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Compatibility of *Metarhizium anisopliae* (Metchnikoff) Sorokin, with various adjuvants

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Abstract
An *in-vitro* study was conducted for compatibility of *Metarhizium anisopliae* an entomopathogenic fungus (EPF) with 8 adjuvants at two concentrations *viz*., 0.1 and 0.5% through poisoned food technique. The outcomes were assessed as radial growth and growth prohibition of the EPF on an adjuvant treated medium. All the adjuvants showed inhibition in mycelial development of the EPF and somewhat depending upon their concentrations. The carboxymethyl cellulose (CMC) showed the maximum radial growth with least growth prohibition (66.50 mm and 7.16%, respectively), followed by Silica gel (60.00 mm and 16.25%, respectively) and Neem soap (58.83 mm, and 17.89%, respectively). The findings indicated that CMC could be used in the formulation of *M.anisopliae* with the most astounding spore load of 4.00 x 10^{10} spores ml^{-1}.

Keywords: Adjuvants, compatibility, radial growth and *M. anisopliae*

1. Introduction
The *M. anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) is an imperative microbial biopesticide that causes the green muscardine disease in insects, which is a proficient performance in the management of a wide range of insect-pests (Borgio and Sahayaraj, 2007) [4]. It is one of the important groups of bio-agents that associate with the insects living in diverse habitats, including fresh water, soil surface and aerial location (Joshi *et al*. 2018) [10]. Spontaneous variability of *M. anisopliae* should be considered as a reserve for selection of this biocontrol agent on high virulence towards pest insects (Serebrov *et al*. 2007) [16]. Although, these microbes can lose their viability under to unfavorable states of temperature, humidity and ultraviolet radiation, by influencing the life of conidia. Therefore, the shelf life of microbes can be enhanced by adding appropriate adjuvants, which leads to growth and viability of the fungus, that may act as nutrient, adhesive, wetting agent *etc*. Unique features like pathogenicity for a wide range of insect, easy production procedure on basic substrates with good feasibility in soil and shelf life, have emerged great interest for this microbial agent (Patil *et al*. 2012) [14]. Therefore, a huge number of mycoinsecticides have reached the market place and millions of hectares are treated annually with EPF around the world (Faria and Wraight, 2007) [6]. The compatibility between EPFs and biopesticides may ease the selection of appropriate products under IPM programs (Neves *et al.* 2001) [13]. Such combined applications can improve the efficacy of control by reducing the applied amounts, minimizing environmental pollution threats and build-up of pest resistance (Usha *et al*. 2014) [20]. Consequently, the objective of the present investigation was to study the compatibility of adjuvant/s which could act as a synergist for improving the shelf life of *M. anisopliae*.

2. Materials and methods
The present investigation was conducted in the Biocontrol Research and Production Center, Department of Entomology, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India. The experiment was carried out in a completely randomized design (CRD) and Factorial CRD with 8 adjuvants at two different concentrations in three replicates along with a control (Table-1).

Table 1: Adjuvants evaluated for compatibility with *M. anisopliae*

Tr.	Adjuvants	Property	References
T_1	Tween - 80	Wetting agent and Emulsifiers	Burges (2012) [5]
T_2	Glycerol	Humectant, Carrier, Osmotic protectant	Stevenson *et al*. (2017) [19]
T_3	Neem oil	Anti-microbial, larvicidal	Simone *et al*. (2015) [18]
T_4	Neem soap		
T_5	Carboxymethyl cellulose (CMC)	Thickner, Sticker, Binder, Stabilizer	Petlamul *et al*. (2017) [15]
T_6	Silica gel	Persistence, Desiccants	Agnes (1971) [1]
T_7	Sunflower oil	Antidessicant, Nutrient, Adhesive, and Encoater	Jyothi *et al*. (2014) [11]
T_8	Groundnut oil		
T_9	Control	---	---

2.1 *M. anisopliae* strain

M. anisopliae strain was isolated from *Helicoverpa armigera* larvae, which was grown on Potato Dextrose Agar (PDA) and the bioassay studies were carried out with a standard concentration of $1x10^{10}$ spores ml^{-1}.

2.2 Trial methodology

Impact of the adjuvants was assessed by poisoned food technique in PDA medium on the basis of radial growth and sporulation of *M. anisopliae* (Moorhouse *et al.* 1992) [12]. Disinfected 20 ml PDA with the added adjuvant at two different concentrations were fused in 25 mm width sterile Petri dishes and were permitted to cement under laminar air current. A 5 mm disc of fungus was taken from seven-day old *M. anisopliae* culture and was kept at the center of the Petri dishes containing PDA fused with various adjuvants. The plates were sealed with parafilm and agonized at room condition for its development. PDA without adjuvants was used as a control. The plates were brooded in BOD at 28^0C and the growth of the developing culture was assessed on the 10^{th} day after inoculation. The information was expressed as development prohibition of *M. anisopliae* in the added adjuvants as proposed by Hokkanen and Kotiluoto, 1992 [9].

$$X = \frac{Y-Z}{Y} \times 100$$

where, X, Y, and Z alludes to the level of growth prohibition, radial growth of *M. anisopliae* in control and radial growth of *M. anisopliae* in the poisoned medium, respectively.

3. Results and discussion

The growth performance of *M. anisopliae* recorded on the 10^{th} day after inoculation in different adjuvants are presented in Table-2 and illustrated in fig.-1.

3.1 Radial growth

The results revealed that the differences in the growth of *M. anisopliae* in different additives at 0.5% and 0.1% concentration were significant. Control recorded maximum radial growth of 71.67 mm at both the concentrations. However, among the additives, CMC (T_5) recorded maximum radial growth (67.33 and 65.67 mm) which significantly superior from the other additives. The next effective additives were Silica gel (T_6) followed by Neem soap (T_4) and Sunflower oil (T_7), whereas minimum radial growth was recorded in Groundnut oil (T_8) (36.00 and 36.33 mm) in both the concentrations.

3.2 Inhibitory growth

Perusal of the data revealed that there was a significant difference in the growth inhibition of *M. anisopliae* among different additives at both the concentration. Treatment CMC (T_5) showed minimum growth inhibition (6.02% and 8.30%) and was significantly superior to the other additives. This was followed by Silica gel (T_6), Neem soap (T_4) and Sunflower oil (T_7). While maximum growth inhibition was recorded in Groundnut oil (T8) (49.79 and 49.33%) at both the concentrations.

The data further revealed that among the various concentrations of the adjuvants, higher concentration (0.5%) of CMC (T_5), showed highest radial growth (67.33mm) with minimum growth prohibition (17.16%) which indicated that the CMC was highly compatible with *M. anisopliae* at both the concentrations.

3.3 Spore viability

Perusal of the data revealed that the differences in the mean spore load of *M. anisopliae* were significant. Highest spore count was recorded in control (T_9) (5.00 x 10^{10} spores ml^{-1}), followed by CMC (T_5) (4.00 x 10^{10} spores ml^{-1}) and Neem soap (T_4) (2.50 x 10^{10} spores ml^{-1}) but they did not differ significantly from each other. The next effective adjuvant was Silica gel (T_6) (3.00 x 10^{10} spores ml^{-1}) which was followed by Sunflower oil (T_7) (2.17 x 10^{10} spores ml^{-1}), Glycerol (T_2) (1.83 x 10^{10} spores ml^{-1}) and Tween-80 (T_1) (1.50 x 10^{10} spores ml^{-1}), but all were at par with each other. However, minimum spore count was recorded in Groundnut oil (T_8) (1.00 x 10^{10} spores ml^{-1}).

3.4 Interaction of adjuvants and their concentrations on *M. anisopliae*

Perusal of the data in Table-2 and fig.-1 revealed that the adjuvants exhibited a significant impact on the *M. anisopliae* radial growth, growth inhibition and spore load, but the interaction with different was found to be non-significant. There is no literature on Interaction effect of *M. anisopliae* strain with adjuvants in the present study. Although, few relevant references in support of the results on the growth of *M. anisopliae* on adjuvants.

In the present investigation, CMC was found to be less toxic as it recorded maximum radial growth with lowest growth inhibition and highest spore load in comparison to the other adjuvants. The present findings are in conformity with the findings of Gade (2015) [8] who also stated that the *M. anisopliae* + CMC @ 0.50% recorded the maximum biomass 8.93 g/40 ml at 10 Days After Inoculation and *Ma* + CMC @ 1.25% recorded highest surface coverage (29.33%) at 3 DAI at spore concentration of $1x10^9$ spores/ml. The maximum growth in CMC might be due to release of cellulolytic and xylanases enzymes by *M. anisopliae* on CMC, which plays an important role in the natural biodegradation process and degradation to carbon, which is essential for microorganisms (Betty *et al*. 2013) [2]. In addition, Sharma *et al*. (1998) [17]

reported that carboxymethyl cellulose to the *M. anisopliae* reduced time to 100 percent mortality by more than a week in first instar larvae of *Holotrichia consanguinea*. Therefore, Carboxymethyl cellulose (CMC) is one of the most important cellulose by-products. It is a linear, long chain, water-soluble, anionic polysaccharide derived from cellulose and used as an adjuvant (Bono *et al*. 2009) [3]. In addition, the assessment of spore's compatibility such as silica gel may be a viable alternative to favor the retention of moisture in the air, which reduces the adsorption of water molecules or a process in which water molecules are trapped in the surface pore desiccants. Therefore, silica gel keeps up the viability of dry conidia of *M. anisopliae* (Freitas *et al*. 2014) [7]. Thus, Further study is needed to be done as a combination, which can be used to develop or enhance the efficacy of fungi.

4. Conclusion

It can be concluded that CMC followed silica gel could be used in the formulations of *M. anisopliae* that will improve its timeframe of shelf-life. However, CMC compatible with *M. anisopliae* have the potential use as biological control agents against insect pests because they were relatively safe on non-target insects, such as natural enemies and beneficial soil insects.

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Table 2: Compatibility of adjuvants on growth and development of *Metarhizium anisopliae* on the 10th day after inoculation.

Tr. codes	Adjuvants (Factor-A)	Performance of *M. anisopliae* in different adjuvants at different concentrations								
		Radial growth (mm)			Growth inhibition (%)			Mean spore count ($1x10^{10}$ spores ml^{-1})		
		Concentrations (Factor-B)								
		0.1%	0.5%	Mean	0.1%	0.5%	Mean	0.1%	0.5%	Mean
T_1	Tween 80	47.67	47.33	47.50	33.52 (35.35)	33.96 (35.63)	33.74 (35.49)	1.67 (1.46)	1.33 (1.34)	1.50 (1.40)
T_2	Glycerol	52.00	48.67	50.33	27.44 (31.55)	32.08 (34.44)	29.76 (33.00)	2.00 (1.58)	1.67 (1.46)	1.83 (1.52)
T_3	Neem oil	42.67	39.67	41.17	40.46 (39.49)	44.73 (41.96)	42.59 (40.72)	1.33 (1.34)	1.33 (1.34)	1.33 (1.34)
T_4	Neem soap	59.33	58.33	58.83	17.15 (24.37)	18.62 (25.47)	17.89 (24.92)	2.67 (1.77)	2.33 (1.68)	2.50 (1.73)
T_5	CMC	65.67	67.33	66.50	8.30 (16.15)	6.02 (14.08)	7.16 (15.12)	4.33 (2.20)	3.67 (2.04)	4.00 (2.12)
T_6	Silica gel	60.67	59.33	60.00	15.35 (23.05)	17.16 (24.38)	16.25 (23.72)	3.33 (1.93)	2.67 (1.76)	3.00 (1.85)
T_7	Sunflower oil	57.00	57.67	57.33	20.48 (26.86)	19.55 (26.21)	20.01 (26.53)	2.33 (1.68)	2.00 (1.56)	2.17 (1.62)
T_8	Groundnut oil	36.33	36.00	36.17	49.33 (44.62)	49.79 (44.88)	49.56 (44.75)	1.00 (1.22)	1.00 (1.22)	1.00 (1.22)
T_9	Control	71.67	71.67	71.67	-	-	-	5.00 (2.32)	5.00 (2.32)	5.00 (2.32)
SEm±		1.42	1.69	0.85	1.58	1.44	0.79	0.14	0.14	0.05
CD(p=0.05)		4.21	5.00	2.73	4.68	4.28	2.51	0.40	0.43	0.17
Interaction of Factor: AxB										
SEm±		1.68			1.65			0.12		
CD(p=0.05)		NS			NS			NS		

NS = Non-significant

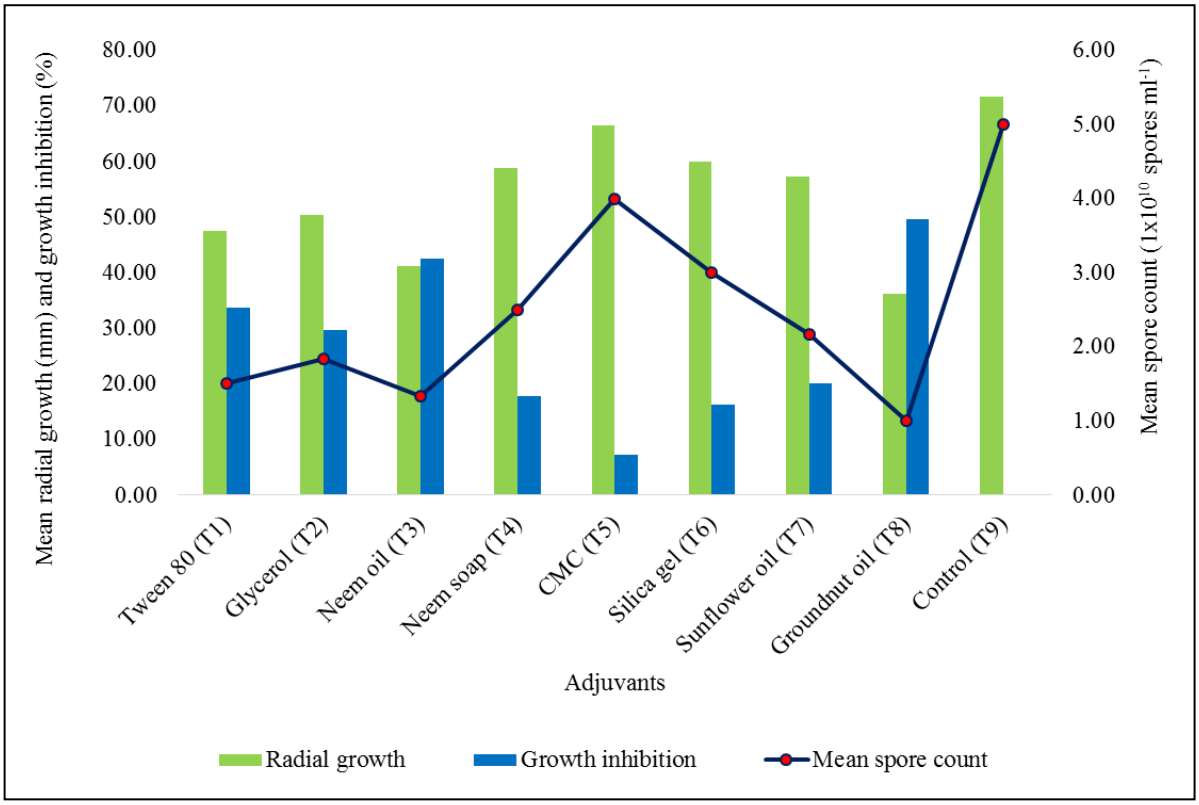

Fig 1: Impact of adjuvants on growth and development of *Metarhizium anisopliae* on 10th day after inoculation

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