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#### RESEARCH PAPER

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# Effect of UVC rays on biomass production by *Nomuraea rileyi* (Farlow) Samson 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, N.r.+SFO 1.0 per cent produced highest (4.97g) biomass when it was in rest of the treatments (2.40 to 4.87g) against 1.80g in control (N.r. alone). The next promising treatments were N.r.+SFO 0.5 per cent (4.87g), N.r.+GNO 1.0 per cent (4.87g) and N.r.+GNO 2.0 per cent (4.77g). The control N.r. alone without UVC exposure produced 7.30g 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.

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# **INTRODUCTION**

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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. 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 (Vimla Devi *et al.*, 2002).



Efficiency of entomopathogens in the field depends upon virulence towards target pest, coverage and persistence on target site. However, major constraints for successful use of such bio-agents are their difficulties in use of pure cultures, survival on crop after application, loosing virulence by ultra violet (UV) rays, short shelf life, and dependability on the prevailing environmental conditions are the problems reported by Kaur *et al.* (1999).

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). In the presence study laboratory tests were undertaken to determine the UV rays protactability 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 with various adjuvants.

# **MATERIAL AND METHODS**

The study was carried out in the Biocontrol Research Laboratory of the Department of Entomology of the University at Mahatma Phule Krishi Vidyapeeth, Rahuri (M.S.). The Sabouraud's dextros broth with yeast extract medium was used for multiplication and growth of the fungus. The 32 formulations of N. rileyi 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 (0.5 and 1.0%), groundnut (0.5, 1.0 and 2.0%), mustard (0.5 and 1.0%), soybean (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 N.rileyi 30 per cent AS. Various concentrations of adjuvants were added to optimum concentration of N.rileyi aqua suspension 30 per cent 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 3<sup>rd</sup>, 7<sup>th</sup> and 10<sup>th</sup> days and fungal biomass on 10<sup>th</sup> day after inoculation were noted. The experimental data were subjected to statistical analysis. These experiments were carried out in Complete Randomized Design with three replications.

# **RESULTS AND DISCUSSION**

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

# Effect of 10 to 50 minutes and 1 to 5 hours UVC rays on growth of the mycoagents :

*N.rileyi* liquid cultures with various adjuvants exposed to UVC rays for 10 to 50 minutes, 2, 3 and 5 hours and observations on per cent surface coverage by the fungal growth on the medium although noted at 3,7 and 10 days after exposure results at 10 DAI are presented in Table 1.

#### UVC exposure -10 minutes:

At 10 DAI, all the treatments with various adjuvants recorded cent per cent growth of fungus except the treatments with tween-80 0.5 per cent (48.33%), tween-80 1.0 per cent (60.0%) and control (*N.r.*alone) (98.33%), respectively. Overall 10 minutes UVC rays exposure was harmless to *N. rileyi*. Detrimental effect of tween-80 might be attributed to breaking surface tension on *N.rileyi* spores due to its wetting agent action which might had permitted adverse impact of UVC rays.

## UVC exposure -20, 30, 40 and 50 minutes:

The trend of results was almost same in all observations except glycerol 1 and 2 per cent comprising treatments which showed slightly low (96.67 to 98.33%), moderately low (83.33 to 95%) and (81.67 to 90%) at 1 to 5 per cent concentrations by the exposure for 20, 30

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(00.00) 00.0 (90.0) 00.06 (90.0) 00.0 (90.0) (0.00) 0.001 00.06 (90.0) 00.06) 00.00 00.06) 0000 00.0 (90.0) 00.00 (90.0) 00.0 (90.0) 00.06) 00.00 00.0 (90.0) 00.0 (90.0) 00.06) 0000 10 min 0.27 0.76 Abbreviations and other details are beneath Table 2 Conc (0%) ipi 0.5 0.5 2 5.0 0.5 1.0 3.0 0.5 1.0 0.5 1.0 0.5 1.0 1.0 2.0 1.0 1.0 2.0 1.0 0.5 1.0 2.0 0.5 1.0 0.5 0.1 0.5 1.0 2.0 3.0 1.0 2.0 Control (N.r. alone) (W.UVC) C.D(P=0.05) S.E+ Control (N.r.alone) N.r.+ Turmeric N.r.+ Turmeric N.r.+ Molasses V.r.+ Molasses N.r.+ TW-80 N.r.+ TW-80 N.r.+ Indigo V.r.+ Indigo V.r.+ MUO N.r.+ MUO N.r.+ CMC N.r.+ CMC V.r.+ GNO Treatments N.r.+GLY N.r.+ GLYN.r.+ GLYN.r.+ GLYN.r.+ Milk N.r.+ Milk V.r.+ GNO N.r.+ GNO V.r.+ SBO V.r.+ SBO N.r.+ SFO V.r.+ SFO V.r.+BAV.r.+ GH N.r.+BAV.r.+ GH N.r.+ BAV.r.+HO N.r.+HO No. T  $\mathbf{T}_{\mathrm{II}}$  $T_{12}$  $T_{\rm B}$  $T_{14}$  $T_{\rm I5}$  $T_{16}$  ${}^{\rm L}_{\rm 2}$  $T_{\rm IS}$  $T_{\rm B}$  $T_{20}$  $T_{21}^{21}$ T.33  $T_{24}$ Tzs  $T_{26}$  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and 40, 50 minutes, respectively. Tween-80 was consistently harmful to the fungal growth on UVC ray exposure, when all the treatments showed cent per cent medium surface coverage.

#### UVC exposure -2, 3 and 5 hrs :

The trend of results was almost similar to that in previous observations. Glycerol 1 to 5 per cent and Tween-80 0.5 and 1 per cent resulted in 78.33 to 90.0, 78.33 to 86.67, 76.67 to 81.67 and 21.67 to 30, 18.33 to 26.67 and 16.67 to 25.0 per cent growth by 2, 3 and 5 hrs exposure, respectively, against complete surface coverage by rest of the treatments. The formulation of *N.r.*alone (without UVC rays exposure) recorded cent per cent surface coverage of medium.

#### Effect of UVC rays on biomass development :

The data on biomass produced by the mycoagent of *N.rileyi* with various adjuvants in culture medium after UVC rays for 10 to 50 minutes, 2, 3 and 5 hours are presented in Table 2. The differences of biomass production in different treatments were significant and trend of performance of adjuvants was more or less similar to that observed for surface coverage.

#### UVC exposure- 10 to 50 minutes :

The biomass produced by the fungus in treatments with *N.r.*+indigo 0.5 per cent was 5.50g. It emerged significantly superior to rest of the treatments, except at par treatments with *N.r.*+SFO 1.0 per cent (5.40g), *N.r.*+SFO 0.5% (5.37g) and *N.r.*+indigo 1.0 per cent (5.33g). The next promising treatments were *N.r.*+BA 2.0 per cent (5.27g), *N.r.*+GNO 0.5 per cent (5.20g), *N.r.*+GLY 3.0 per cent (5.13g), *N.r.*+BA 3.0 per cent (5.10g) and *N.r.*+GLY 2.0 per cent (5.10g). The lowest (3.40g) biomass was recorded in control (*N.r.* alone)

After 20 minutes UVC rays exposure the significantly maximum (5.50g) biomass developed in *N.r.*+SFO 1.0 per cent. The next promising and at par treatments were *N.r.*+GNO 1.0 per cent (5.30g), *N.r.*+SFO 0.50 per cent (5.27g), *N.r.*+GNO 2.0 per cent (5.27g), *N.r.*+indigo 0.5 and 1.0 per cent (5.20g). The treatments in decending order of superiority for the biomass were *N.r.*+GNO 0.5 per cent (5.13g), *N.r.*+BA 3.0 per cent (5.10g), *N.r.*+GLY 2.0 per cent (5.00g) and *N.r.*+BA 2.0 per cent (4.97g). The fungus culture with adjuvant *N.r.*+tween 80 and *N.r.*+molasses produced

least biomass (3.30 to 4.03g) when the fungus culture without adjuvants produced 3.20g biomass in culture medium.

After 30 minutes UVC rays exposure, the treatment with adjuvants *N.r.*+SFO 1.0 per cent produced 5.27g biomass. It was significantly highest than rest of treatments except at par treatments with *N.r.*+SFO 0.5 per cent (5.20g), *N.r.*+GNO 1.0 per cent and *N.r.*+GNO 0.5 per cent (5.17 and 4.97g) for production of biomass. The results of 40 min. UVC rays exposure were more or less similar to that of 30 minutes UVC rays exposure.

After 50 minutes of UVC exposure, SFO 1.0 per cent produced significantly maximum (5.27g) biomass over the rest of the treatments with and without adjuvants. It recorded more or less doubled biomass over control (2.90g). The next promising treatments for biomass development were N.r.+SFO 0.5 per cent (5.10g), N.r.+GNO 1.0 per cent (5.03g), N.r.+GNO 0.5 per cent (4.97g) and N.r.+BA 2.0 per cent (4.93g).

## UVC exposure 2 hours :

There were significant differences among the treatments for production of fungal biomass. The adjuvant N.r.+SFO 1.0 per cent (5.20g) showed its superiority for biomass production over the adjuvants (2.70 to 5.10g) and control (2.50g). However, it was at par with N.r.+SFO 0.5 per cent (5.10g). N.r.+Tween-80, N.r.+CMC, N.r.+turmeric and N.r.+molasses produced least biomass (2.70 to 3.97g) when control produced 2.50g biomass. Among the chemical adjuvants biomass production was 2.70 to 4.73g. The other edible substrate produced biomass in 3.73 to 4.63g. The results of 3 hours UVC rays exposure were more or less similar to that of 2 hours UVC rays exposure.

#### UVC exposure 5 hours :

There were significant differences among treatments for fungal biomass production. The treatment with adjuvant N.r.+SFO 1.0 per cent produced highest (4.97g) biomass when it was in rest of the treatments (2.40 to 4.87g) against 1.80g in control (N.r. alone). The next promising treatments were N.r.+SFO 0.5 per cent (4.87g), N.r.+GNO 1.0 per cent (4.87g) and N.r.+GNO 2.0 per cent (4.77g). The control N.r. alone without UVC exposure produced 7.30g of fungal biomass.

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

Tr.	Treatments	Conc. (%)		Biomass (g) produced after indicated exposure							
No.		of Adj.	10min	20min	30min	40min	50min	2 hrs	3hrs	5 hrs	
$T_1$	N.r.+GLY	1.0	4.83	4.77	4.63	4.53	4.50	4.07	4.00	3.80	
$T_2$	N.r.+GLY	2.0	5.10	5.00	4.80	4.67	4.63	4.20	4.17	3.90	
<b>T</b> <sub>3</sub>	N.r.+GLY	3.0	5.13	4.83	4.73	4.60	4.47	4.20	4.03	3.80	
$T_4$	N.r.+GLY	5.0	4.83	4.67	4.53	4.50	4.53	4.30	4.00	3.80	
$T_5$	N.r.+TW-80	0.5	3.57	3.50	3.43	3.27	3.20	2.97	2.80	2.60	
$T_6$	N.r.+TW-80	1.0	3.45	3.30	3.00	3.10	3.00	2.70	2.60	2.40	
$T_7$	N.r.+BA	1.0	5.07	5.03	4.87	4.93	4.87	4.67	4.53	4.30	
$T_8$	N.r.+BA	2.0	5.27	4.97	4.97	4.97	4.93	4.73	4.57	4.40	
T9	N.r.+BA	3.0	5.10	5.10	5.03	4.93	4.87	4.63	4.47	4.27	
T <sub>10</sub>	N.r.+CMC	0.5	4.27	4.27	4.07	4.10	4.10	3.97	3.87	3.70	
T11	N.r.+CMC	1.0	4.13	4.10	4.07	3.93	3.70	3.50	3.40	3.23	
T <sub>12</sub>	N.r.+Indigo	0.5	5.50	5.20	5.00	4.93	4.87	4.67	4.43	4.23	
T <sub>13</sub>	N.r.+Indigo	1.0	5.33	5.20	4.97	4.87	4.70	4.47	4.40	4.13	
$T_{14}$	N.r.+Turmeric	0.5	4.67	4.63	4.10	3.97	3.93	3.67	3.40	3.13	
T <sub>15</sub>	N.r.+Turmeric	1.0	4.47	4.20	3.93	3.83	3.77	3.57	3.30	2.93	
T <sub>16</sub>	N.r.+Molasses	1.0	4.20	4.00	3.97	3.90	3.77	3.47	3.37	3.17	
T <sub>17</sub>	N.r.+Molasses	2.0	4.37	4.03	3.87	3.73	3.73	3.37	3.13	3.03	
T <sub>18</sub>	N.r.+Honey	0.5	4.80	4.63	4.53	4.57	4.40	4.07	4.00	3.97	
T <sub>19</sub>	N.r.+Honey	1.0	4.90	4.70	4.60	4.60	4.50	4.17	4.10	4.07	
$T_{20}$	N.r.+Milk	1.0	4.70	4.57	4.63	4.57	4.50	4.33	4.20	4.07	
T <sub>21</sub>	N.r.+Milk	2.0	4.63	4.77	4.77	4.67	4.63	4.43	4.30	4.10	
T <sub>22</sub>	N.r.+SFO	0.5	5.37	5.27	5.20	5.20	5.10	5.10	4.97	4.87	
T <sub>23</sub>	N.r.+SFO	1.0	5.40	5.50	5.27	5.27	5.27	5.20	5.03	4.97	
T <sub>24</sub>	N.r.+ GNO	0.5	5.20	5.13	4.97	4.97	4.97	4.93	4.83	4.77	
T <sub>25</sub>	N.r.+ GNO	1.0	5.10	5.30	5.17	5.13	5.03	4.97	4.93	4.87	
T <sub>26</sub>	N.r.+GNO	2.0	5.10	5.27	5.13	5.10	5.10	4.97	4.90	4.77	
T <sub>27</sub>	N.r.+SBO	0.5	4.63	4.73	4.63	4.67	4.57	4.47	4.37	4.30	
T <sub>28</sub>	N.r.+SBO	1.0	4.50	4.63	4.53	4.47	4.40	4.30	4.23	4.10	
T <sub>29</sub>	N.r.+MUO	0.5	4.70	4.63	4.53	4.27	4.37	4.07	3.93	3.73	
T <sub>30</sub>	N.r.+MUO	1.0	4.67	4.43	4.37	4.30	4.27	4.13	4.00	3.77	
T <sub>31</sub>	N.r.+GH	0.5	4.80	4.63	4.53	4.47	4.37	4.23	4.07	3.87	
T <sub>32</sub>	N.r.+GH	1.0	4.83	4.77	4.73	4.67	4.37	4.17	3.97	3.93	
T <sub>33</sub>	Control (N.r. alone)	-	3.40	3.20	3.10	3.00	2.90	2.50	2.40	1.80	
T <sub>34</sub>	Control (N.r. alone)	-	6.90	6.90	7.20	7.20	7.30	7.30	7.00	7.30	
	(W.UVC)										
	S.E <u>+</u>		0.06	0.05	0.05	0.05	0.06	0.07	0.06	0.13	
	C.D (P=0.0	5)	0.18	0.15	0.15	0.14	0.18	0.19	0.18	0.36	

\*Figures in parentheses indicate arcsin values. N.r. = Nomuraea rileyi

SFO = Sunflower oil

GNO = Groundnut oil

DAI = Days after inoculation TW= Tween-80

TX = Triton-X-100

CMC = Carboxymethyl Cellulose

W.UVC = without UVC

GLY= Glycerol CNO = Coconut oil SBO = Soybean oil

BA = Boric acidMUO = Mustered oil GH = Ghee

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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 5h 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. Similarly Sharmila et al. (2015); Kachhadiya et al. (2014); Barad et al. (2014 a&b) and Sharmila and Manjula (2015) also worked on the related topic.

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