisture for the fungus to remain active for longer period since adjuvants doesn't evaporate faster (Kaaya. B. P, et al., 2011). Adjuvants might helps in maintaining the quality of conidia, i.e., maintain vigour of conidia as well as other propagules**.**

Table 2. Effect of vegetable oils on liquid bioformulation of *M. anisopliae*

Treatments	Concentration (%)	Surface area covered (%) by *M. anisopliae* after					Biomass on 15th day/40 ml (g)
		3 days	5 days	7 days	10 days	15 days	
Control (*M. anisopliae* alone)		2.00	7.67	20.00	35.00	50.00	1.35
Sunflower oil	0.1	4.00	21.67	33.33	78.33	83.33	2.52
	0.5	4.67	40.00	90.00	100.00	100.00	4.06
	1.0	4.33	35.00	85.00	93.33	98.67	3.59
Safflower oil	0.1	2.00	15.00	20.00	35.00	65.17	1.55
	0.5	3.00	20.33	43.33	85.17	98.67	3.97
	1.0	3.27	31.67	86.67	95.00	99.67	4.04
Soybean oil	0.1	2.00	9.00	31.67	33.33	35.00	1.63
	0.5	2.00	24.80	41.67	50.00	70.33	2.03
	1.0	5.00	25.07	61.67	91.67	98.50	2.55
Mustard oil	0.1	2.00	10.00	21.67	50.00	60.25	1.70
	0.5	2.00	21.67	30.00	50.00	83.33	2.06
	1.0	3.00	30.00	40.00	50.00	91.67	2.12
Arachnid oil	0.1	1.00	5.00	10.00	45.00	66.67	1.78
	0.5	1.00	15.00	35.00	55.00	83.33	2.56
	1.0	2.00	21.17	80.00	85.17	95.17	3.20
Coconut oil	0.1	2.00	19.33	31.67	40.00	63.33	1.55
	0.5	3.00	20.25	34.83	40.00	78.33	2.14
	1.0	5.00	30.00	85.00	90.00	95.00	2.42
SEd(±)		0.08	0.04	1.43	1.95	0.11	0.05
$CD_{0.05}$		0.23	0.08	2.91	3.58	0.23	0.10

Data are mean of three replications

Observations on surface area covered (%) at 3 days after treatment revealed that all treatment combinations of sunflower oil were significantly superior (4.00 to 4.67%) to liquid formulation alone (2.00%) for coverage of surface growth (Table 2). On 15th day after treatment, highest surface area was covered (100.0%) by *M. anisopliae* treated with sunflower oil @ 0.5 per cent followed by safflower oil @ 1.0 per cent (99.67%). Both the treatments were significantly superior then rest of the treatments. All the oil treated liquid formulation covered higher surface area than untreated control (50.00%). Hunt. T. R, et al., 1994; Moore. D, et al., 1993 and Shah. P. A, et al., 1998 reported that addition of oils in the bioformulations is one of the strategies for protecting microbial biopesticides in liquid formulations from solar degradation. Similarly, Legand. J. E. (2001) reported that use of oil-based formulations appear to provide three important advantages over water-based formulations, these include: 1) *M. anisopliae* var. *acridum* is more infective at low humidity in oil-based formulations (Bateman. R. P, et al., 1993 and Prior. C, et al., 1988); 2) oil-based formulations allow the fungal biopesticide to be applied in a desiccated state, which reduces effects of thermal stress (McClatchie. G, et al., 1994; Hedgecock. S, et al., 1995 and Hong. T. D, et al., 1997, 1998). Observations on combined effect of adjuvant (Glycerol @ 10.0%) and oils (Sunflower oil @ 0.5 and 1.0%) revealed that liquid formulation amended with 10.0 per cent glycerol and 0.5 per cent sunflower oil was found significantly effective covering highest surface area of 25.25 per cent (Table 3).

Efficacy of the liquid bioformulation of *M. anisopliae* in pot condition

At 30 days of spraying we have not observed any increase of leaf and shoot infestation in plants sprayed with liquid formulation supplemented with Glycerol 10.0 per cent and Sunflower oil 0.5 per cent. Highest protection of leaf (88.11%) and shoot infestation (95.96%) was recorded in treatment of liquid formulation + (Glycerol 10.0% + Sunflower oil 0.5%) which was found to be the best followed by liquid formulation + Sunflower oil 0.5% with protection of leaf and shoot infestation up to 86.14 and 93.93 per cent respectively (Table 4). Plants sprayed with bioformulation of *M. anisopliae* could escape more aphid infestation as compared to control plants.

Table 3. Effect of supplementation of adjuvants and oils on liquid biofurmulation of *M. anisopliae*

Treatments	Surface area covered (%) by *M. anisopliae* after					Biomass on 15th day/40 ml (g)
	3 days	5 days	7 days	10 days	15 days	
Liquid formulation (LF)	5.40	20.50	45.00	50.00	50.00	0.21
LF + Adjuvant (Glycerol 10.0 %)	21.00	48.50	75.00	80.50	95.00	3.57
LF + Oil (Sunflower oil 0.5 %)	25.00	50.00	70.00	85.00	95.55	4.00
LF + [Adjuvant (Glycerol 10.0 %) + Oil (Sunflower oil 0.5 %)]	25.25	60.00	80.00	88.90	98.95	4.15
SEd(±)	0.31	0.68	1.19	1.31	0.22	0.02
$CD_{0.05}$	0.83	1.20	2.34	2.87	0.76	0.04

Data are mean of three replications

Data presented in Table 5 showed that liquid formulation amended with Glycerol 10.0 per cent + Sunflower oil 0.5 per cent was highly pathogenic to aphid and killed 80 per cent of the test population at 30 days after spraying. It was followed by liquid formulation amended + sunflower oil 0.5 per cent with aphid mortality of 50.00 per cent. Greenish coloured mycelial growth of the fungus, *M. anisopliae* was observed over the surface of the aphid body covering head, thorax, abdomen and all other appendages. Infected aphids remained adhered to the shoot tips, leaf surfaces as well as stems of cow pea plant.

Table 4. Efficacy of biofurmulations on *M. anisopliae* on aphid infestation in pot condition

Treatments	Per cent leaf infestation				Per cent shoot infestation			
	Leaf infestation		Protection over control		Shoot infestation		Protection over control	
	15 DAS	30 DAS	15 DAS	30 DAS	15 DAS	30 DAS	15 DAS	30 DAS
Control (Water spray)	67.22 (55.07)	84.16 (66.54)	-	-	56.66 (48.82)	82.50 (65.27)	-	-
Liquid formulation (LF) alone	13.33 (21.41)	13.33 (21.41)	80.16 (63.54)	84.16 (66.54)	11.66 (19.96)	11.66 (19.96)	79.42 (63.02)	85.86 (67.91)
LF + Adjuvant (Glycerol 10.0 %)	11.66 (19.96)	11.66 (19.96)	82.65 (65.38)	86.14 (68.14)	6.66 (14.94)	6.68 (14.97)	88.24 (69.94)	91.90 (73.46)
LF + Oil (Sunflower oil 0.5 %)	11.60 (19.91)	12.50 (20.70)	82.74 (65.45)	86.14 (68.14)	5.00 (12.92)	5.00 (12.92)	91.17 (72.71)	93.93 (75.73)
LF + [Adjuvant (Glycerol 10.0 %)+ Oil (Sunflower oil 0.5 %)]	10.00 (18.43)	10.00 (18.43)	85.12 (49.67)	88.11 (69.82)	3.33 (10.51)	3.33 (10.51)	94.12 (75.96)	95.96 (78.40)
SEd(±)	0.029	0.242			0.264	0.185		
$CD_{0.05}$	0.052	0.412			0.567	0.481		

DAS: Days after spraying; Data are mean of three replications; Data within parenthesis are angular transformed values

This might be due to the reason that addition of oils and adjuvants increases infectivity of entomopathogens by enhancing conidial adherence and prolonged persistence (Meyer. U, et al, 2002 and Visalakshy. P. N, et al, 2006). Somervaille. A, et al., 2012 reported that adjuvants lower the surface tension of spray droplet which will help in better retention of spray droplet on the plant surface. To ascertain the cow pea mosaic disease incidence in pot condition, per cent disease incidence was calculated and found that there was no disease incidence in plants treated with liquid formulation amended with Glycerol 10.0 per cent + Sunflower oil 0.5 per cent which could protect the crop from cow pea mosaic disease up to 100 per cent (Table 6).

Table 5. Efficacy of bioformulation of *M. anisopliae* against Cow pea aphid

Treatments	Corrected mortality (%) ± SD	
	15 DAS	30 DAS
Control (Water spray)	0.00 ± 0.00 (0.28)	0.00 ± 0.00 (0.28)
Liquid formulation (LF) alone	15.00 ± 0.00 (22.78)	15.25 ± 0.41 (22.98)
LF + Adjuvant (Glycerol 10.0 %)	26.33 ± 0.49 (30.87)	30.16 ± 0.40 (33.31)
LF + Oil (Sunflower oil 0.5 %)	42.50 ± 0.49 (40.68)	50.00 ± 0.00 (45.00)
LF + [Adjuvant (Glycerol 10.0 %) + Oil (Sunflower oil 0.5 %)]	50.00 ± 0.00 (45.00)	80.00 ± 0.00 (63.43)
SEd(±)	0.612	0.194
$CD_{0.05}$	1.282	0.396

DAS: Days after spraying; Data are mean of three replications; Data within parenthesis are angular transformed values

Table 6. Efficacy of bioformulation of *M. anisopliae* on incidence of Cow pea mosaic disease

Treatments	Disease incidence			
	PDI		Protection over control (%)	
	15 DAS	30 DAS	15 DAS	30 DAS
Control (Water spray)	16.66 (4.08)*	69.16 (56.26)**	-	-
Liquid formulation (LF) alone	12.41 (3.52)	13.33 (21.41)	25.51 (30.34)**	80.72 (63.95)**
LF + Adjuvant (Glycerol 10.0 %)	1.00 (0.50)	1.66 (7.40)	100.00 (90.00)	97.59 (81.07)
LF + Oil (Sunflower oil 0.5 %)	1.66 (1.28)	0.83 (5.22)	90.03 (71.59)	98.79 (83.68)
LF + [Adjuvant (Glycerol 10.0 %) + Oil (Sunflower oil 0.5 %)]	0.00 (0.50)	0.00 (1.65)	100.00 (90.00)	100.00 (90.00)
SEd(±)	0.166	0.192		
$CD_{0.05}$	0.404	0.395		

PDI: Per cent disease incidence; DAS: Days after spraying; Data are mean of three replications; *Data within parenthesis are square root transformed value; **Data within parenthesis are angular transformed value

Efficacy of bioformulations of *M. anisopliae* in field condition

Under field condition after 15 days of spraying, significantly lowest aphid incidence (0.33%) was recorded in plants sprayed with liquid formulation amended with 10.0 per cent Glycerol + 0.5 per cent Sunflower oil (Table 7). At 30 days after spraying significantly lowest aphid incidence of 1.99 per cent was recorded in liquid formulation amended with Glycerol 10.0 per cent + Sunflower oil 0.5 per cent followed by liquid formulation amended with glycerol 10 per cent with 10.33 per cent aphid incidence. In our observation we have found that about 89.67 per cent of the aphids were killed when plants were sprayed with liquid formulation supplemented with glycerol 10.0 per cent + sunflower oil 0.5 per cent. We also observed that the rate of aphid mortality was comparatively lower under field condition than in the pot experiment. Plants sprayed with bioformulation of *M. anisopliae* could escape more aphid incidence as compared to untreated control.

Oils and adjuvants reduce evaporation of spray droplets and extend the active life of the bioagent (Kaaya. G. P, et al, 2011). Adjuvants and oils may also affect the uptake of biopesticides across insect cuticle, physically disrupting the surface by dissolving waxy deposits (Somervaille. A, et al., 2012) resulted in death of the insect. Result presented in Table 8 revealed that at 30 days after spraying, liquid formulation amended with Glycerol 10.0 per cent + Sunflower oil 0.5 per cent was found to be best treatment which could protect the crop from cow pea mosaic disease up to 96.03 per cent followed by liquid formulation amended with Sunflower oil 0.5 per cent with protection of 69.73 per cent. It was evident that highest disease incidence was recorded in control plants. Data on pod yield of cow pea as influenced by different treatments of bioformulations along with cost- benefit ratio over control were worked out and presented in terms of weight (q/ha) in the Table 9. The highest yield (24.03q/ha) with cost- benefit ratio of 1: 7.95 was recorded in the plot treated with liquid formulation amended with glycerol 10.0 per cent + sunflower oil 0.5 per cent. It was followed by spraying of liquid formulation amended with glycerol 10.0 per cent which resulted in yield and cost-benefit ratio of 15.61 q/ha and 1: 4.87 respectively. The yield obtained from the untreated control was significantly lower than that of treated plots (11.20 q/ha).

Table 7. Efficacy of *M. anisopliae* bioformulation against Cow pea aphid under field condition

Treatments	Per cent incidence of Aphid ±SD		Corrected mortality (%) ±SD	
	15 DAS	30 DAS	15 DAS	30 DAS
Control (Water spray)	50.47 ± 0.25 (45.26)	51.08 ± 0.46 (45.61)	0.00 ± 0.00 (0.28)	0.00 ± 0.00 (0.28)
Liquid formulation (LF) alone	16.28 ± 0.27 (23.79)	10.56 ± 0.51 (18.96)	15.33 ±0.57 (23.05)	40.00 ±0.00 (39.23)
LF + Adjuvant (Glycerol 10.0 %)	20.00 ± 0.00 (26.56)	10.33 ± 0.27 (18.74)	30.00 ±0.00 (33.21)	55.00 ±0.00 (47.86)
LF + Oil (Sunflower oil 0.5 %)	27.42 ± 0.25 (31.57)	20.48 ± 0.09 (26.90)	41.67 ±1.52 (40.20)	79.67 ±0.57 (63.19)
LF + [Adjuvant (Glycerol 10.0 %) + Oil (Sunflower oil 0.5 %)]	0.33 ± 3.30 (3.29)	1.99 ± 0.00 (8.10)	50.00 ±0.00 (45.00)	89.67 ±0.57 (71.25)
SEd(±)	0.970	0.067	0.850	0.380
$CD_{0.05}$	2.071	0.125	1.810	0.820

DAS: Days after spraying; Data are mean of three replications; Data within parenthesis are angular transformed values

Table 8. Efficacy of *M. anisopliae* bioformulation on Cow pea mosaic disease incidence under field condition

Treatments	Disease incidence			
	PDI		Protection over control (%)	
	15 DAS	30 DAS	15 DAS	30 DAS
Control (Water spray)	50.17 (45.09)	54.68 (47.68)	-	-
Liquid formulation (LF) alone	16.17 (23.71)	16.46 (23.93)	67.76 (55.40)	69.89 (56.72)
LF + Adjuvant (Glycerol 10.0 %)	20.00 (26.56)	20.66 (27.03)	60.13 (50.84)	62.21 (52.07)
LF + Oil (Sunflower oil 0.5 %)	15.33 (23.05)	16.55 (24.00)	69.44 (56.44)	69.73 (56.62)
LF + [Adjuvant (Glycerol 10.0 %) + Oil (Sunflower oil 0.5 %)]	2.28 (8.68)	2.17 (8.47)	95.45 (77.68)	96.03 (78.50)
SEd(±)	0.191	0.340	0.384	0.330
$CD_{0.05}$	0.412	0.618	0.830	1.146

PDI: Per cent disease incidence; DAS: Days after spraying; Data are mean of three; replications; Data within parenthesis are angular transformed values

Table 9. C:B ratio of different treatments for the management of Cow pea mosaic disease

Treatment	Yield (q/ha)	C:B
Control (Water spray)	11.20	-
Liquid formulation (LF) alone	14.42	1:4.42
LF + Adjuvant (Glycerol 10.0 %)	15.61	1:4.87
LF + Oil (Sunflower oil 0.5 %)	14.07	1:4.22
LF + [Adjuvant (Glycerol 10.0 %) + Oil (Sunflower oil 0.5 %)]	24.03	1:7.95
SEd(±)	0.360	
$CD_{0.05}$	0.772	

Data are mean of three replications

CONCLUSION

From our experiment we can conclude that liquid formulation of *M. anisopliae* supplemented with 10.0 per cent Glycerol and 0.5 per cent Sunflower oil was found significantly effective covering highest surface area and highest biomass production which will enhance storability of the formulations. Addition of adjuvants and oils also reduces conidial desiccation and ultimately increase efficacy of the formulation against Cow pea aphid with mortality of about 80 per cent and the bioformulatio which can also protect cow pea crop from cow pea mosaic disease upto 96.03 per cent.

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