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Research Article

Effect of UVC rays on biomass production by *Metarhizium anisopliae* (Metschinikoff) Sorokin when mixed with various adjuvants

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ABSTRACT : The effect of UVC rays on the viability of entomopathogenic fungus, *Metarhizium anisopliae* (Metschinikoff) Sorokin, in the presence of various concentrations of adjuvants comprising glycerol (1.0, 2.0, 3.0 and 5.0%), tween-80 (0.5 and 1.0%), boric acid (1.0, 2.0 and 3.0%), carboxymethyl cellulose (0.5 and 1.0%), indigo (0.5 and 1.0%), turmeric (0.5 and 1.0%), molasses (0.5 and 1.0%), honey (1.0 and 2.0%), milk (1.0 and 2.0%), sunflower oil (0.5 and 1.0%), groundnut oil (0.5, 1.0 and 2.0%), mustard oil (0.5 and 1.0%), soybean oil (0.5 and 1.0%) and ghee (0.5 and 1.0%) and formulations without adjuvants, when exposed for 10 to 50 minutes, 2,3 and 5 hours was studied under laboratory conditions. The UVC rays proved detrimental to the fungus and the effect increased with increase in exposure period. After 5 hours exposure to UVC rays, the fungal culture with *M.a.*+sunflower oil 1.0 per cent produced maximum (5.0g) biomass to rest of the treatments. However, it was at par with *M.a.*+sunflower oil 0.5 per cent (4.90g). The next effective treatment for fungal biomass production was *M.a.*+groundnut oil 0.5 per cent (4.73g), *M.a.*+groundnut oil 1.0 per cent (4.67g) and *M.a.*+groundnut oil 2.0 per cent (4.60g). The *M.anisopliae* without adjuvants exposed to UVC rays produced biomass of 2.23g while, the control *M.a.*alone without UVC exposure produced 6.50g of fungal biomass. Among the various oils sunflower and groundnut oil, among chemical adjuvant glycerol 2.0 per cent, CMC 0.50 per cent, boric acid and among nutrient sources honey, milk act as appreciable UVC protectant.

KEY WORDS : *Metarhizium anisopliae*, Media, Yeast extract, Ultraviolet formulations, Biomass

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INTRODUCTION

Metarhizium anisopliae (Metschinikoff) Sorokin is one of the main fungal candidates for use in microbial control of pests. Special attributes such as pathogenicity for wide group of insects easy mass production on simple substrates and good viability in soil and shelf life have emerged great interest in this mycoagent. The efficacy of pathogens in the field depends on environmental conditions. The extreme temperatures and light including ultraviolet (UV) may influence the distribution of micro-organisms and their persistence in nature (Zimmermann and Butin, 1973 and Ignoffo *et al*., 1977). Roberts and Campbell (1977) reported a rapid decrease of viable spores exposed to direct sunlight and they suggested that the spore mortality was caused by UV radiation. The solar radiation (UV-B radiation) are the major challenges to mycoinsecticide viability. Several reports are available on effect of temperature on growth and activity of fungi (Lomer *et al*., 2001 and Leland, 2005).

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EXPERIMENTAL METHODS

The study was carried out in the Biocontrol Research Laboratory of the Department of Entomology of the University at MPKV, Rahuri, Maharashtra. The Sabouraud's dextrose broth with yeast extract medium was used for multiplication and

growth of the fungus. The 32 formulations of *M.anisopliae* with the adjuvants comprising glycerol (1.0, 2.0, 3.0 and 5.0%), tween-80 (0.5 and 1.0%), boric acid (1.0, 2.0 and 3.0%), carboxymethyl cellulose (0.5 and 1.0%), indigo (0.5 and 1.0%), turmeric (0.5 and 1.0%), molasses (0.5 and 1.0%), honey (1.0 and 2.0%), milk (1.0 and 2.0%), sunflower oil (0.5 and 1.0%), groundnut oil (0.5, 1.0 and 2.0%), mustard oil (0.5 and 1.0%), soybean oil (0.5 and 1.0%) and ghee (0.5 and 1.0%) and formulations without adjuvants were evaluated in C.R.D. with 3 replications for their UVC rays protectability along with *M.anisopliae* 30 per cent AS. Various concentrations of adjuvants were added to optimum concentration of *M.anisopliae* aqua suspension 30 per cent v/v to prepare various formulations. Each formulation was kept in 100ml saline glass bottle. Each formulation was kept in 50 ml glass beaker and such formulations were exposed to UVC rays through UV light source of Phillips TUV lamp for 10, 20, 30, 40, 50 minutes, 2, 3 and 5 hours. The distance between exposed suspension and UV light source was 0.30 m.

One ml of such exposed formulation was added to 40ml Sabouraud's dextrose (SD) broth + Yeast extract medium and observed for growth and development up to 10 days.

EXPERIMENTAL RESULTS AND ANALYSIS

The data of biomass produced by the mycoagent of *M. anisopliae* with various adjuvants in culture medium after UVC rays exposure for 10 to 50 minutes, 2, 3 and 5 hours are presented in Table 1. The difference of biomass production in different treatments were significant and trend of performance of adjuvants was more or less similar

UVC exposure 10 to 50 minutes:

The biomass produced in sunflower oil 1.0 per cent was 5.77g which emerged significantly superior to remaining treatments. However, it was at par with *M.a.*+groundnut oil 2.0 per cent (5.50g), *M.a.*+boric acid 2.0 per cent (5.43g), *M.a.*+sunflower oil 0.5 per cent (5.40g), *M.a.*+boric acid 3.0 per cent (5.37g), *M.a.*+groundnut oil 1.0 per cent (5.37g), *M.a.*+boric acid 1.0 per cent (5.33g), *M.a.*+soybean oil 1.0 per cent (5.30g), *M.a.*+milk 2.0 per cent (5.27g), *M.a.*+soybean oil 0.5 (5.23g), *M.a.*+groundnut oil 0.5 per cent (5.20g), *M.a.*+mustard oil 1.0 per cent (5.13g) and *M.a.*+molasses 2.0 per cent (5.10g). The lowest biomass (2.97g) in treatments with control was recorded.

After 20 minutes UVC rays exposure, significantly maximum biomass (5.73g) with *M.a.*+sunflower oil 1.0 per cent was registered. The next treatments in their decending order were *M.a.*+sunflower oil 0.5 per cent (5.37g), *M.a.*+groundnut oil 2.0 per cent (5.33g), *M.a.*+groundnut oil 1.0 per cent (5.27g), *M.a.*+boric acid 2.0 per cent (5.23g), *M.a.*+soybean oil 1.0 per cent (5.20g) and *M.a.*+milk 2.0 per cent (5.17g). Among the treatments with various adjuvants, the fungus culture with adjuvants produced fungal biomass in ranged from 3.07 to 5.73g.

After 30 minutes UVC rays exposure, sunflower oil 1.0 per cent produced 5.30g fungal biomass which was significantly highest than rest of the treatments. However, it was at par with *M.a.*+sunflower oil 0.5 per cent (5.13g) and *M.a.*+groundnut oil 2.0 per cent (5.13g).

Among treatments of fungus culture with chemical adjuvants, the biomass production was ranged from 2.87 to 5.07g. The least biomass of 2.60g in treatment with turmeric 1.0 per cent was produced.The results of 40 and 50 minutes UVC

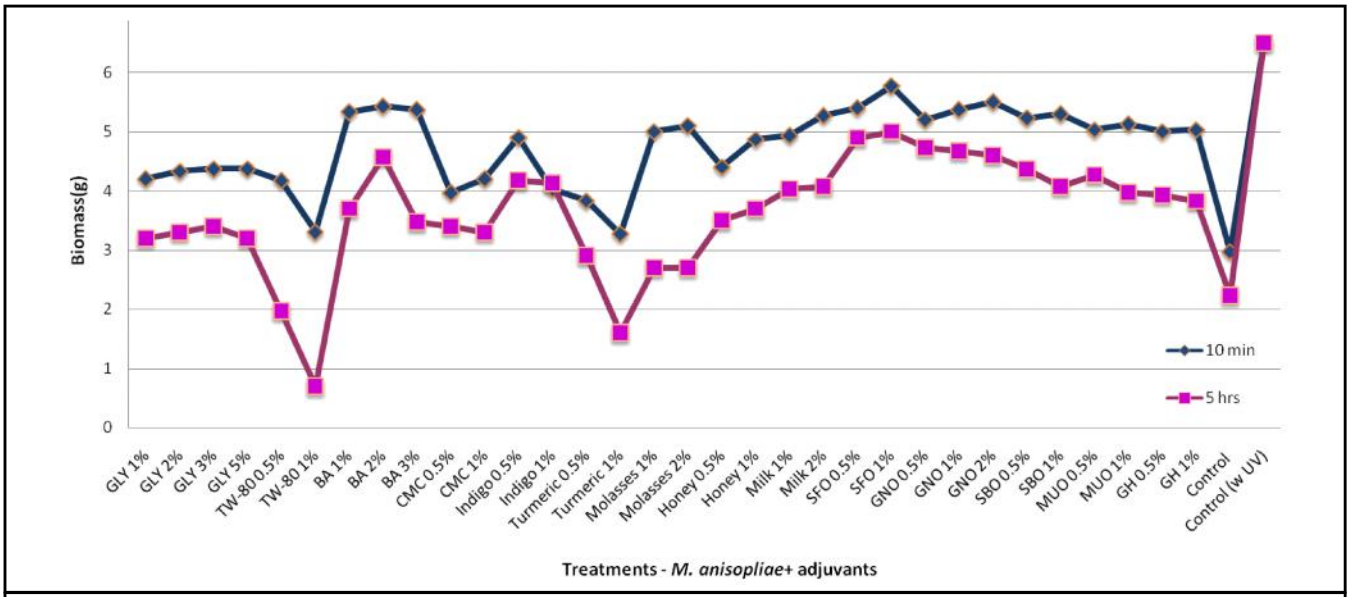

Fig. 1 : Inorganic forms of P (Mean values)

rays exposure were more or less similar to that of 30 minutes UVC rays exposure. The fungus culture with sunflower oil 0.5 and 1.0 per cent produced significantly highest fungal biomass of 5.10 and 5.03g at 40 and 50 minutes exposure, respectively.

UVC exposure 2, 3 and 5 hours :

After 2 hours UVC rays exposure, sunflower oil 1.0 per cent (5.03g) showed its superiority for biomass production to remaining treatments with adjuvants (1.20g to 5.00g) and control (2.33g). *M.a.*+Tween-80, *M.a.*+turmeric and *M.a.*+molasses produced least biomass (1.20 to 3.20g) whereas control produced 2.33g biomass. The results of 3 hours UVC rays exposure was more or less similar to that of 2 hours UVC rays exposure.

Table 1 : Effect of UVC treatment on biomass production by *M.anisopliae* in the presence of some adjuvants

Tr. No.	Treatments	Conc. (%)	Biomass (g) produced after indicated exposure							
			10min	20min	30min	40min	50min	2hrs	3hrs	5hrs
T_1	*M.a.*+Glycerol	1.0	4.20	3.97	3.67	3.60	3.53	3.50	3.30	3.20
T_2	*M.a.*+Glycerol	2.0	4.33	4.20	4.00	3.43	3.40	3.40	3.30	3.30
T_3	*M.a.*+Glycerol	3.0	4.37	4.30	3.97	3.40	3.40	3.73	3.57	3.40
T_4	*M.a.*+Glycerol	5.0	4.37	4.17	3.97	3.47	3.40	3.40	3.37	3.20
T_5	*M.a.*+Tween-80	0.5	4.17	3.90	3.77	2.97	2.97	2.67	2.27	1.97
T_6	*M.a.*+Tween-80	1.0	3.30	3.67	2.87	2.67	2.43	1.20	0.80	0.70
T_7	*M.a.*+Boric acid	1.0	5.33	5.07	4.77	3.97	3.93	3.80	3.80	3.70
T_8	*M.a.*+Boric acid	2.0	5.43	5.23	5.07	4.90	4.73	4.70	4.63	4.57
T_9	*M.a.*+Boric acid	3.0	5.37	4.93	4.80	4.10	4.03	4.00	3.70	3.47
T_{10}	*M.a.*+CMC	0.5	3.97	3.97	3.77	3.50	3.50	3.47	3.47	3.40
T_{11}	*M.a.*+CMC	1.0	4.20	3.97	3.77	3.40	3.33	3.30	3.30	3.30
T_{12}	*M.a.*+Indigo	0.5	4.90	4.70	4.33	4.30	4.30	4.23	4.27	4.17
T_{13}	*M.a.*+Indigo	1.0	4.03	4.73	4.37	4.37	4.37	4.30	4.23	4.13
T_{14}	*M.a.*+Turmeric	0.5	3.83	3.63	3.30	3.20	3.23	3.20	3.17	2.90
T_{15}	*M.a.*+Turmeric	1.0	3.27	3.07	2.60	1.97	1.80	1.70	1.70	1.60
T_{16}	*M.a.*+Molasses	1.0	5.00	4.90	4.87	4.27	4.20	2.93	2.87	2.70
T_{17}	*M.a.*+Molasses	2.0	5.10	5.00	4.87	4.30	4.30	2.90	2.70	2.70
T_{18}	*M.a.*+Honey	0.5	4.40	4.33	4.00	3.90	3.80	3.70	3.60	3.50
T_{19}	*M.a.*+Honey	1.0	4.87	4.77	4.53	4.10	4.00	3.90	3.80	3.70
T_{20}	*M.a.*+Milk	1.0	4.93	4.77	4.53	4.17	4.17	4.30	4.20	4.03
T_{21}	*M.a.*+Milk	2.0	5.27	5.17	4.83	4.63	4.47	4.47	4.33	4.07
T_{22}	*M.a.*+Sunflower oil	0.5	5.40	5.37	5.13	5.10	5.03	5.00	5.00	4.90
T_{23}	*M.a.*+Sunflower oil	1.0	5.77	5.73	5.30	5.10	5.03	5.03	5.00	5.00
T_{24}	*M.a.*+Groundnut oil	0.5	5.20	5.17	4.90	4.80	4.80	4.77	4.73	4.73
T_{25}	*M.a.*+Groundnut oil	1.0	5.37	5.27	5.07	5.00	5.00	4.77	4.73	4.67
T_{26}	*M.a.*+Groundnut oil	2.0	5.50	5.33	5.13	4.90	4.90	4.90	4.73	4.60
T_{27}	*M.a.*+Soybean oil	0.5	5.23	5.07	4.73	4.70	4.73	4.77	4.50	4.37
T_{28}	*M.a.*+Soybean oil	1.0	5.30	5.20	4.73	4.53	4.43	4.40	4.30	4.07
T_{29}	*M.a.*+Mustard oil	0.5	5.03	4.97	4.77	4.50	4.40	4.33	4.30	4.27
T_{30}	*M.a.*+Mustard oil	1.0	5.13	4.97	4.67	4.33	4.23	4.20	4.13	3.97
T_{31}	*M.a.*+Ghee	0.5	5.00	4.83	4.37	4.30	4.23	4.20	4.07	3.93
T_{32}	*M.a.*+Ghee	1.0	5.03	4.93	4.57	4.33	4.23	4.00	3.90	3.83
T_{33}	Control (*M.a.*alone)	-	2.97	2.83	2.63	2.40	2.37	2.33	2.30	2.23
T_{34}	Control (*M.a.*alone) (W.UVC)	-	6.50	6.40	6.40	6.50	6.50	6.50	6.60	6.50
	S.E $\pm$		0.08	0.05	0.08	0.06	0.08	0.05	0.07	0.05
	C.D(P=0.05)		0.23	0.15	0.24	0.17	0.23	0.16	0.21	0.14

DAI = Days after inoculation *M.a. = Metarhizium anisopliae* W.UVC= without UVC
CMC = Carboxymethyl cellulose

After 5 hours exposure to UVC rays, the fungal culture with *M.a.*+sunflower oil 1.0 per cent produced maximum (5.0g) biomass to rest of the treatments. However, it was at par with *M.a.*+sunflower oil 0.5 per cent (4.90g). The next effective treatment for fungal biomass production was *M.a.*+groundnut oil 0.5 per cent (4.73g), *M.a.*+groundnut oil 1.0 per cent (4.67g) and *M.a.*+groundnut oil 2.0 per cent (4.53g). The control *M.a.*alone without UVC exposure produced 6.50g of fungal biomass (Fig.1).

It is indicated that surface coverage and biomass produced by *M.anisopliae* with or without adjuvants in culture medium after exposure to UVC rays for 10 to 50 minutes, 2, 3 and 5 hours decreased with increase in exposure period. The adjuvants reacted variably for their UVC rays protecting capacity for *M.anisopliae.* However, higher concentrations of the adjuvants were better than their lower ones except turmeric and CMC. Among the various oils sunflower and groundnut oil, among chemical adjuvant glycerol 2.0 per cent, CMC 0.50 per cent, boric acid and among nutrient sources honey, milk act as appreciable UVC protectant.

According to Hunt *et al.* (1994), the chemical sunscreen incorporated in oil formulations of the *Metarhizium* spp. gave protection after solar radiation of 2 h but increased exposure upto 5 h failed to offer protection. Moore *et al.* (1993) pointed out that the conidial viability of *Metarhizium* spp. decreased with increased UV exposure. Similarly Alves *et al.* (1998) reported that germination of *Metarhizium anisopliae* decreased with increasing exposure time to solar radiation. Peanut oil enhanced the conidial tolerance against UV light for upto 6 h of exposure compared to unformulated and tween-80. Reduction in relative per cent culturability of *M.anisopliae* with increased UV exposure from 1 to 8 h reported by Braga *et al.* (2001). Rangel and Roberts (2007) reported that any carbon source plus 1 per cent NaCl or KCl with high alkalinity had the highest UVB tolerance. Francisco *et al*. (2008) found that conidia of *M.anisopliae* with oil emulsion had higher survival after 3h of UV exposure. These findings are in line with the present investigation.

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