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Effect of various adjuvants on growth and development of the entomopathogenic fungi *Nomuraea rileyi* (Farlow) Samson

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ABSTRACT

Studies on the effect of various adjuvants on growth and development of *Nomuraea rileyi* (Farlow) Samson was undertaken with a view to select suitable adjuvant for developing formulation. It was revealed that the *N.rileyi* formulations with combination of adjuvants helped in increasing production of fungal biomass at 10 DAI. Based on the results it is concluded that the overall performance of the adjuvants for growth and development of *N.rileyi* in series of lab experimentation out of 96 test formulations. 10 formulation comprising 1) *N.r.*+HO (1%), 2)*N.r*+SFO(1%), 3) *N.r.*+GH(0.5%), 4) *N.r.*+TW(0.5%)+ GH(0.5%), 5)*N.r.*+GLY(2%)+SFO(1%), 6)*N.r.*+ GLY(2%)+GH (0.5%), 7) *N.r.*+SFO (1%)+GH(0.5%), 8)*N.r.*+TW(0.5%)+ GLY(2%)+ SFO(1%) + CMC (0.5%), 9) *N.r.*+ TW(0.5%)+GLY(2%)+HO (1%) and 10) *N.r.*+TW (0.5%) + GLY (2%) + CMC(0.5%) were emerged out as most promising and advanced stage formulations of *N. rileyi.*

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INTRODUCTION

Biological control is an important, effective ecofriendly and economical component of IPM in almost all important crops for the development of sustainable cropping systems. There is ample scope for microbial control of pests of cereals, pulses, vegetables and horticultural crops. So the insect viruses, bacteria, nematodes and fungi are emerging as potential bioagents (Pandey and Kanujia, 2005).

At the end of 2001, there were approximately 195 registered biopesticides and 780 formulated products for the control of insects (38.10 %), bacteria (37.00 %), nematodes (15.7 %), fungi (4.7 %), viruses (2.85 %) and protozoa (2.14 %) (Anonymous, 2003). Among the pathogens used in microbial control, entomopathogenic fungi have played an important role in the history of insect pathology and microbial control of insects. More than 750 species of entomopathogenic fungi, representing 100 genera are currently known (Hajek and Lager, 1994). The entomopathogenic fungi causing diseases to the insects are practically more significant

as they are epizootic in nature. Also they have the advantage of ease of production and contact action which allow direct penetration of the host cuticle without ingestion.

Nomuraea rileyi (Farlow) Samson Moniliales, Moniliaceae is a fungus of cosmopolitan nature. *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 (Lingappa and Patil, 2002). *N.rileyi* frequently cause epizootics in nature, is one promising because of its wide spread occurrence and relative abundance due to its wide host range which included many catterpiller pests. The pathogenicity of fungi towards insects has been mainly attributed to various hydrolytic enzymes such as chitinase, proteases and lipases. 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 (Vimladevi *et al.*, 2002).

The formulation of the fungi still awaits a serious efforts in formulation technology. Exploring formulation of *N.rileyi* 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. Presently crude suspensions of the fungi with short shelf-life of around one to two months for liquid and 5 to 6 months for WP are marketed. For developing wettable powder formulation, basic research on standardization of bioactive ingredient and suitable adjuvants is necessary before the formulation. There are many examples were fungi have been formulated with various adjuvants. The addition of nutrients to a spore spray did improve control of aphids and white flies in green house cucumber, compared with spores applied in water alone (Hall, 1982). Humectants prolong the viability of *Alternaria cassia* (Shabana *et al.*, 1977). *V. lecanii* formulated with arachnid oil showed significantly better control of powdery mildew than without oil (Verhaar *et al.*, 1999).

In the present study a range of adjuvants and vegetable oil were screened for their growth and development of *N.rileyi* on culture medium with a view to select suitable adjuvants for developing the formulations of entomopathogen.

MATERIAL 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 in completely randomized design were carried out in the biological control laboratory, Dept. of Entomology, MPKV, Rahuri during 2009 to 2012.

Medium :

The medium used for multiplication and growth of the fungus was Sabourauds dextrose broth with yeast extract.

Methodology:

***Effect of various adjuvants with inoculum on growth and development of N.rileyi* :**

Various formulations with adjuvants were made and tested for growth and development of *N.rileyi.*

The effect of combination of chemical adjuvants :

Optimum concentration of promising chemical adjuvants comprising glycerol (2.0%), tween-80 (0.50%), triton-X-100 (0.03%), boric acid (2.0%) and carboxymethyl cellulose (0.50%) were selected and mixed with each other in combination with optimum concentration 30 per cent AS of *N.rileyi* ($1.97x10^9$cfu/ml) and 14 liquid formulations with multiple adjuvants and 5 liquid formulations with individual adjuvants were prepared. The 19 formulations were tested for growth and development of *N.rileyi*.

The effect of combination of chemicals and edible oils :

Optimum concentration of promising chemical and oil adjuvants comprising glycerol (2.0%), tween-80 (0.50%), triton-X-100 (0.03%), boric acid (2.0%) and carboxymethyl cellulose (0.50%) and oils as sunflower (1.0%), groundnut (0.5%), coconut (1.0%), mustard (0.5%), soybean (0.5%) and *Ghee* (0.5%) were selected and mixed with each other in combination with optimum concentrations 30 per cent AS of *N.rileyi* ($1.97x10^9$cfu/ml) and 39 liquid formulations with multiple adjuvants

and 11 liquid formulationss with individual adjuvants were prepared. The 50 formulations were tested for growth and development of *N.rileyi*

The effect of combination of edible oils :

Optimum concentration of edible oils as adjuvants compring sunflower (1.0%), groundnut (0.5%), coconut (1.0%), mustard (0.5%), soybean (0.5%) and *Ghee* (0.5%) were selected and mixed with each other in combination with optimum concentrations 30 per cent AS of *N. rileyi* ($1.97x10^9$ cfu/ml) and 13 liquid formulations with multiple adjuvants and 6 liquid formulations with individual adjuvants were prepared. The 19 formulations were tested for growth and development of *N.rileyi.*

The effect of combination of chemicals, oils and other substraes :

Optimum concentration of various adjuvants comprising glycerol (2.0%), tween-80 (0.50%), triton-x-100 (0.03%), boric acid (2.0%) and carboxyl methyl cellulose (0.50%), sunflower (1.0%), groundnut (0.5%), coconut (1.0%), mustard (0.5%), soybean (0.5%), *Ghee* (0.5%) and honey (1.0%) were selected and mixed with each other in combination with optimum concentrations 30 per cent AS of *N.rileyi* ($1.97x10^9$cfu/ml) and 17 formulations with multiple adjuvants and 12 formulations with individual adjuvants were prepared. The 29 formulations were tested for growth and development of *N.rileyi.*

These adjuvants with different concenstrations were added to 100 ml optimum concentration of 30 per cent AS of *N. rileyi* ($1.97x10^9$cfu/ml) in 500 ml saline glass bottle and 31 liquid formulations were made.

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.

RESULTS AND DISCUSSION

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

Influence of multiple adjuvants with inoculum on growth and development of *N.rileyi* :

***Influence of combination of chemical adjuvants* :**

The combinations of glycerol, tween-80, triton-X-100, boric acid and carboxymethyl cellulose added in aqua suspension of the mycoagent of *N.rileyi* was evaluated.

Effect on growth :

The results on growth and development of *N.rileyi* during 10 days are presented in Table 1. At 3 DAI, for surface coverage T_8-*N.r.*+GLY+BA and T_9-*N.r.*+GLY+CMC (90% each) were significantly superior to remaining treatments (0.0 to 40%). However, T_{10}-*N.r.*+BA+CMC was at par with it recording 86.67 per cent surface coverage. T_{15}-*N.r.*+TW and T_{16}-*N.r.*+TX, which completely prevented the growth of the fungus upto 3 days.

At 7 DAI, the growth and development of fungus ranged from 10 to 100 per cent in all treatments. The treatments with T_3-*N.r.*+TW+BA, T_4-*N.r.*+TW+CMC, T_8-*N.r.*+GLY+BA, T_9-*N.r.*+GLY+CMC, T_{10}-*N.r.*+BA+CMC, T_{12}-*N.r.*+TW+GLY+BA+CMC, T_{17}-*N.r.*+GLY, T_{18}-*N.r.*+BA and T_{19}-*N.r.*+CMC recorded significantly higher surface coverage (100%). At 10 DAI, all the treatments covered cent per cent surface of the medium.

Effect on biomass :

Corresponding observations on biomass produced in gram per 40 ml liquid medium showed that treatment T_{13}-*N.r.*+TW+GLY+CMC proved its superiority, producing 12.17g fungal biomass. It was followed by T_{12}-*N.r.*+TW+GLY+BA+CMC (11.20g). Other adjuvant in combinations and alone developed 7.80 to 10.97g biomass. The control (*N.r.*alone) could produced 7.70g biomass. It was least among all the treatments.

The pH of the fungal culture developed from 20 treatment formulations ranged from 7.15 in adjuvant with 2 per cent boric acid to 8.58 in adjuvant with Triton-X-100 0.03% +Glycerol 2% registering biomass of 9.90 and 8.97g, respectively. The pH of formulation producing maximum biomass (12.17g) was 8.10 comparing adjuvants Tween-80 0.5% +Glycerol 2% +CMC 0.5%, when it was 8.48 in control producing biomass of 7.70g

per 40 ml medium. These observations clarified that there is no relation between fungal development and pH of the culture at 10 DAI. Further, it is evident that *N.rileyi* tried to produce as far as possible more biomass probably appropriating favourable micro conditions varied as per various adjuvants. So, wherever possible higher biomass was developed. Generally, the pH of the developed culture in present study is above 7. Balardin and Loch (1989) found that a pH above 7 enhance the growth of *N.rileyi* isolates.

The influence of combinations of chemicals and oils adjuvants :

The adjuvant combination treatments comprised of duly evaluated promising concentration of glycerol, tween-80, triton-X-100, boric acid and carboxymethyl cellulose; edible oils, sunflower, coconut, mustard, groundnut, soybean oil and *Ghee*. These were added in aqua suspension of *N.rileyi* to get advanced test formulations and inoculated in the SDY culture medium. The results on the influence of the multiple adjuvant treatments on growth and development of the mycoagent upto 10 days are presented in Table 2 and 3.

Effect on growth :

Significant differences in surface coverage in multiple and single adjuvant, the treatments were registered, at 3 and 7 DAI (Table 2). At 3 DAI, T_{23}-*N.r.*+GH+BA, T_{24}-*N.r.*+GH+CMC, T_{45}-*N.r.*+SFO recorded signiciantly highest per cent surface growth (86.67%) over remaining treatments (5 to 80%) except T_{50}-*N.r.*+GH (85.0%). It was followed by T_{48}-*N.r.*+GNO (80.00%) which was at par with T_{50}-*N.r.*+GH.

Effect on biomass and pH :

T_{18}-*N.r.*+GLY+GH produced highest (12.07g) fungal biomass. It was at par with T_6- *N.r.*+TW+GH (11.90), T_{33}- *N.r.*+TW+GLY+SFO+CMC (11.87g) and T_{13}- *N.r.*+GLY+SFO (11.83g). The next promising treatments in their descending order of superiority for biomass production were T_{50}- *N.r.*+GH, T_{38}- *N.r.*+ TW+ GLY+GRO+CMC and T_{39}- *N.r.*+ TW + GLY+

Table 1 : Effect of combinations of chemical adjuvants with inoculum in *N.rileyi* 30 per cent AS on growth and biomass of the mycoagent

Tr. No.	Treatments	Conc. (%) of adjuvant	Surface coverage (%)			Biomass g/40ml medium	pH at 10 DAI
			3 DAI	7 DAI	10 DAI		
T_1	*N.r.*+ TW + TX	0.5 + 0.03	10.00 (18.44)*	66.67 (54.76)	100.00 (90.00)	9.37	8.22
T_2	*N.r.*+ TW + GLY	0.5 + 2.0	6.67 (15.00)	70.00 (56.79)	100.00 (90.00)	8.07	8.53
T_3	*N.r.*+ TW + BA	0.5 + 2.0	5.00 (12.92)	100.00 (90.00)	100.00 (90.00)	10.53	7.54
T_4	*N.r.*+ TW + CMC	0.5 + 0.5	6.67 (15.00)	100.00 (90.00)	100.00 (90.00)	10.63	7.52
T_5	*N.r.*+ TX + GLY	0.03 + 2.0	6.67 (15.00)	83.33 (65.88)	100.00 (90.00)	8.97	8.58
T_6	*N.r.*+ TX + BA	0.03 + 2.0	10.00 (18.44)	28.33 (32.14)	100.00 (90.00)	9.80	7.35
T_7	*N.r.*+ TX + CMC	0.03 + 0.5	6.67 (15.00)	26.67 (31.11)	100.00 (90.00)	8.20	7.37
T_8	*N.r.*+ GLY + BA	2.0 + 2.0	90.00 (71.56)	100.00 (90.00)	100.00 (90.00)	9.03	7.50
T_9	*N.r.*+ GLY + CMC	2.0 + 0.5	90.00 (71.56)	100.00 (90.00)	100.00 (90.00)	10.10	8.36
T_{10}	*N.r.*+ BA + CMC	2.0 + 0.5	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	10.90	8.12
T_{11}	*N.r.*+ TW + TX + GLY	0.5 + 0.03 +2	6.67 (15.00)	26.67 (31.11)	100.00 (90.00)	10.20	8.03
T_{12}	*N.r.*+ TW+GLY+BA+CMC	0.5+2+2+0.5	6.67 (15.00)	100.00 (90.00)	100.00 (90.00)	11.20	7.70
T_{13}	*N.r.*+ TW+GLY+CMC	0.5+2+0.5	8.33 (16.78)	53.33 (46.89)	100.00 (90.00)	12.17	8.10
T_{14}	*N.r.*+ TW+GLY+BA	0.5+2+2	6.67 (15.00)	63.33 (52.71)	100.00 (90.00)	10.97	7.42
T_{15}	*N.r.*+ TW	0.50	0.00 (0.00)	80.00 (63.44)	100.00 (90.00)	8.83	7.78
T_{16}	*N.r.*+ TX	0.03	0.00 (0.00)	10.00 (18.44)	100.00 (90.00)	7.80	7.26
T_{17}	*N.r.*+ GLY	2.00	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	9.90	7.85
T_{18}	*N.r.*+ BA	2.00	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	9.90	7.15
T_{19}	*N.r.*+ CMC	0.50	26.67 (31.18)	100.00 (90.00)	100.00 (90.00)	9.97	7.71
T_{20}	Control (*N.r.*alone)	-	30.00 (33.21)	41.67 (40.22)	100.00 (90.00)	7.70	8.48
	S.E. $\pm$		1.88	3.20	-	0.07	-
	C.D.(P=0.05)		5.37	9.15	-	0.21	-

*Figures in parentheses are arcsin values. DAI = Days after inoculation *N.r.* = *Nomuraea rileyi* TW = Tween-80
GLY= Glycerol BA = Boric acid TX = Triton-X-100 CMC = Carboxymethyl Cellulose

Table 2 : Effect of combinations of multiple chemicals and oils with inoculum in *N.rileyi* 30 per cent as on growth and biomass of the mycoagent

Tr. No.	Treatments	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium	pH
			3 DAI	7 DAI	10 DAI		
T_1	*N.r.*+ TW + SFO	0.5 + 1.0	6.67 (15.00)*	88.33 (70.00)	100.00 (90.00)	10.83	7.45
T_2	*N.r.*+ TW + CNO	0.5 + 1.0	5.00 (12.92)	100.00 (90.00)	100.00 (90.00)	10.23	8.34
T_3	*N.r.*+ TW + MUO	0.5 + 0.5	5.00 (12.92)	100.00 (90.00)	100.00 (90.00)	9.73	7.56
T_4	*N.r.*+ TW + GNO	0.5 + 0.5	6.67 (15.00)	66.67 (54.76)	100.00 (90.00)	11.10	7.75
T_5	*N.r.*+ TW + SBO	0.5 + 0.5	8.33 (16.78)	93.33 (75.00)	100.00 (90.00)	11.00	7.22
T_6	*N.r.*+ TW + GH	0.5 + 0.5	11.67 (20.00)	100.00 (90.00)	100.00 (90.00)	11.90	7.69
T_7	*N.r.*+ TX + SFO	0.03 + 1.0	5.00 (12.92)	46.67 (43.11)	100.00 (90.00)	8.50	7.90
T_8	*N.r.*+ TX + CNO	0.03 + 1.0	6.67 (15.00)	50.00 (45.00)	100.00 (90.00)	8.38	8.15
T_9	*N.r.*+ TX + MUO	0.03 + 0.5	6.67 (15.00)	26.67 (31.11)	100.00 (90.00)	8.33	7.90
T_{10}	*N.r.*+ TX+ GNO	0.03 + 0.5	6.67 (15.00)	28.33 (32.14)	100.00 (90.00)	8.32	7.92
T_{11}	*N.r.*+ TX + SBO	0.03 + 0.5	5.00 (12.92)	28.33 (32.14)	100.00 (90.00)	8.07	8.30
T_{12}	*N.r.*+ TX + GH	0.03 + 0.5	6.67 (15.00)	28.33 (32.14)	100.00 (90.00)	8.47	7.45
T_{13}	*N.r.*+ GLY + SFO	2.0 + 1.0	11.67 (20.00)	100.00 (90.00)	100.00 (90.00)	11.83	7.44
T_{14}	*N.r.*+ GLY + CNO	2.0 + 1.0	6.67 (15.00)	100.00 (90.00)	100.00 (90.00)	10.90	7.60
T_{15}	*N.r.*+ GLY + MUO	2.0 + 0.5	5.00 (12.92)	100.00 (90.00)	100.00 (90.00)	10.30	8.05
T_{16}	*N.r.*+ GLY + GNO	2.0 + 0.5	15.00 (22.79)	100.00 (90.00)	100.00 (90.00)	11.17	7.47
T_{17}	*N.r.*+ GLY + SBO	2.0 + 0.5	15.00 (22.79)	83.33 (65.88)	100.00 (90.00)	11.17	7.28
T_{18}	*N.r.*+ GLY + GH	2.0 + 0.5	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	12.07	7.58
T_{19}	*N.r.*+ SFO+ BA	1.0 + 2.0	11.67 (20.00)	100.00 (90.00)	100.00 (90.00)	9.90	7.52
T_{20}	*N.r.*+ SFO+ CMC	1.0 + 0.5	15.00 (22.79)	100.00 (90.00)	100.00 (90.00)	9.93	7.53
T_{21}	*N.r.*+ GNO + BA	0.5 + 2.0	33.33 (35.24)	100.00 (90.00)	100.00 (90.00)	9.27	7.63
T_{22}	*N.r.*+ GNO + CMC	0.5 + 0.5	33.33 (35.24)	100.00 (90.00)	100.00 (90.00)	11.20	7.74
T_{23}	*N.r.*+ GH + BA	0.5 + 2.0	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	9.70	7.80
T_{24}	*N.r.*+ GH + CMC	0.5 + 0.5	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	10.63	7.97
T_{25}	*N.r.*+TW+TX+GLY+SFO	0.5 + 0.03+2+ 1	6.67 (15.00)	33.33 (35.24)	100.00 (90.00)	8.50	7.67
T_{26}	*N.r.*+ TW + TX+ GLY + FO +CNO	0.5 + 0.03+2 +1+1	6.67 (15.00)	31.67 (34.27)	100.00 (90.00)	8.17	7.10
T_{27}	*N.r.*+ TW + TX+ GLY + SFO +CNO+MUO	0.5+0.03+2+1+1+0.5	8.33 (16.78)	31.67 (34.27)	100.00 (90.00)	8.50	7.50
T_{28}	*N.r.*+ TW + TX +GLY + SFO +CNO +MUO+GNO	0.5+0.03+2+1+ 1+ 0.5+0.5	6.67 (15.00)	43.33 (41.15)	100.00 (90.00)	8.33	7.28
T_{29}	*N.r.*+TW +TX+GLY+ SFO + CNO+MUO+GNO+ SBO	0.5+0.03+2+1+1+ 0.5+0.5+0.5	6.67 (15.00)	40.00 (39.23)	100.00 (90.00)	8.50	7.70
T_{30}	*N.r.*+TW+TX+GLY+SFO + CNO+MUO+GNO+SBO+GH	0.5+0.03+2+1+1+0.5 + 0.5+o.5+0.5	6.67 (15.00)	68.33 (55.73)	100.00 (90.00)	8.40	7.21
T_{31}	*N.r.*+ TW + TX + GLY + SFO +CNO +MUO+GNO +SBO+GH+BA	0.5+0.03+2+1+1+ 0.5+ 0.5+0.5+0.5+2	8.33 (16.78)	53.33 (46.89)	100.00 (90.00)	8.40	7.25
T_{32}	*N.r.*+ TW + TX + GLY + SFO +CNO +MUO+GNO +SBO+GH+BA+CMC	0.5+0.03+2+1+1+ 0.5+0.5+ 0.5+0.5+2+0.5	8.33 (16.78)	33.33 (35.24)	100.00 (90.00)	8.33	7.31
T_{33}	*N.r.*+ TW+GLY+SFO+CMC	0.5+2+1+0.5	8.33 (16.78)	96.67 (79.53)	100.00 (90.00)	11.87	7.21
T_{34}	*N.r.*+TW+GLY+CNO+CMC	0.5+2+1+0.5	6.67 (15.00)	80.00 (63.44)	100.00 (90.00)	11.00	7.20
T_{35}	*N.r.*+TW+GLY+MUO+CMC	0.5+2+0.5+0.5	6.67 (15.00)	100.00 (90.00)	100.00 (90.00)	10.57	7.52
T_{36}	*N.r.*+ TX+GLY+SFO+CMC	0.03+2+1+0.5	8.33 (16.78)	23.33 (28.86)	100.00 (90.00)	10.07	8.19
T_{37}	*N.r.*+ TW+GLY+GH	0.5+2+0.5	6.67 (15.00)	100.00 (90.00)	100.00 (90.00)	10.67	8.81
T_{38}	*N.r.*+TW+GLY+GNO+CMC	0.5+2+0.5+0.5	5.00 (12.92)	86.67 (68.61)	100.00 (90.00)	11.30	7.75
T_{39}	*N.r.*+TW+GLY+SBO+CMC	0.5+2+0.5+0.5	10.00 (18.44)	96.67 (79.53)	100.00 (90.00)	11.30	7.69

Table 2: Contd….

Table 2 contd...

T_{40}	*N.r.*+ TW	0.50	0.00 (0.00)	80.00 (63.44)	100.00 (90.00)	8.80	7.78
T_{41}	*N.r.*+ TX	0.03	0.00 (0.00)	10.00 (18.44)	100.00 (90.00)	7.73	7.26
T_{42}	*N.r.*+ GLY	2.00	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	9.80	7.85
T_{43}	*N.r.*+ BA	2.00	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	9.75	7.15
T_{44}	*N.r.*+ CMC	0.50	26.67 (31.18)	100.00 (90.00)	100.00 (90.00)	9.90	7.71
T_{45}	*N.r.*+ SFO	1.00	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	12.40	7.16
T_{46}	*N.r.*+ CNO	1.00	46.67 (43.11)	90.00 (71.56)	100.00 (90.00)	9.70	7.26
T_{47}	*N.r.*+ MUO	0.50	35.00 (36.27)	100.00 (90.00)	100.00 (90.00)	10.30	7.52
T_{48}	*N.r.*+ GNO	0.50	80.00 (63.44)	100.00 (90.00)	100.00 (90.00)	11.00	7.63
T_{49}	*N.r.*+ SBO	0.50	55.00 (47.87)	100.00 (90.00)	100.00 (90.00)	10.20	7.33
T_{50}	*N.r.*+ GH	0.50	85.00 (67.21)	100.00 (90.00)	100.00 (90.00)	11.70	7.21
T_{51}	Control (*N.r.*alone)		30.00 (33.21)	46.67 (43.11)	100.00 (90.00)	7.60	8.40
	S.E ±		1.70	2.91	-	0.09	-
	C.D. (P=0.05)		4.77	8.16	NS	0.25	-

*Figures in parentheses are arcsin value DAI = Days after inoculation *N.r. = Nomuraea rileyi*
GH = Ghee TW = Tween-80 SFO = Sunflower oil GLY= Glycerol CNO = Coconut oil
TX = Triton-X-100 GNO = Groundnut oil BA = Boric acid SBO= Soybean oil
CMC=Carboxymethyl Cellulose MUO = Mustered oil NS=Non-significant

Table 3 : Effect of combinations of edible oils as adjuvants with inoculum in *N.rileyi* 30 per cent as on growth and biomasss of the mycoagent

Tr. No.	Treatments	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium	pH at 10 DAI
			3 DAI	7 DAI	10 DAI		
T_1	*N.r.*+ SFO + CNO	1.0 + 1.0	8.33 (16.78)*	100.00 (90.00)	100.00 (90.00)	10.90	7.88
T_2	*N.r.*+ SFO + GNO	1.0 + 0.5	11.67 (20.00)	100.00 (90.00)	100.00 (90.00)	11.00	7.38
T_3	*N.r.*+ SFO + GH	1.0 + 0.5	53.33 (46.89)	100.00 (90.00)	100.00 (90.00)	11.97	7.67
T_4	*N.r.*+ SFO + SBO	1.0 +0.5	33.33 (35.24)	93.33 (75.00)	98.33 (82.51)	9.33	7.80
T_5	*N.r.*+ SFO + MUO	1.0+0.5	11.67 (20.00)	85.00 (67.21)	95.00 (77.08)	9.87	7.60
T_6	*N.r.*+ GNO+CNO	0.5+1	10.00 (18.44)	80.00 (63.44)	96.67 (79.53)	10.53	7.65
T_7	*N.r.*+ GNO+GH	0.5+0.5	41.67 (40.22)	93.33 (75.00)	96.67 (79.53)	11.23	7.60
T_8	*N.r.*+ GNO+SBO	0.5+0.5	31.67 (34.27)	83.33 (65.88)	95.00 (77.08)	9.17	7.90
T_9	*N.r.*+ GNO+MUO	0.5+0.5	18.33 (25.33)	66.67 (54.76)	91.67 (73.26)	9.33	7.80
T_{10}	*N.r.*+ SFO+GNO+GH	1.0+0.5+0.5	18.33 (25.33)	66.67 (54.76)	96.67 (79.53)	9.77	7.75
T_{11}	*N.r.*+ SFO+GNO+GH+CNO	1.0+0.5+0.5+1.0	16.67 (24.12)	66.67 (54.76)	96.67 (79.53)	10.17	7.80
T_{12}	*N.r.*+SFO+GNO+GH+MUO	1.0+0.5+0.5+0.5	18.33 (25.33)	68.33 (55.73)	96.67 (79.53)	10.20	7.82
T_{13}	*N.r.*+SFO+GNO+GH+MUO	1.0+0.5+0.5+	18.33 (25.33)	63.33 (52.71)	98.33 (82.51)	10.27	7.85
	+SBO	0.5+0.5					
T_{14}	*N.r.*+ SFO	1.00	88.33 (70.00)	100.00 (90.00)	100.00 (90.00)	12.47	7.16
T_{15}	*N.r.*+ CNO	1.00	41.67 (40.22)	90.00 (71.56)	100.00 (90.00)	9.60	7.26
T_{16}	*N.r.*+ MUO	0.50	33.33 (35.24)	100.00 (90.00)	100.00 (90.00)	10.20	7.52
T_{17}	*N.r.*+ GNO	0.50	75.00 (60.00)	100.00 (90.00)	100.00 (90.00)	10.90	7.63
T_{18}	*N.r.*+ SBO	0.50	50.00 (45.00)	100.00 (90.00)	100.00 (90.00)	10.30	7.33
T_{19}	*N.r.*+ GH	0.50	80.00 (63.44)	100.00 (90.00)	100.00 (90.00)	11.80	7.24
T_{20}	Control (*N.r.*alone)	-	20.00 (26.56)	50.00 (45.00)	100.00 (90.00)	7.80	8.40
	S.E. ±		1.53	2.14	1.81	0.11	-
	C.D.(P=0.05)		4.38	6.13	5.20	0.32	-

*Figures in parentheses are arcsin values. DAI = Days after inoculation *N.r. = Nomuraea rileyi*

SBO+CMC which produced 11.70, 11.30 and 11.30g biomass, respectively.

The least growth and development of the fungus was observed in T_2- *N.r.*+TW+CNO and T_3- *N.r.*+ TW+

MUO (5% each). Nevertheless, the triton-X-100 alone or with other adjuvants was found to be detrimental to the fungal growth and development. It was evident from less production of fungal biomass (7.73 to 8.50g). The biomass in control was 7.60g which was significantly lower than adjuvant comprising treatments except that of T_{41}- *N.r.*+TX (7.73g).

The pH of the fungal culture developed from 51 treatment formulations ranged from 7.10 in treatment T_{26} *N.r.*+ TW + TX + GLY + SFO +CNO to 8.81 in T_{37} *N.r.*+ TW + GLY+ GH registering biomass of 8.17 and 10.67g, respectively. The pH of formulation producing maximum biomass (12.07g) was 7.58 comparing treatment adjuvants Glycerol 2% +GH 0.5%, when it was 8.40 in control producing biomass of 7.60g per 40 ml medium.

These observations clarified that there is no relation between fungal development and pH of the culture at 10 DAI. Further, it is evident that *N.rileyi* tried to produce as far as possible more biomass probably appropriating favourable micro conditions varied as per various adjuvants. So, wherever possible higher biomass was developed. Generally, the pH of the developed culture in present study is above 7. Balardin and Loch (1989) found that a pH above 7 enhance the growth of *N.rileyi* isolates.

Thus, among combination of chemical and edible oils as adjuvants, the advanced stage formulations of *N.rileyi* with T_{18}-*N.r.*+GLY+GH, T_6-*N.r.*+TW+GH, T_{33}-*N.r.*+TW+GLY+SFO+CMC, T_{13}-*N.r.*+GLY+SFO were highly promising for the growth and development of the fungi.

Mixture of oils as adjuvants :

The combinations of mixtures of sunflower, coconut, mustard, groundnut, soybean and *Ghee* were added to aqua suspension of *N.rileyi* as test formulation bases. One ml of respective formulation was inoculated in the culture medium. The results on growth and development of the mycoagent as influenced by the treatments at 3, 7 and 10 days are presented in Table 3 and 4.

Effect on growth :

Data presented in Table 3 revealed that T_{14}-*N.r.*+SFO registered significantly highest (88.33 %) surface coverage over the rest of the treatments except the treatments with T_{19}- GH (80%).

Later was followed by T_{17}-*N.r.*+GNO (75.0%), T_3-*N.r.*+SFO+GH (53.33%) at 3 DAI. Among the treatments with combination of oils, the treatment with T_3-*N.r.*+SFO+GH recorded highest (53.33%) surface coverage followed by T_7-*N.r.*+GNO+GH (41.67%). At 7 DAI, treatments recorded 63.33 to 100 per cent surface coverage.

Effect on biomass and pH :

T_{14}-*N.r.*+SFO alone registered significantly higher (12.47g) yield of fungal biomass than rest of the treatments. Among the mixtures of oils, T_3-*N.r.*+SFO+ GH produced maximum (11.97g) biomass. However, it was at par with T_{19}-*N.r.*+GH alone (11.80g). The lowest (7.80g) biomass was recorded in T_{20} control (*N.r.*alone).

The pH of the fungal culture developed from 20 treatment formulations ranged from 7.16 in treatment T_{14} *N.r.*+SFO to 7.90 in T_8 *N.r.*+GNO+SBO registering biomass of 12.47 and 9.17g, respectively. The pH of formulation producing maximum biomass (12.47g) was 7.16 comparing treatment adjuvants sunflower oil 1 per cent when it was 8.40 in control producing biomass of 7.80g per 40 ml medium. These observations clarified that there is no relation between fungal development and pH of the culture at 10 DAI. Further, it is evident that *N.rileyi* tried to produce as far as possible more biomass probably appropriating favourable micro conditions varied as per various adjuvants. So, wherever possible higher biomass was developed. Generally, the pH of the developed culture in present study is above 7. Balardin and Loch (1989) found that a pH above 7 enhance the growth of *N.rileyi* isolates.

Considering performance of the oil adjuvants based combinations at10 DAI T_3-*N.r.*+SFO+GH yielded highest (11.97g) biomass. But it was at par with T_{19}-*N.r.*+GH (11.80g). Among sole oil adjuvants T_{14}-*N.r.*+SFO produced maximum biomass (12.47g). Hence, T_3- *N.r.*+SFO+GH, T_{14}-*N.r.*+ SFO and T_{19}-*N.r.*+GH were selected as input for advancing *N.rileyi* was selected which produced highest biomass of 10.60g. There is no published information on aspect of study.

The combinations of chemical, oils and few other edible substrates as adjuvants :

Effect on growth :

At 3 DAI, T_6- *N.r.*+GH+HO and T_{23}- *N.r.*+HO

recorded significantly highest (90.0 % each) growth of *N.rileyi* (Table 4). However, it was at par with T_{24}- *N.r.*+SFO (86.67%) and T_{29}- *N.r.*+GH (85.00%), when the growth in control was 25.0 per cent.

The lowest surface coverage (6.67%) among multiple adjuvant treatments was observed in T_1- *N.r.*+TW+HO, T_7-*N.r.*+BA+HO, T_9- *N.r.*+TW+GLY +BA+CMC+HO,T_{11}-*N.r.*+GLY+SFO+CMC+Ho, T_{12}- *N.r.*+TW+GLY+HO, T_{13}-*N.r.*+TX+GLY+GH+HO and T_{15}- *N.r.*+BA+CMC+HO (6.67% each).

At 7 DAI, the surface coverage was 21.67 to 100 per cent among the treatments with adjuvant

Table 4 : Effect of combinations of chemicals, oils and other edible substrates as adjuvants with inoculum in *N.rileyi* 30 per cent AS on growth and biomass of mycoagent

Tr. No.	Treatment details	Conc. (%) of adj.	Surface coverage (%)			Biomass g/40ml medium	pH at 10 DAI
			3 DAI	7 DAI	10 DAI		
T_1	*N.r.*+ TW + HO	0.5 + 1.0	6.67 (15.00)*	100.00(90.00)	100.00(90.00)	11.00	7.37
T_2	*N.r.*+ TX + HO	0.03 + 1.0	10.00(18.44)	26.67(31.11)	100.00(90.00)	10.37	8.29
T_3	*N.r.*+ GLY + HO	2.0 + 1.0	28.33(32.14)	100.00(90.00)	100.00(90.00)	9.47	8.19
T_4	*N.r.*+ SF + HO	1.0 + 1.0	36.67(37.29)	100.00(90.00)	100.00(90.00)	10.27	7.64
T_5	*N.r.*+ GNO + HO	0.5 + 1.0	25.00(30.00)	100.00(90.00)	100.00(90.00)	10.30	7.77
T_6	*N.r.*+ GH + HO	0.5 + 1.0	90.00(71.56)	100.00(90.00)	100.00(90.00)	9.93	8.11
T_7	*N.r.*+ BA + HO	2.0 + 1.0	6.67(15.00)	100.00(90.00)	100.00(90.00)	9.90	8.75
T_8	*N.r.*+ CMC + HO	0.5 + 1.0	26.67(31.11)	100.00(90.00)	100.00(90.00)	9.10	8.50
T_9	*N.r.*+TW+TX +GLY+	0.5+0.03+2+1+1	6.67 (15.00)	56.67 (48.85)	100.00 (90.00)	10.57	7.34
	SFO+CNO+MUO+GNO+	+0.5+0.5+0.5+0.5					
	SBO+GH+BA+CMC+HO	+2+0.5+1					
T_{10}	*N.r.*+ TW+GLY+BA+CMC+HO	0.5+2+2+0.5+1	6.67(15.00)	100.00(90.00)	100.00(90.00)	11.30	7.45
T_{11}	*N.r.*+ GLY+SFO+CMC+HO	2+1+0.5+1	6.67(15.00)	100.00(90.00)	100.00(90.00)	10.97	8.21
T_{12}	*N.r.*+ TW+GLY+HO	0.5+2+1	6.67(15.00)	83.33(65.88)	100.00(90.00)	12.28	7.37
T_{13}	*N.r.*+ TX+GLY+GH+HO	0.03+2+0.5+1	6.67(15.00)	33.33(35.24)	100.00(90.00)	11.03	7.42
T_{14}	*N.r.*+ TX+CMC+HO	0.03+0.5+1	8.33 (16.78)	21.67 (27.76)	100.00 (90.00)	9.27	7.96
T_{15}	*N.r.*+ BA+CMC+HO	2+0.5+1	6.67 (15.00)	100.00 (90.00)	100.00 (90.00)	9.63	7.43
T_{16}	*N.r.*+GH+HO+GLY	0.5+1+2	16.67 (24.12)	100.00 (90.00)	100.00 (90.00)	9.03	8.94
T_{17}	*N.r.*+ GH+HO+GLY+CMC	0.5+1+2+0.5	16.67 (24.12)	100.00 (90.00)	100.00 (90.00)	9.03	8.92
T_{18}	*N.r.*+ TW	0.50	0.00 (0.00)	80.00 (63.44)	100.00 (90.00)	8.70	7.78
T_{19}	*N.r.*+ TX	0.03	0.00 (0.00)	10.00 (18.44)	100.00 (90.00)	7.80	7.26
T_{20}	*N.r.*+ GLY	2.00	38.33 (38.23)	100.00 (90.00)	100.00 (90.00)	9.90	7.85
T_{21}	*N.r.*+ BA	2.00	40.00 (39.23)	100.00 (90.00)	100.00 (90.00)	9.70	7.15
T_{22}	*N.r.*+ CMC	0.50	26.67 (31.18)	100.00 (90.00)	100.00 (90.00)	9.80	7.71
T_{23}	*N.r.*+ HO	1.00	90.00 (71.56)	100.00 (90.00)	100.00 (90.00)	11.90	7.70
T_{24}	*N.r.*+ SFO	1.00	86.67 (68.61)	100.00 (90.00)	100.00 (90.00)	12.17	7.16
T_{25}	*N.r.*+ CNO	1.00	46.67 (43.11)	90.00 (71.56)	100.00 (90.00)	9.50	7.26
T_{26}	*N.r.*+ MUO	0.50	35.00 (36.27)	100.00 (90.00)	100.00 (90.00)	10.35	7.52
T_{27}	*N.r.*+ GNO	0.50	80.00 (63.44)	100.00 (90.00)	100.00 (90.00)	11.10	7.63
T_{28}	*N.r.*+ SBO	0.50	55.00 (47.87)	100.00 (90.00)	100.00 (90.00)	10.00	7.33
T_{29}	*N.r.*+ GH	0.50	85.00 (67.21)	100.00 (90.00)	100.00 (90.00)	11.60	7.24
T_{30}	Control (*N.r.*alone)		25.00 (30.00)	50.00 (45.00)	100.00 (90.00)	7.50	8.40
	S.E. $\pm$		1.73	2.30	-	0.11	-
	C.D.(P=0.05)		4.91	6.51	NS	0.33	-

*Figures in parentheses are arcsin value DAI = Days after inoculation *N.r.* = *Nomuraea rileyi*
SFO = Sunflower oil TW= Tween-80 CNO = Coconut oil GLY= Glycerol GNO = Groundnut oil
GH = Ghee TX = Triton-X-100 SBO = Soybean oil BA = Boric acid MUO = Mustered oil
CMC = Carboxymethyl cellulose NS=Non-significant

combinations.

The lowest per cent surface coverage was recorded in T_{14}- *N.r.*+TX +CMC+HO (21.67%). At 10 DAI, all the treatments showed cent per cent growth.

Effect on biomass and pH :

At 10 DAI, the biomass in the treatments was 7.80 to 12.28g against 7.50g in control. T_{12}- *N.r.*+TW+GLY+ HO produced significantly highest (12.28g) fungal biomass. However, it was at par with T_{24}- *N.r.*+SFO (12.17g) and T_{23}- *N.r.*+HO (11.90g).

Next highly promising treatments were T_{29}- *N.r.*+GH, T_{10}- *N.r.*+TW+GLY+BA+CMC+HO and T_{27}- *N.r.*+GNO which produced 11.60, 11.30 and 11.10g biomass, respectively. The pH of the fungal culture developed from 30 treatment formulations ranged from 7.16 in treatment T_{24} *N.r.*+ SFO to 8.75 in T_7 *N.r.*+ *BA* + HO registering biomass of 12.17 and 9.90g, respectively. The pH of formulation producing maximum biomass (12.28g) was 7.37 comparing treatment adjuvants tween 80 0.5% + Glycerol 2% + Honey 1%, when it was 8.40 in control producing biomass of 7.50g per 40 ml medium.

It was established that the *N.rileyi* formulations with combination of adjuvants helped in increasing production of fungal biomass at 10 DAI. T_{12}-*N.r.*+TW+GLY+HO (12.28g) produced highest biomass and T_{23}-*N.r.*+HO (11.90g) and T_{24}-*N.r.*+SFO (12.17g) were at par to it.

Considering the overall performance of the adjuvants for growth and development of *N.rileyi* in series of lab experimentation out of 96 test formulations. 10 formulation comprising *N.r.*+HO (1%),*N.r*+SFO (1%), *N.r.*+GH(0.5%), *N.r.*+TW(0.5%)+ GH(0.5%), *N.r.*+ GLY(2%)+SFO(1%), *N.r.*+GLY(2%)+ GH(0.5%), *N.r.*+SFO(1%)+GH(0.5%), *N.r.*+TW(0.5%) +GLY(2%) +SFO(1%)+CMC(0.5%), *N.r.*+TW(0.5%) +GLY (2%) +HO(1%) and *N.r.*+TW(0.5%)+GLY(2%) +CMC (0.5%) were emerged out as most promising and advanced stage formulations of *N. rileyi.*

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.

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