Evaluation of some adjuvants for UVC rays protectability for *Beauveria bassiana* (Balsamo) Vuillemin

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ABSTRACT

The effect of UVC rays on biomass development of *Beauveria bassiana* (Balsamo) Vuillemin combination with chemicals, vegetable oils and other substrates as adjuvants were evaluated by exposing the combination for 10 to 50 minutes and 2, 3 and 5 hours under laboratory conditions. It was established that the biomass produced by *B. bassiana* with or without adjuvants in culture medium after exposure to UVC rays decreased with increase in the exposure periods. After 5 hrs UVC rays exposure, the treatment with adjuvant sunflower oil 1.0 per cent maintained its superiority over rest of the treatments and developing highest (6.13 g) biomass/40 ml medium of *B. bassiana* fungal mat. It was at par with sunflower oil 0.5 % (6.07 g). The next effective treatments for the UVC protectability reflecting in fungal biomass production were groundnut oil 1.0 % (5.70 g) and 0.5 % (5.63 g). Treatment with Tween 80 0.5 % (2.63 g), molasses 2.0 % (3.13 g) and Tween 80 1.0 % (zero g) consistently proved to be inefficient for UVC rays protectability for *B. bassiana*. As adjuvants vegatableoils, ghee and indigo 0.5 % and 1% gave considerable protection to *B. bassiana* from the UVC rays producing the biomass 4.47 to 6.13 g against 6.20 g and 2.92 g biomass development in the unexposed and exposed control, respectively.

Key words : B. bassiana, biomass, adjuvants, UVC rays treatment

Introduction

Beauveria bassiana (Balsamo) Vuillemin is one of the main fungal Candidate for the use in the microbiar control of paste. Entomopathogenic fungi have played an important role in the history of insect pathology and microbial control of insects (Sundarababu, 1992). Agastino Bassi, 1835 was the first to demonstrate that entomopathogenic fungus, *B. bassiana* could cause an infectious disease in silkworm and suggested the concept that, an infectious micro-organism might be used to control insect pests. Steinhaus, 1965 reported that *B. bassiana* causes mycosis in 175 host insects from order

Lepidoptera, Coleoptera and Hemiptera. *B. bassiana* is cosmopolitan fungus useful for the control of various insect pests of different crops. The efficacy of pathogen in field depends on environmental conditions. The extreme temprature and light including ultraviolet may influence the distribution of microorganisms and their persistance in nature (Zimmermann and Butin, 1973). Rapid decrease of viable spores exposed to direct sunlight was reported by Roberts and Campbell, 1977. Efficiency of entomopathogens in the field depends upon virulence towards target pest, coverage and persistence on target site. However, one of the major constraints for successful use of insect pathogens is

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their loosing virulence by ultra violet (UV) rays. Ramle et al. (2004) reported that the short (254 nm) ultraviolet radiation was more detrimental to the conidia compared to long (365nm) ultraviolet radiation. Cagan and Svercel (2001) reported that radial growth of the UV variants were slower with increasing time of exposure. Chavan and Kadam (2010 b) reported that detrimental effect of UV rays increased with increase in exposure period. They also reported that glycerol, boric acid and Tween 80 as chemical adjuvants gave good UV protection to V. lecanii. Vegetables oils and some other edible substrates are emerging as promising adjuvants for fungal biopesticides. Hence, in the present investigation the attempt is made to find out the UV protecting ability of such substrates for B. bassiana.

Material and Methods

The study was carried out at Biocontrol Research Laboratory, Department of Agricultural Entomology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra State, India during year 2009-2011. The potato dextrose broth was used for growth and multiplication of the fungus. 31 combinations as test formulations of *B. bassiana* and adjuvants were evaluated with exposed and unexposed *B. bassiana* alone (Table 1) in CRD with 3 replications to study their UV rays protecting ability. The respective quantities as per concentrations of adjuvants were

added to optimum concentration of *B. bassiana* aqua suspension culture to prepare different formulations. Each formulation was kept in 50 ml beaker and was exposed to UV rays for 10, 20, 30, 40, 50 min and also 2, 3 and 5 hrs. The distance between exposed suspensions and UV light source was 0.3 m. One ml of such exposed formulation was added to duly autoclaved 40 ml PDB medium and observed for the medium surface coverage (%) and biomass development up to 10 days. The data was subjected to statistical analysis.

Results and Discussion

The data on biomass development by the mycoagent with various adjuvants in culture medium after UVC irradiation for 10 to 50 minutes, 2 hrs, 3 hrs and 5 hrs are presented in Table 1. The differences in biomass production in the treatments were significant and trend of performance of

adjuvants for UVC rays protectability was more or less similar to that was observed for surface area covered at 3, 7 and 10 DAI on exposure of the 5 hrs UVC rays (Table 1).

After 10 minutes of UVC exposure, the highest (6.80 g) biomass was produced in sunflower oil 1.0 per cent which was found at par with sunflower oil 0.5 per cent producing 6.63 g fungal biomass. The next highly promising and at par treatments showing high UVC protectability, reflecting in biomass production were groundnut oil 1.0 per cent (6.43 g) and groundnut oil 0.5 per cent (6.33 g). The rest of treatments produced significantly higher biomass than control (4.10 to 6.00 g) except Tween 80 0.5 per cent, molasses 2.0 per cent and control (3.97 g). The lowest biomass (1.87 g) was produced in Tween 80 1.0 per cent.

After 20 minutes UVC rays exposure significantly maximum biomass (6.70 g) was recorded in sunflower oil 1.0 per cent which was at par with that of 0.5 per cent (6.57 g). The next highly promising and at par treatments were groundnut oil 1.0 per cent (6.33 g) and 0.5 per cent (6.27 g). The biomass was in rest of treatments was 4.03 to 5.87 g, when it was 3.93 g in control (*B. b.* alone). Molasses 2.0 per cent (3.93 g) and Tween 80 0.5 per cent (3.60 g) were at par to control. The lowest (1.63 g) biomass was produced in Tween 80 1.0 per cent.

After 30 minutes UVC rays exposure, the best and at par adjuvants for UVC rays protectability were sunflower oil 1.0 (6.60 g) and 0.5 per cent (6.53 g). The next highly promising at par treatments were groundnut oil 1.0 per cent (6.23 g) and 0.5 per cent (6.17 g). The biomass was adversely affected in molasses 2.0 per cent (3.80 g), Tween 80 0.5 (3.40 g) and 1.0 per cent (1.53 g).

The results of 40 and 50 minutes UVC rays exposure were more or less similar to those of 30 minutes UVC rays exposure. The fungus culture with sunflower oil 1.0 per cent produced significantly highest fungal biomass of 6.43 and 6.27 g at 40 and 50 minutes UVC rays exposure, respectively.

After 2 hrs UVC rays exposure, the significant differences among treatments for production of fungal biomass were noticed. The treatment with adjuvant sunflower oil 1.0 per cent recorded highest (6.23 g) biomass. It was at par with sunflower oil 0.5 per cent recording 6.17 g biomass. It was followed by groundnut oil 1.0 and 0.5 per cent recording 5.90 and 5.87 g biomass, respectively. All the rest of the

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| Tr. No. | Treatments* | Conc. (%) of adjuvants | Biomass (g) produced after UVC exposure period | | | | | | | |
|------------|---------------------|------------------------------|--|-------|-------|-------|-------|------|------|------|
| | | | 10 min | 20min | 30min | 40min | 50min | 2 hr | 3 hr | 5 hr |
| T1 | B.b. + GLY | 1.0 | 4.57 | 4.47 | 4.37 | 4.30 | 4.20 | 3.87 | 3.73 | 3.67 |
| T2 | B.b. + GLY | 2.0 | 4.73 | 4.53 | 4.40 | 4.33 | 4.27 | 3.93 | 3.77 | 3.70 |
| T3 | B.b. + GLY | 3.0 | 4.57 | 4.50 | 4.20 | 4.13 | 4.13 | 3.83 | 3.73 | 3.67 |
| T4 | B.b. + GLY | 5.0 | 4.77 | 4.60 | 4.47 | 4.30 | 4.17 | 4.00 | 3.97 | 4.13 |
| T5 | B.b. + TW | 0.5 | 3.97 | 3.60 | 3.40 | 3.20 | 3.10 | 2.80 | 2.73 | 2.63 |
| T6 | B.b. + TW | 1.0 | 1.87 | 1.63 | 1.53 | 1.23 | 1.10 | 0.80 | 0.00 | 0.00 |
| T7 | B.b. + BA | 1.0 | 5.33 | 5.17 | 5.10 | 5.07 | 5.00 | 4.73 | 4.30 | 4.17 |
| T8 | B.b. + BA | 2.0 | 5.67 | 5.50 | 5.40 | 5.27 | 5.17 | 4.97 | 4.67 | 4.50 |
| T9 | B.b. + BA | 3.0 | 5.47 | 5.40 | 5.37 | 5.23 | 5.13 | 4.90 | 4.60 | 4.47 |
| T10 | B.b. + Indigo | 0.5 | 5.57 | 5.47 | 5.33 | 5.23 | 5.13 | 4.97 | 4.77 | 4.57 |
| T11 | B.b. + Indigo | 1.0 | 5.53 | 5.37 | 5.30 | 5.20 | 5.13 | 4.80 | 4.73 | 4.63 |
| T12 | B.b. + Molasses | 1.0 | 4.10 | 4.03 | 3.97 | 3.93 | 3.90 | 3.70 | 3.60 | 3.50 |
| T13 | B.b. + Molasses | 2.0 | 3.97 | 3.93 | 3.80 | 3.73 | 3.63 | 3.47 | 3.37 | 3.13 |
| T14 | B.b. + Turmeric | 0.5 | 5.57 | 5.43 | 5.33 | 5.23 | 5.13 | 4.87 | 4.73 | 4.53 |
| T15 | B.b. + Turmeric | 1.0 | 5.43 | 5.33 | 5.33 | 5.17 | 5.07 | 4.87 | 4.67 | 4.37 |
| T16 | B.b. + Honey | 0.5 | 5.93 | 5.73 | 5.67 | 5.53 | 5.13 | 4.90 | 4.90 | 4.73 |
| T17 | B.b. + Honey | 1.0 | 6.00 | 5.87 | 5.77 | 5.43 | 5.37 | 5.10 | 5.00 | 4.80 |
| T18 | B.b. + CMC | 0.5 | 5.70 | 5.57 | 5.47 | 5.17 | 5.10 | 4.87 | 4.73 | 4.53 |
| T19 | B.b. + CMC | 1.0 | 5.33 | 5.27 | 5.20 | 5.13 | 5.03 | 4.83 | 4.67 | 4.50 |
| T20 | B.b. + Milk | 1.0 | 5.53 | 5.43 | 5.37 | 5.13 | 5.03 | 4.80 | 4.73 | 4.53 |
| T21 | B.b. + Milk | 2.0 | 5.37 | 5.30 | 5.23 | 5.13 | 5.07 | 4.90 | 4.80 | 4.53 |
| T22 | B.b. + SFO | 0.5 | 6.63 | 6.57 | 6.53 | 6.37 | 6.23 | 6.17 | 6.13 | 6.07 |
| T23 | B.b. + SFO | 1.0 | 6.80 | 6.70 | 6.60 | 6.43 | 6.27 | 6.23 | 6.17 | 6.13 |
| T24 | B.b. + GNO | 0.5 | 6.33 | 6.27 | 6.17 | 6.10 | 6.03 | 5.87 | 5.77 | 5.63 |
| T25 | B.b. + GNO | 1.0 | 6.43 | 6.33 | 6.23 | 6.20 | 6.10 | 5.90 | 5.83 | 5.70 |
| T26 | B.b. + SBO | 0.5 | 5.53 | 5.43 | 5.27 | 5.23 | 5.17 | 4.90 | 4.83 | 4.60 |
| T27 | B.b. + SBO | 1.0 | 5.53 | 5.50 | 5.33 | 5.27 | 5.17 | 5.03 | 4.93 | 4.67 |
| T28 | B.b. + MUO | 0.5 | 5.40 | 5.43 | 5.17 | 5.17 | 5.07 | 4.80 | 4.70 | 4.63 |
| T29 | B.b. + MUO | 1.0 | 5.53 | 5.47 | 5.30 | 5.20 | 5.13 | 4.87 | 4.73 | 4.73 |
| T30 | B.b. + GH | 0.5 | 5.33 | 5.27 | 5.13 | 5.17 | 5.13 | 4.97 | 4.83 | 4.73 |
| T31 | B.b. + GH | 1.0 | 5.43 | 5.37 | 5.30 | 5.30 | 5.20 | 5.07 | 4.97 | 4.90 |
| T32 | Control (B.b. alone | | 3.97 | 3.93 | 3.87 | 3.76 | 3.50 | 3.24 | 3.10 | 2.92 |
| T33 | Control (WUV) | - | 6.23 | 6.20 | 6.27 | 6.17 | 6.18 | 6.23 | 6.21 | 6.20 |
| S.E. ± | | | 0.07 | 0.05 | 0.06 | 0.06 | 0.04 | 0.06 | 0.05 | 0.08 |
| C.D. a | | | 0.22 | 0.15 | 0.17 | 0.17 | 0.13 | 0.17 | 0.15 | 0.23 |

Table 1. Effect of UVC treatment on biomass production by *B. bassiana* in the presence of some adjuvants

**B.b.= Beauveria bassiana*, GLY = Glycerol, TW = Tween 80, BA = Boric acid, SFO = Sunflower oil, CMC = Carboxymethyl cellulose, GNO = Groundnut oil, SBO = Soybean oil, MUO = Mustard oil, GH = Ghee, WUV = treatment without UV exposure

adjuvants recorded significantly higher biomass (3.47 to 5.10 g) than control (3.24 g) except treatment with Tween 80 1.0 and 0.5 per cent (2.80 and 0.80 g). The trend of 3 hrs UVC rays exposure was more or less similar to that of the 2 hrs. The treatment with Tween 80 1.0 per cent recorded zero per cent growth of the mycoagent.

After 5 hrs UVC rays exposure (Table 1 and Fig), the treatment with sunflower oil 1.0 per cent maintained its superiority over rest of the treatments and recording highest (6.13 g) biomass. It was at par that with sunflower oil 0.5 per cent (6.07 g). The next effective treatments for the UVC protectability reflecting in fungal biomass production were groundnut oil 1.0 per cent (5.70 g) and 0.5 per cent (5.63 g). All the rest of the treatments recorded significantly higher biomass (3.67 to 4.90 g) than control (2.92 g). Treatment with Tween 80 0.5 per cent (2.63 g), molasses 2.0 per cent (3.13 g) and Tween 80 1.0 per cent (zero g) consistently proved to

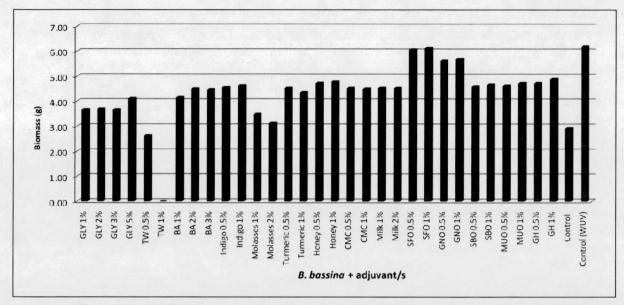


Fig. 1. Effect of 5 hrs UVC treatment on biomass production by B. bassiana in presence of some adjuvants

be inefficient for UVC rays protectability for *B. bassiana*.

It was established that biomass produced by *B*. bassiana with or without adjuvants in culture medium after exposure to UVC rays for 10 to 50 minutes, 2 hrs, 3 hrs and 5 hrs decreased with increase in exposure period. The adjuvants responded variably for their UVC rays protectability for B. bassiana. However, higher concentrations of the adjuvants were better than lower ones except indigo, turmeric and CMC. Thus among the oils sunflower oil and groundnut oil were proved to be best UVC rays protectant. Among chemical adjuvants glycerol, boric acid, indigo, CMC; among oils soybean oil, mustard oil and ghee; and among nutrient substrates turmeric, honey and milk were proved to have appreciable UVC protectability. Glycerol, molasses and Tween 80 favoured UVC rays harms and affected growth and development of B. bassiana as compared to unexposed and exposed control.

The UV protecting ability was studied by irradiation of UVC rays having shorter wavelength of 200 to 290 nm than that of 290 to 320 and 320 to 400 nm wavelengths in respect of UVB and UVA, respectively. Ramle *et al.* (2004) reported that the short (254 nm) ultraviolet radiation was more detrimental to the conidia compared to long (365nm) ultraviolet radiation. Therefore the results of UV protectability by using UVC rays in the present study are more precise. Morley *et al.* (1996) explored the conidia of *B. bassiana* to UV light for 4,8,16 and 24 hrs and found that conidial viability decreased with increase in UV exposure. Tobar *et al.* (1998) reported that isolate Bb9218 was resistant to 10, 30 and 60 minutes exposure to UV light. Cagan and Svercel (2001) reported that radial growth of the UV variants were slower with increasing time of exposure. Chavan and Kadam (2010 b) reported that detrimental effect of UV rays increased with increase in exposure period. They also reported that glycerol, boric acid and Tween 80 give good UV protection to *V. lecanii*, which is contradictory for *B. bassiana* in present study due to variation in *spp.* of EPF.

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