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Research Article

Effect of UVC Rays on Aqua Suspension Formulations of *Metarhizium Anisopliae* (Metschnikoff) Sorokin

S. D. Patil[1]*, R. S. Jadhav[2], and S. A. Raut[3]

[1]Department of Entomology, Agricultural Research Station, Niphad, At Post, Tal.- Niphad, Dist.-Nasik
[2]Department of Entomology, College of Horticulture, Malegaon, Tal. Malegaon, Dist. Nasik
[3]Department of Plant Pathology, College of Agriculture, Babhulgaon, Tal. Yeola, Dist. Nasik

Abstract

The effect of UVC rays on the viability of entomopathogenic fungus**,** *Metarhizium anisopliae* (Metschnikoff) Sorokin, in eight various formulations comprising with *M.a.*+ tweeen-80+ carboxymethyl cellulose (*M.a.*+ TW(0.5%)+CMC (0.5%), *M.a.*+ sunflower oil+ carboxymethyl cellulose (*M.a.*+ SFO (1.0%)+CMC(0.5%), *M.a.*+ sunflower oil+honey (*M.a.*+ SFO(1.0%)+HO(1.0%), *M.a.*+ groundnut oil + boric acid (*M.a.*+ GNO(0.5%)+BA(2.0%), *M.a.*+ groundnut oil + carboxymethyl cellulose (*M.a.*+GNO(0.5%)+CMC(0.5%), *M.a.*+ groundnut oil + ghee(*M.a.*+ GNO(0.5%)+GH(0.5%), *M.a.*+ ghee + honey (*M.a.*+ GH(0.5%)+HO(1.0%) and control without adjuvants (*M.anisopliae* alone) of *M. anisopliae* when exposed for 10 to 50 minutes, 2,3 and 5 hours were 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, *M.a.*+ SFO(1.0%)+HO(1.0%), produced highest (4.70g) biomass when it was in rest of the treatments (3.93 to 4.70g) against 2.07g in control (*M.a.*alone). The next promising treatments were *M.a.*+ SFO(1.0%)+CMC(0.5% (4.63g) and *M.a.* +GNO(0.5%)+BA(2.0%) (4.53g). The control *M.a.*alone without UVC exposure produced 6.47g of fungal biomass.

Keywords: *Metarhizium anisopliae*, media, yeast extract, Ultraviolet, formulations, biomass

***Correspondence**
Author: S.D.Patil
Email: saurushrutu@gmail.com

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. The fungus *M.anisopliae* from the larvae of grain weevil was isolated and also first to demonstrate entomopathogenic nature of the fungus against chrysomelid, curculonid and scarabaeid beetles [1].

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 [2]. A rapid decrease of viable spores exposed to direct sunlight and they suggested that the spore mortality was caused by UV radiation [3]. 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 [4, 5]. In the presence study laboratory study were undertaken to determine the UV rays protecting ability of various adjuvants used for entomopathogenic fungal formulation. Therefore, the study was carried out to determine the impact of UV rays on the surface growth and biomass production of fungus by various formulations after exposure to UVC rays.

Material and methods

The pure fungus culture of *M.anisopliae* was made, available from isolates in Biocontrol Lab of Entomological centre, Mahatma Phule Krishi Vidyapeeth, Rahuri.

Laboratory study was carried out in the Biocontrol Research Laboratory of the Department of Entomology of the University at MPKV, Rahuri, Maharashtra during 2009 to 2012. The Sabouraud's dextros broth with yeast extract medium was used for multiplication and growth of the fungus. The highly promising eight formulations 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) control without adjuvants (*M.anisopliae* alone) of *M.anisopliae* were evaluated in C.R.D. with 3 replications for their UVC rays protecting ability along with *M.anisopliae* 30% AS. One formulation without adjuvant and without UVC rays exposure was also kept for determine the effect of UVC rays. Various concentrations of adjuvants were added to optimum concentration of *M.anisopliae* aqua suspension 30% v/v to prepare various formulations. Each formulation was kept in 100ml saline glass bottle. Each formulation were 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. 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.

Result and discussion

Effect of UVC rays on growth and development of inoculum of formulations

Effect on growth (surface coverage) after exposure to UVC rays

The data on the advanced promising AS formulations of *M.anisopliae* exposed to UVC rays for 10 to 50 minutes and 2, 3 and 5 hours are presented in **Tables 1** to **3**.

Table 1 Influence of AS formulations of M.anisopliae exposed to UVC rays for 10 and 20 minutes on growth of inoculums

Tr. No.	Treatment	Conc. of adj. (%)	Surface coverage (%)					
			10 min. UVC exposure			20 min. UVC exposure		
			3 DAI	7 DAI	10 DAI	3 DAI	7 DAI	10 DAI
T1	*M.a.*+ TW+CMC	0.5+0.5	18.33 (25.33)*	38.33 (38.23)	83.33 (65.88)	15.00 (22.79)	35.00 (36.27)	80.00 (63.44)
T2	*M.a.*+ SFO+CMC	1.0+0.5	26.67 (31.11)	86.67 (68.61)	100.00 (90.00)	23.33 (28.86)	83.33 (65.88)	100.00 (90.00)
T3	*M.a.*+ SFO+HO	1.0+1.0	28.33 (32.14)	95.00 (77.08)	100.00 (90.00)	25.00 (30.00)	91.67 (73.26)	100.00 (90.00)
T4	*M.a.*+ GNO+BA	0.5+2.0	25.00 (30.00)	95.00 (77.08)	100.00 (90.00)	21.67 (27.76)	90.00 (71.56)	100.00 (90.00)
T5	*M.a.*+ GNO+CMC	0.5+0.5	23.33 (28.86)	86.67 (68.61)	100.00 (90.00)	18.33 (25.33)	83.33 (65.88)	100.00 (90.00)
T6	*M.a.*+ GNO+GH	0.5+0.5	21.67 (27.76)	83.33 (65.88)	100.00 (90.00)	20.00 (26.56)	78.33 (62.24)	100.00 (90.00)
T7	*M.a.*+ GH+HO	0.5+1.0	21.67 (27.76)	68.33 (55.73)	100.00 (90.00)	20.00 (26.56)	66.67 (54.76)	100.00 (90.00)
T8	Control (*M.a.*alone)	-	3.33 (10.47)	33.33 (35.24)	46.67 (43.11)	3.33 (10.47)	30.00 (33.21)	40.00 (39.23)
T9	Control (*M.a.*alone) (wt.UVC)	-	33.33 (35.24)	100.00 (90.00)	100.00 (90.00)	36.67 (37.29)	100.00 (90.00)	100.00 (90.00)
	S.E +		**1.54**	**1.96**	**1.94**	**1.24**	**1.41**	**1.08**
	C.D (P=0.05)		**4.57**	**5.82**	**5.78**	**3.69**	**4.20**	**3.22**

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

Table 2 Influence of AS formulations of *M.anisopliae* exposed to UVC rays for 30, 40 and 50 minutes on growth of inoculum

Tr No	Treatment	Conc. of adj. (%)	Surface coverage (%)								
			30 min. UVC exposure			40 min. UVC exposure			50 min. UVC exposure		
			3 DAI	7 DAI	10 DAI	3 DAI	7 DAI	10 DAI	3 DAI	7 DAI	10 DAI
T1	*M.a.*+ TW+ CMC	0.5+0.5	13.33 (21.39)*	31.67 (34.27)	76.67 (61.14)	10.00 (18.44)	28.33 (32.14)	71.67 (57.86)	8.33 (16.78)	25.00 (30.00)	66.67 (54.76)
T2	*M.a.*+SFO+ CMC	1.0+0.5	18.33 (25.33)	81.67 (64.67)	100.00 (90.00)	15.00 (22.79)	78.33 (62.24)	100.00 (90.00)	11.67 (20.00)	73.33 (58.89)	100.00 (90.00)
T3	*M.a.*+ SFO +HO	1.0+1.0	21.67 (27.76)	86.67 (68.61)	100.00 (90.00)	18.33 (25.33)	83.33 (65.88)	100.00 (90.00)	13.33 (21.39)	80.00 (63.44)	100.00 (90.00)
T4	*M.a.*+ GNO +BA	0.5+2.0	20.00 (26.56)	86.67 (68.61)	100.00 (90.00)	20.00 (26.56)	81.67 (64.67)	100.00 (90.00)	16.67 (24.12)	76.67 (61.14)	100.00 (90.00)
T5	*M.a.*+ GNO +CMC	0.5+0.5	18.33 (25.33)	78.33 (65.24)	100.00 (90.00)	15.00 (22.79)	73.33 (58.89)	100.00 (90.00)	13.33 (21.39)	70.00 (56.79)	100.00 (90.00)
T6	*M.a.*+ GNO +GH	0.5+0.5	16.67 (24.12)	73.33 (58.89)	100.00 (90.00)	15.00 (22.79)	70.00 (56.79)	100.00 (90.00)	11.67 (20.00)	65.00 (53.73)	100.00 (90.00)
T7	*M.a.*+ GH +HO	0.5+1.0	16.67 (24.12)	63.33 (52.71)	100.00 (90.00)	13.33 (21.39)	58.33 (49.79)	100.00 (90.00)	11.67 (20.00)	53.33 (46.89)	100.00 (90.00)
T8	Control (*M.a.*alone)	-	3.33 (10.47)	26.67 (31.11)	36.67 (37.29)	1.67 (7.49)	25.00 (30.00)	31.67 (34.27)	1.67 (7.49)	18.33 (25.33)	26.67 (31.11)
T9	Control(*M.a.* alone) (W.UVC)	-	35.00 (36.27)	100.0 (90.0)	100.0 (90.0)	38.33 (38.23)	100.0 (90.0)	100.0 (90.0)	36.67 (37.29)	100.0 (90.0)	100.0 (90.0)
	S.E ±		**1.55**	**1.48**	**1.30**	**1.65**	**1.24**	**1.51**	**1.77**	**1.47**	**1.16**
	C.D (P=0.05)		**4.59**	**4.41**	**3.85**	**4.90**	**3.69**	**4.48**	**5.26**	**4.38**	**3.45**

*Figures in parentheses are arcsin values, DAI = Days after inoculation, *M.a.*. = *Metarhizium anisopliae*, W.UVC=without UVC, SFO = Sunflower oil, GNO = Groundnut oil, GH = Ghee, HO = Honey, TW = Tween-80, BA = Boric acid, CMC = Carboxymethyl Cellulose

Table 3 Influence of AS formulations of *M.anisopliae* exposed to UVC rays for 2, 3 and 5 hours on growth of inoculums

Tr. No	Treatment	Concof adj. (%)	Surface coverage (%)								
			2 hrs. UVC exposure			3 hrs. UVC exposure			5 hrs. UVC exposure		
			3 DAI	7 DAI	10 DAI	3 DAI	7 DAI	10 DAI	3 DAI	7 DAI	10 DAI
T1	*M.a.*+TW+ CMC	0.5+0.5	6.67 (15.00)*	21.67 (27.76)	58.33 (49.79)	0.00 (0.00)	16.67 (24.12)	51.67 (45.97)	0.00 (0.00)	11.67 (20.00)	41.67 (39.82)
T2	*M.a.*+SFO+ CMC	1.0+0.5	8.33 (16.78)	66.67 (54.76)	91.67 (73.26)	0.00 (0.00)	61.67 (51.77)	81.67 (64.67)	0.00 (0.00)	53.33 (46.89)	75.00 (60.00)
T3	*M.a.*+SFO+ HO	1.0+1.0	10.00 (18.44)	73.33 (58.89)	93.33 (75.00)	0.00 (0.00)	65.00 (53.73)	83.33 (65.88)	0.00 (0.00)	58.33 (49.79)	75.00 (60.00)
T4	*M.a.*+GNO+ BA	0.5+2.0	11.67 (20.00)	70.00 (56.79)	90.00 (71.56)	0.00 (0.00)	60.00 (50.77)	81.67 (64.67)	0.00 (0.00)	53.33 (46.89)	71.67 (57.86)
T5	*M.a.*+GNO+ CMC	0.5+0.5	10.00 (18.44)	63.33 (52.71)	91.67 (73.26)	0.00 (0.00)	58.33 (49.79)	83.33 (65.88)	0.00 (0.00)	48.33 (44.03)	75.00 (60.00)
T6	*M.a.*+ NO+ GH	0.5+0.5	8.33 (16.78)	58.33 (49.79)	90.00 (71.56)	0.00 (0.00)	51.67 (45.97)	80.00 (63.44)	0.00 (0.00)	46.67 (43.11)	68.33 (55.73)
T7	*M.a.*+ GH+ HO	0.5+1.0	8.33 (16.78)	46.67 (43.11)	88.33 (70.00)	0.00 (0.00)	41.67 (40.22)	81.67 (64.67)	0.00 (0.00)	33.33 (35.24)	68.33 (55.73)
T8	Control (*M.a.*alone)	-	0.00 (0.00)	13.33 (21.39)	20.00 (26.56)	0.00 (0.00)	11.67 (20.00)	13.33 (21.39)	0.00 (0.00)	8.33 (16.78)	10.00 (18.44)
T9	Control (*M.a* alone) (W.UVC)	-	35.00 (36.27)	100.0 (90.0)	100.0 (90.0)	36.67 (37.29)	100.0 (90.0)	100.0 (90.0)	38.33 (38.23	100.0 (90.0)	100.0 (90.0)
	S.E ±		**1.44**	**1.22**	**2.38**	**-**	**1.16**	**1.81**	**-**	**1.43**	**1.74**
	C.D (P=0.05)		**4.28**	**3.61**	**7.08**	**-**	**3.44**	**5.37**	**-**	**2.26**	**5.16**

*Figures in parentheses are arcsin values, DAI = Days after inoculation, *M.a.*. = *Metarhizium anisopliae*, W.UVC=without UVC, SFO = Sunflower oil, GNO = Groundnut oil, GH = Ghee, HO = Honey, TW = Tween-80, BA = Boric acid, CMC = Carboxymethyl Cellulose

UVC exposure- 10 to 50 minutes

After 10 minutes exposure to UVC rays, T3- SFO + HO recorded highest (28.33%) growth of fungus at 3 DAI. However, it was at par with T2- *M.a.*+SFO+CMC (26.67%), it was followed by T4-*M.a.*+GNO+ BA (25.0%), T5-*M.a.*+GNO+CMC (23.33%), T6- *M.a.*+GNO+GH and T7- *M.a.*+GH+HO (21.67%each). Least (3.33%) surface coverage by fungus was registered in T11-control. Similar trend of growth was observed at 7 DAI. At 10 DAI, formulations except T1- *M.a.*+TW+CMC (83.33%) and T8- Control (46.67%) registered cent per cent surface coverage. The coverage in the 20, 30, 40 and 50 minutes treatments at 3 DAI was in the range of 3.33 to 25.00, 3.33 to 21.67, 1.67 to 20.00 and 1.67 to 16.67 against corresponding figures of 30.0 to 91.67, 26.67 to 86.67, 25.0 to 83.33 and 18.33 to 80.00 at 7 DAI and at 10 DAI, it was 40.0 to 100, 36.67 to 100, 31.67 to 100 and 26.67 to 100, respectively.

UVC exposure- 2, 3 and 5 hours

Data presented in Table 3 revealed that all the aqua suspension formulations exposed to UVC rays prevented the growth of fungus at 3 DAI after exposure to UVC rays for 3 and 5 hours. The surface coverage in the 2, 3 and 5 hours exposure was in the range 13.33 to 73.33, 11.67 to 65.0 and 8.33 to 58.33 at 7 DAI against 20.0 to 93.33, 13.33 to 83.33 and 10.0 to 75.0 at 10 DAI, respectively.

Effect on develpoment (biomass production)

The biomass produced by *M.anisopliae* with various adjuvants in medium after UVC irradiation for 10 to 50 minutes and 2, 3 and 5 hours are presented in **Table 4** and depicted in **Figure 1**. The differences in biomass production in various promising formularies were significant and trend of performance of formulations were more or less similar to that was observed for surface coverage at 3 and 7 DAI.

Table 4 Effect of UVC treatment on biomass production by *M.anisopliae* AS formulations

Tr. No.	Treatments	Conc.(%) Of adj.	Biomass (g) at 10 DAI after indicated exposure							
			10 min	20 min	30 min	40 min	50 min	2 hrs	3 hrs	5 hrs
T1	*M.a.*+ TW+CMC	0.5+0.5	4.73	4.63	4.57	4.52	4.47	4.30	4.20	3.93
T2	*M.a.*+ SFO+CMC	1.0+0.5	5.23	5.18	5.13	5.10	5.03	4.93	4.83	4.63
T3	*M.a.*+ SFO+HO	1.0+1.0	5.33	5.27	5.22	5.13	5.10	5.00	4.90	4.70
T4	*M.a.*+ GNO+BA	0.5+2.0	5.17	5.12	5.07	4.77	4.90	4.80	4.70	4.53
T5	*M.a.*+ GNO+CMC	0.5+0.5	5.10	5.07	5.02	4.70	4.83	4.73	4.63	4.43
T6	*M.a.*+ GNO+GH	0.5+0.5	4.97	4.93	4.88	4.80	4.72	4.60	4.50	4.30
T7	*M.a.*+ GH+HO	0.5+1.0	4.87	4.83	4.78	4.72	4.63	4.50	4.37	4.30
T8	Control (*M.a.*alone)	-	2.98	2.93	2.87	2.82	2.77	2.57	2.47	2.07
T9	Control (*M.a.*alone) (W.UVC)	-	6.47	6.50	6.50	6.60	6.63	6.60	6.50	6.47
	S.E ±		**0.04**	**0.03**	**0.03**	**0.04**	**0.05**	**0.04**	**0.04**	**0.05**
	C.D (P=0.05)		**0.11**	**0.09**	**0.09**	**0.11**	**0.14**	**0.13**	**0.13**	**0.14**

After 10 minutes, UVC rays exposure the biomass produced by various promising formulations and control was in the range of 2.98 to 5.33g. T3- *M.a.*+SFO+HO produced significantly highest (5.33g) biomass. However, it was at par with T2- *M.a.*+SFO+CMC (5.23g). The next best formularies were T4- *M.a.*+GNO+BA (5.17g) and T5-*M.a.*+GNO+CMC (5.10g). T3- *M.a.*+SFO+HO recorded highest (5.27g) biomass. However, it was at par with T2-*M.a.*+SFO+CMC (5.18g). The next promising formulations were T4- *M.a.*+GNO+BA (5.12g) and T5-*M.a.*+GNO+CMC (5.07g) when exposed for 20 minutes to UVC rays. The least biomass (2.93g) was in control. After 30 minutes exposure, T3- *M.a.*+SFO+HO produced highest (5.22g) biomass. However, it was at par with T2-*M.a.*+SFO+CMC (5.13g).

The trend of results of 40, 50 minutes, 2, 3 and 5 hours exposure on biomass development was more or less similar to that of 30 minutes UVC rays exposure. The biomass production decreased with increase in time of exposure to UVC rays. The formulations T3- *M.a.*+SFO+HO produced significantly highest biomass of 5.13, 5.10, 5.00, 4.90

and 4.70g; however, it was at par with T2- *M.a.*+SFO+CMC which produced 5.10, 5.03, 4.93, 4.83 and 4.63g of biomass after 40, 50 minutes, 2, 3 and 5 hours UVC rays exposure, respectively. The next promising formulations for better production of biomass on exposure of 40 minutes to 5 hrs were T4- *M.a.*+GNO+BA (5.13 to 4.78g) and T5- *M.a.*+GNO+CMC (4.70 to 4.43g). The lowest biomass of 2.82, 2.77, 2.57, 2.47 and 2.07g developed in adjuvants devoid in T8- control (*M.a.*alone) was registered on the exposure for 40,50 min, 2,3 and 5 hrs, respectively.

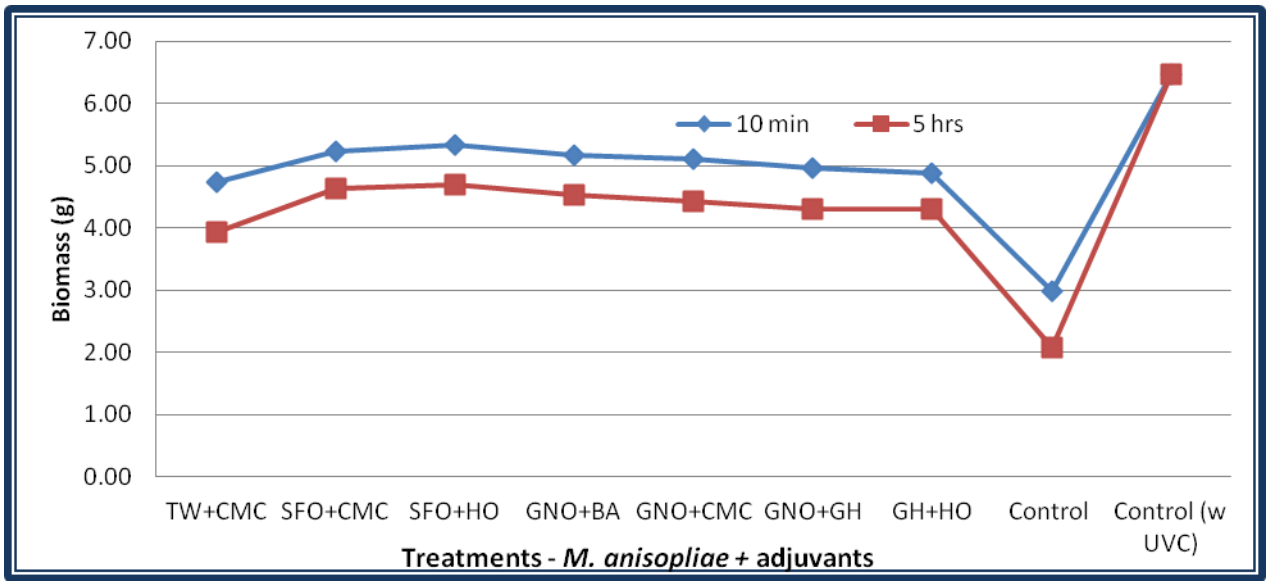

Figure 1 Effect of 10 minutes and 5 hrs UVC exposure on biomass production by M. anisopliae with adjuvants

It is indicated that medium surface coverage and biomass development of *M.anisopliae* in culture medium on inoculation of formulations either with or without adjuvants after exposure to UVC rays for 10 to 50 minutes, 2, 3 and 5 hours decreased with increase in exposure period. The UVC protecting ability of *M.anisopliae* by adding various adjuvants have been discussed.

According to [6] the chemical sunscreen incorporated in oil formulations of the *Metarhizium spp. g*ave protection after solar radiation of 2 h but increased exposure upto 5h failed to offer protection. The conidial viability of *Metarhizium spp.* decreased with increased UV exposure [7]. Similarly [8] 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 [9]. A carbon source plus 1% NaCl or KCl with high alkalinity had the highest UVB tolerance [10]. The conidia of *M.anisopliae* with oil emulsion had higher survival after 3h of UV exposure. These findings are in line with the present investigation [11].

Conclusion

It is concluded from the results that UV rays were detrimental to the growth and development of *M.anisopliae* and the effect increased with increasing exposure period. However, the formulations *M.anisopliae* + Sunflower oil + Carboxyl methyl cellulose and *M.anisopliae* + Sunflower oil + Honey proved to be effective UV protectants for this entomolpathogen.

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