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Research Article

# Role of vegetable oil as UV light protector for *Beauveria bassiana* and *Metarhizium anisopliae* in management of *Bactrocera zonata*

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## ABSTRACT

Entomopathogenic fungi (EPF), *Metarhizium anisopliae* and *Beauveria bassiana,* are used in the field against different insect pests because they are best candidates to include in integrated pest management. But the efficacy of these EPF is limited in the field conditions because different environmental conditions like; sunlight, UV light, visible light, temperature and humidity affects the conidial viability, shelf life and pathogenicity of EPF. The solution to this problem is the use of vegetable oils as liquid carriers to coat and shield the conidia. In the present study three different oils were used like, Sesame oil, sunflower oil and corn oil. The conidia of *M. anisopliae* and *B. bassiana* were exposed to three different intervals of UV light 15, 30 and 45 minutes. Conidia exposed to UV light after the addition of oil and making oil water emulsion with fungus. The germination percentage and pathogenicity of *M. anisopliae* and *B. bassiana* against adult and pupae (through immersion method) of *Bactrocera zonata* were recorded after exposure to UV light. Sesame oil showed highest conidia germination, adult mortality for *M. anisopliae* and *B. bassiana* after UV light exposure. In case of *M. anisopliae* germination rate reached up to 79.5% (48–51 h) and mortality 84.4% (7th day), lower rates in aqueous suspensions (germination: 43.3%; mortality: 35.6%). In case of *B. bassiana* germination rate reached up to 69.17% (48–51 h) and mortality 64.4% (7th day), lower rates in aqueous suspensions (germination: 33.3%; mortality: 35.6%). Adult emergence was highest in the aqueous suspensions and lowest in the sesame oil based formulation. Overall, *M. anisopliae* performed better than *B. bassiana* with sesame oil recommended to protect conidia from UV radiation.

**Keywords:** Oil water emulsion, EPF, Sesame oil, Sunflower oil, Corn oil, UV light, Conidia coat, Conidia shield, Liquid carriers.

## INTRODUCTION

The fruit fly, *Bactrocera zonata* is one of the most destructive pests of the mango, guava, citrus and peach. *B. zonata* causes major economic losses in the fruit production (Bilal *et al.,* 2021). The female of the fruit fly lays its eggs inside the fruit, after hatching larvae start feeding causes direct damage resulting in rotting of the fruit (Li *et al.,* 2024). Traditionally different synthetic insecticides are used to manage *B. zonata*. Extensive use of synthetic insecticides possesses a series of challenges including resistance in insect, risk of secondary pest outbreak, effects on non-target organisms, residues in the fruit, environmental pollution and serious human health concerns (Sarfaraz *et al.,* 2023).

Need for environmental safe alternatives to synthetic insecticides have shifted the attention of the researcher to explore biological control method including predator,

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parasitoid, and biopesticides (Zhou *et al.,* 2024). Among different available biological alternative entomopathogenic fungi (EPF) *Metarhizium anisopliae* and *Beauveria bassiana* have emerged as most effective's solution for management of *B. zonata* (Qayyum *et al.,* 2024). These fungi infect the insect when it comes in contact with it, penetrating into the cuticle causing nutrients depletion and death of insect due to production of certain toxin (Hong *et al.,* 2024).

Field application of entomopathogenic fungi possess certain limitation particularly their susceptibility to the environmental factors such as Ultraviolet radiation (UV) Exposure of the UV light can reduce the persistence of the fungal conidia in the field conditions (Ghorui *et al.,* 2024). Sun light contain radiation of a wide range of wavelength especially UV-A, UV-B. Due to ozone layer depletion the portion of UV rays in the sun light is increasing day by day (Liaqat *et al.,* 2024).

Therefore it is crucial to develop a strategy to protect conidia of fungi from UV degradation. Biopesticide when properly formulated can perform better in field conditions and even in unfavorable environmental conditions (Chaudhuri *et al.,* 2024). To overcome adverse effects of UV radiations on conidia of EPF, different strategies have been explored such as UV protectants. Vegetable oils have drawn much interest as potential UV protectants in the formulations of Entomopathogenic fungi, which has thus enhanced the performance of microbial biopesticides against *B. zonata* in realistic scenarios (Thirumeni et al., 2024). Not only do vegetable oils help in UV protection, but they also increase the sticking ability of conidia towards the insect cuticle; hence, their performance in those formulations is enhanced (Mascarin *et al.,* 2024).

This study tested the protective effect of various vegetable oils on the *B. bassiana* and *M. anisopliae* conidia subjected to UV light. Determine if the use of different vegetable oils as protection against UV degradation of fungal can influence its pathogenicity against the EPF of *B. zonata*. The study assessed the efficacy of the formulation in germination rates of conidia and bioassay of the formulations regarding their biocontrol activity towards the pupae and adult stages of *B. zonata*.

## MATERIALS AND METHODS

### Preparation of Fungal Culture

*B. bassiana* and *M. anisopliae* pre-maintained cultures were obtained from the Insect Pathology Lab of the Institute of Plant Protection at the MNS-University of Agriculture Multan. Commercially available potato dextrose agar (PDA @39 g/litre) (Merck KGaA, Darmstadt, Germany) was used to maintain the culture. Colonies on freshly prepared media plates were purified using a single spore purification procedure described by Zhang *et al*. (2013) and Noman *et al*. (2018).

The prepared media was autoclaved for 15 minutes at 121°C and 15 psi pressure to prevent contamination. After autoclaved the media was left for some time to cooldown. To prevent contamination, pouring of media was performed in the laminar air flow chamber, which was first UV light treated for 15 minutes and sterilized with methylated spirit. *B. bassiana* and *M. anisopliae* conidia were inoculated onto solidified PDA plates using a sterile, autoclaved inoculation needle. To prevent contamination, the inoculated media plates were sealed with parafilm tape strips. To obtained fungal growth, Petri Plates were cultured for 14 days in an incubator at conditions like, 25±1ºC temperature and Relative Humidity 60±10%.

### Preparation of oil-in-water emulsions

Selected vegetable oils (Sesame oil, sunflower oil and corn oil) and Tween 80 (non-ionic surfactant) were disinfected at 60°C. Each vegetable oil was mixed separately with 0.05% Tween 80 to prepare oil phase. *B. bassiana* and *M. anisopliae* conidia were harvested by scraping the culture surface with a sterile loop. Add fungal conidia to oil phase (1 g of conidia per 10 ml of oil) and mix it thoroughly. Prepare aqueous phase by adding 0.05% Tween 80 in distilled water. To prepare oil in water emulsion, 10 ml of oil phase containing conidia of EPF were pipetted into 90 ml aqueous phase, which was stirring vigorously at 200 rpm by homogenizer. All formulations were kept overnight to allow conidia to become sensitized to the effects of vegetable oils (Lei *et al.,* 2022).

### UV light exposure chamber

The specially designed chamber was made entirely of wood. The UV light was positioned around 25 cm above the samples. There was an on/off switch for UV lamp. There was a space in the chamber on the lower portion where the samples were kept. The inner temperature was tracked via a temperature sensor. A flux meter was placed in the chamber to observe the flux of the UV light. The thermometer, a door for transferring the samples, and buttons to turn on and off the lighting was all in the front section.

**UV light exposure to formulation of EPF**

The effect of UV light exposure on formulation (oil in water emulsion) of EPF was assessed by pipetting them in 9cm petri plates. These Petri plates were subjected to a UV lamp radiation of around 250±5 nm range that was achieved through a 6 watt UV lamp. The plates were kept away from the radiation source at 25 cm. The duration of exposure to UV was 15, 30 and 45 minutes. Every treatment was replicated three times. After exposure to UV radiations these the petri plates were placed in incubator at temperature of about 25ºC.

**Conidial germination test of EPF formulation after exposure of UV light:**

Aliquots of 0.1 ml from each formulation were pipetted over a PDA surface that was thinly distributed in a Petri dish with a 9cm diameter. All the plates were incubated at 25°C in incubator. After 24 and 48 hours, conidial germination was recorded. Conidia (minimum 200 per Petri dish) were examined at a 400x magnification to observe germination through visibility of germ tube (Bernardo *et al.,* 2018).

**Bioassay on *B. zonata* pupae to observe the Pathogenicity of formulations after UV exposure**

After the evaluation of the viability, bioassay on pupa of *B. zonata* was carried out to assess the pathogenicity of formulation of EPF. Pupae are immobile making it easier for fungal conidia to penetrate the cuticle. In field condition pupae is the stage of fruit fly that mostly come in contact with EPF in soil, where EPF naturally proliferate (Cruz-Miralles *et al.,* 2024). Selection of pupae in bioassay mimics the ecological interaction more closely. For the bioassay 3-day old pupae were used. Using the immersion method, virulence of conidial formulation was assessed against a batch of 15 *B. zonata* pupae. Pupae were dipped for 15 sec in Petri dish with a concentration of $1\times10^{8}$ conidia $mL^{-1}$. The treatments used against pupae were; SFO-UV15, SFO-UV30, SFO-UV45, SSO-UV15, SSO-UV30, SSO-UV45, CO-UV15, CO-UV30, CO-UV45, AQ-UV15, AQ-UV30, AQ-UV45, Formulated product (Control as no exposure of UV Light). So, the total treatments were 13 with 3 replications of each treatment by using CRD. The adult emergence was evaluated at 3, 5, and 7 days after treatment. Experiment was terminated after 8 days from the start of experiment. For treatments light and dark period was maintained at 16:8, relative humidity was between 60±5%, and temperature was 27±2 °C.

Table 1. List of Abbreviation for oil in water emulsion of *M. anisopliae*

| Sr. No | Abbreviation | Full form |
|---|---|---|
| 1 | SFO-Ma-UV15 | Prepare Oil in Water Emulsion Using Sunflower Oil, Conidia of *M. anisopliae* after 15 Minutes of UV Light Exposure |
| 2 | SFO-Ma-UV30 | Prepare Oil in Water Emulsion Using Sunflower Oil, Conidia of *M. anisopliae* after 30 Minutes of UV Light Exposure |
| 3 | SFO-Ma-UV45 | Prepare Oil in Water Emulsion Using Sunflower Oil, Conidia of *M. anisopliae* after 45 Minutes of UV Light Exposure |
| 4 | SSO-Ma-UV15 | Prepare Oil in Water Emulsion Using Sesame Oil, Conidia of *M. anisopliae* after 15 Minutes of UV Light Exposure |
| 5 | SSO-Ma-UV30 | Prepare Oil in Water Emulsion Using Sesame Oil, Conidia of *M. anisopliae* after 30 Minutes of UV Light Exposure |
| 6 | SSO-Ma-UV45 | Prepare Oil in Water Emulsion Using Sesame Oil, Conidia of *M. anisopliae* after 45 Minutes of UV Light Exposure |
| 7 | CO-Ma-UV15 | Prepare Oil in Water Emulsion Using Corn Oil, Conidia of *M. anisopliae* after 15 Minutes of UV Light Exposure |
| 8 | CO-Ma-UV30 | Prepare Oil in Water Emulsion Using Corn Oil, Conidia of *M. anisopliae* after 30 Minutes of UV Light Exposure |
| 9 | CO-Ma-UV45 | Prepare Oil in Water Emulsion Using Corn Oil, Conidia of *M. anisopliae* after 45 Minutes of UV Light Exposure |
| 10 | AQ-Ma-UV15 | Prepare Aqueous suspension Using distilled water, Conidia of *M. anisopliae* after 15 Minutes of UV Light Exposure |
| 11 | AQ-Ma-UV30 | Prepare Aqueous suspension Using distilled water, Conidia of *M. anisopliae* after 30 Minutes of UV Light Exposure |
| 12 | AQ-Ma-UV45 | Prepare Aqueous suspension Using distilled water, Conidia of *M. anisopliae* after 45 Minutes of UV Light Exposure |

Table 2. List of Abbreviation for the oil in water emulsion of *B. bassiana*

| Sr. No | Abbreviation | Full form |
|---|---|---|
| 1 | SFO-Bb-UV15 | Prepare Oil in Water Emulsion Using Sunflower Oil, Conidia of *B. bassiana* after 15 Minutes of UV Light Exposure |
| 2 | SFO-Bb-UV30 | Prepare Oil in Water Emulsion Using Sunflower Oil, Conidia of *B. bassiana* after 30 Minutes of UV Light Exposure |
| 3 | SFO-Bb-UV45 | Prepare Oil in Water Emulsion Using Sunflower Oil, Conidia of *B. bassiana* after 45 Minutes of UV Light Exposure |
| 4 | SSO-Bb-UV15 | Prepare Oil in Water Emulsion Using Sesame Oil, Conidia of *B. bassiana* after 15 Minutes of UV Light Exposure |
| 5 | SSO-Bb-UV30 | Prepare Oil in Water Emulsion Using Sesame Oil, Conidia of *B. bassiana* after 30 Minutes of UV Light Exposure |
| 6 | SSO-Bb-UV45 | Prepare Oil in Water Emulsion Using Sesame Oil, Conidia of *B. bassiana* after 45 Minutes of UV Light Exposure |
| 7 | CO-Bb-UV15 | Prepare Oil in Water Emulsion Using Corn Oil, Conidia of *B. bassiana* after 15 Minutes of UV Light Exposure |
| 8 | CO-Bb-UV30 | Prepare Oil in Water Emulsion Using Corn Oil, Conidia of *B. bassiana* after 30 Minutes of UV Light Exposure |
| 9 | CO-Bb-UV45 | Prepare Oil in Water Emulsion Using Corn Oil, Conidia of *B. bassiana* after 45 Minutes of UV Light Exposure |
| 10 | AQ-Bb-UV15 | Prepare Aqueous suspension Using distilled water, Conidia of *B. bassiana* after 15 Minutes of UV Light Exposure |
| 11 | AQ-Bb-UV30 | Prepare Aqueous suspension Using distilled water, Conidia of *B. bassiana* after 30 Minutes of UV Light Exposure |
| 12 | AQ-Bb-UV45 | Prepare Aqueous suspension Using distilled water, Conidia of *B. bassiana* after 45 Minutes of UV Light Exposure |

**Bioassay on *B. zonata* adult to observe the Pathogenicity of formulation of EPF after UV exposure**

After the evaluation of the viability, bioassay on adult of *B. zonata* was carried out to assess the pathogenicity of formulation of EPF. For bioassay of adult small, sterilized boxes of 23 cm height and 9 cm diameter were used. Virulence of conidial formulation was assessed against a batch of 15 *B. zonata* (1-day old) adults. For adult a concentration of $1\times10^8$ conidia $mL^{-1}$ was used, 1ml of the same concentration was sprayed on to the adult colony using hand atomizer. The treatments used against adult were; SFO-UV15, SFO-UV30, SFO-UV45, SSO-UV15, SSO-UV30, SSO-UV45, CO-UV15, CO-UV30, CO-UV45, AQ-UV15, AQ-UV30, AQ-UV45, Formulated product (Control as no exposure of UV Light). So, the total treatments were 13 with 3 replications of each treatment by using CRD. The adults were fed on artificial diets when in bioassay boxes. The diet used was sugar solution and water solution in small diet cups by soaking absorbent cotton. The mortality of the adult was evaluated at 3, 5, and 7 days after treatment. Experiment was terminated at 8th days from the start of bioassay. Light and dark period was maintained at 16:8, relative humidity was between 60±5%, and temperature was 27±2 °C.

**Statistical Analysis**

Abbott formula was used to calculate the corrected mortality (Abbott, 1925). The collected data was analyzed using STATIX 8.1 to determine significance of all treatments means. The analysis was carried out using Analysis of Variance under completely randomized design to compare the means at the 5% probability level. Tukey's HSD was used in multiple comparison tests to differentiate in means in different treatments.

## RESULTS

**Percentage germination of conidia**

The germination percentage of two EPF *M. anisopliae* and *B. bassiana* was evaluated after the addition of three vegetable oils like; Sunflower, Sesame and Corn oil then treated the conidial formulations with UV light for three time durations 15, 30 and 45 minutes (SFO-UV15, SFO-UV30, SFO-UV45, SSO-UV15, SSO-UV30, SSO-UV45, CO-UV15, CO-UV30, CO-UV45, AQ-UV15, AQ-UV30, AQ-UV45, Formulated product (Control as no exposure of UV Light), there are total 13 treatments including a formulated product as a control, and counting 200 conidia under the

microscope for conidial growth, to check the viability of the fungal conidia after coating with oil to confirm whether the oil protect the conidia against the negative effects of UV lights or not. Highest germination means highest survival and lowest means lowest survival of conidia.

There were significant differences for conidial germination of *M. anisopliae* at all intervals for 24-27 hour ($F_{12, 26}$ = 72.30, α=0.05, P=0.000) and for 48-51 hour ($F_{12, 26}$ = 87.40, α=0.05, P=0.000). Germination percentage is in the sequence of: Formulated Ma > SSO-Ma-UV15 > SSO-Ma-UV30 > SSO-Ma-UV45 > SFO-Ma-UV15 > SFO-Ma-UV30 > SFO-Ma-UV45 > CO-Ma-UV15 > CO-Ma-UV30 > CO-Ma-UV45 > AQ-Ma-UV15 > AQ-Ma-UV30 > AQ-Ma-UV45. In case of *M. anisopliae* the highest percentage of germination of conidia after treatments was 60.50% after 24-27 hours and 91.17% after 48-51 hour for formulated product (Control as no exposure of UV Light)) of *M. anisopliae*. In case of oil protection used on conidia the highest protection was given by sesame oil, when exposed with UV light for 45 minutes, the germination rates were 50.17% after 24-27 hours and 79.50% after 48-51 hour. And lowest was in case of AQ-Ma-UV45 treatment after 45 minutes which has only water and fungus and not any oil, 16.33% after 24-27 hour and 43.33% after 48-51 hour (Fig.1).

There were significant differences for conidial germination of *B. bassiana* at all intervals for 24-27 hour ($F_{12, 26}$ = 67.06, α=0.05, P=0.000) and for 48-51 hour ($F_{12, 26}$ = 92.19, α=0.05, P=0.000). Same trend was seen for percentage germination of *B. bassiana*. Germination percentage is in the sequence of: Formulated Bb > SSO-Bb-UV15 > SSO-Bb-UV30 > SSO-Bb-UV45 > SFO-Bb-UV15 > SFO-Bb-UV30 > SFO-Bb-UV45 > CO-Bb-UV15 > CO-Bb-UV30 > CO-Bb-UV45 > AQ-Bb-UV15 > AQ-Bb-UV30 > AQ-Bb-UV45. In case of *B. bassiana* the highest percentage of germination of conidia after treatments was 48.17% after 24-27 hours and 73.50% after 48-51 hour for formulated product (Control as no exposure of UV Light) of *B. bassiana*. In case of oil protection used on conidia the highest protection was given by sesame oil, when exposed with UV light for 45 minutes, the germination rates were 37.50% after 24-27 hours and 69.17% after 48-51 hour. And lowest was in case of AQ-Bb-UV45 treatment after 45 minutes which has only water and fungus and not any oil, 8.33% after 24-27 hour and 33.33% after 48-51 hour. The results show that the percentage germination for *M. anisopliae* was more than *B. bassiana* (Fig.2).

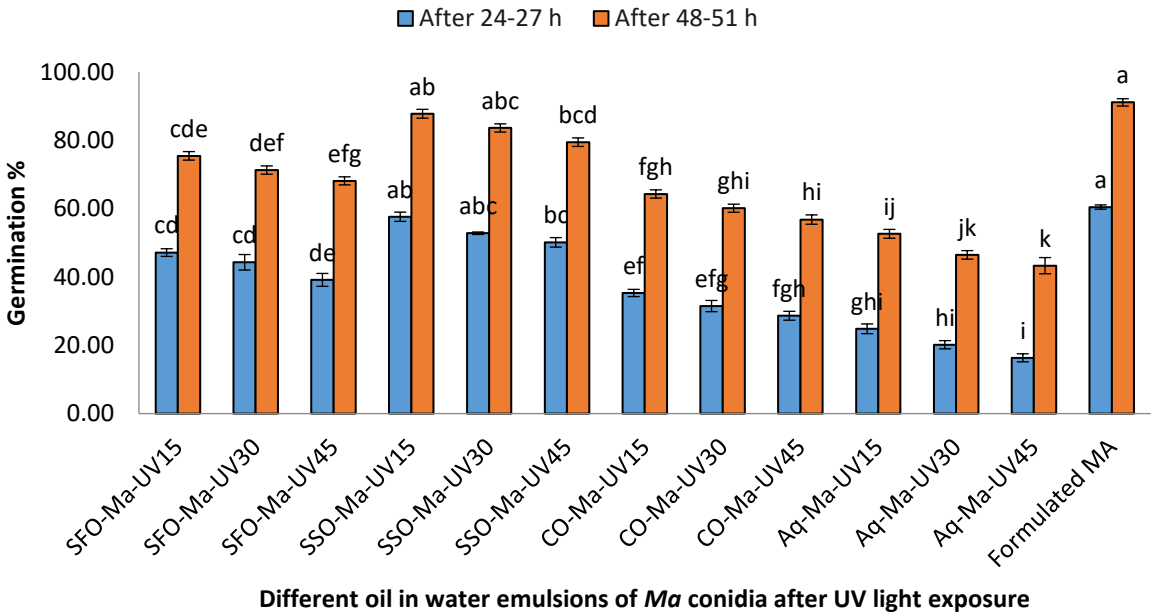


Figure 1. Percentage germination of *Ma* conidia (Mean±SE) at two different intervals 24-27 and 48-51 after UV light exposure on oil in water emulsions of *Ma* conidia
(Bars with the same letter indicates their means are not significantly different at α=0.05).

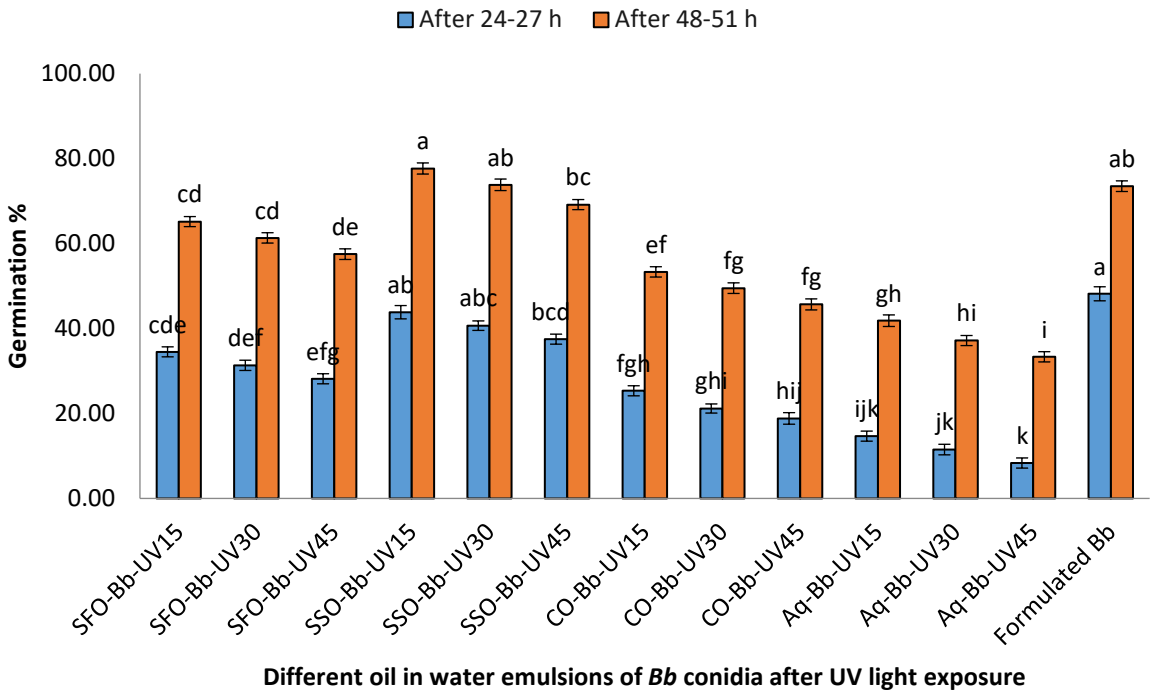


Figure 2. Percentage germination of Bb conidia (Mean±SE) at two different intervals 24-27 and 48-51 after UV light exposure on oil in water emulsion of Bb conidia
(Bars with the same letter indicates their means are not significantly different at α=0.05)

**Adult bioassay for *B. zonata***

After the evaluation of the viability, bioassay on adult of *B. zonata* was carried out to assess the pathogenicity of formulation of EPF. 1 day old adult of *B. zonata* were treated with *B. bassiana* and *M. anisopliae.* Virulence of conidia was assessed against a batch of 15 *B. zonata* adults. The treatments used against adult were; Sunflower, Sesame and Corn oil with UV light for three time durations 15, 30 and 45 minutes (SFO-UV15, SFO-UV30, SFO-UV45, SSO-UV15, SSO-UV30, SSO-UV45, CO-UV15, CO-UV30, CO-UV45, AQ-UV15, AQ-UV30, AQ-UV45, Formulated product) there are total 13 treatments including a formulated product (Control as no exposure of UV Light) as a control. The mortality of the adult was evaluated at 3, 5, and 7 days after treatment.

There were significant differences for adult mortality of *B. zonata* at all intervals after 3 days ($F_{12, 26}$ = 31.40, α=0.05, P=0.000), after 5 days ($F_{12, 26}$ = 27.23, α=0.05, P=0.000) and after 7 days ($F_{12, 26}$ = 28.69, α=0.05, P=0.000). Mortality percentage was in the sequence of: Formulated Ma > SSO-Ma-UV15 > SSO-Ma-UV30 > SSO-Ma-UV45 > SFO-Ma-UV15 > SFO-Ma-UV30 > SFO-Ma-UV45 > CO-Ma-UV15 > CO-Ma-UV30 > CO-Ma-UV45 > AQ-Ma-UV15 > AQ-Ma-UV30 > AQ-Ma-UV45. In case of *M. anisopliae* the highest percentage adult mortality after treatments was 55.6% after 3 days, 71.1% after 5 days and 84.4% after 7 days for formulated product (Control as no exposure of UV Light) of *M. anisopliae*. In case of oil protection used on conidia the highest protection was given by sesame oil, when exposed with UV light for 45 minutes, so the high mortality was achieved when conidia protected with sesame oil, the mortality was 37.8% after 3 days, 66.7% after 5 days and 75.6% after 7 days. And lowest was in case of AQ-Ma-UV45 treatment after 45 minutes which has only water and fungus and not any oil, 8.9% after 3 days, 28.9% after 5 days and 35.6% after 7 days (Fig. 3).

There were significant differences for adult mortality of *B. zonata* at all intervals after 3 days ($F_{12, 26}$ = 13.30, α=0.05, P=0.000), after 5 days ($F_{12, 26}$ = 15.84, α=0.05, P=0.000) and after 7 days ($F_{12, 26}$ = 16.97, α=0.05, P=0.000). Mortality percentage suppressed in the sequence of: Formulated Bb > SSO-Bb-UV15 > SSO-Bb-UV30 > SSO-Bb-UV45 > SFO-Bb-UV15 > SFO-Bb-UV30 > SFO-Bb-UV45 > CO-Bb-UV15 > CO-Bb-UV30 > CO-Bb-UV45 > AQ-Bb-UV15 > AQ-Bb-UV30 > AQ-Bb-UV45. In case of *B. bassiana* the highest percentage adult mortality after treatments was 40.00% after 3 days, 51.1% after 5 days and 73.3% after 7 days for formulated product (Control as no exposure of UV Light) of *B. bassiana*. In case of oil protection used on conidia the highest protection was given by sesame oil, when exposed with UV light for 45 minutes, so the high mortality was achieved when conidia protected with sesame

oil, the mortality was 31.1% after 3 days, 37.8% after 5 days and 64.4% after 7 days. And lowest was in case of AQ-Bb-UV45 treatment after 45 minutes which has only water and fungus and not any oil, 6.7% after 3 days, 13.3% after 5 days and 35.6% after 7 days. The results show that the percentage adult mortality for *M. anisopliae* was more than *B. bassiana* (Fig. 4).

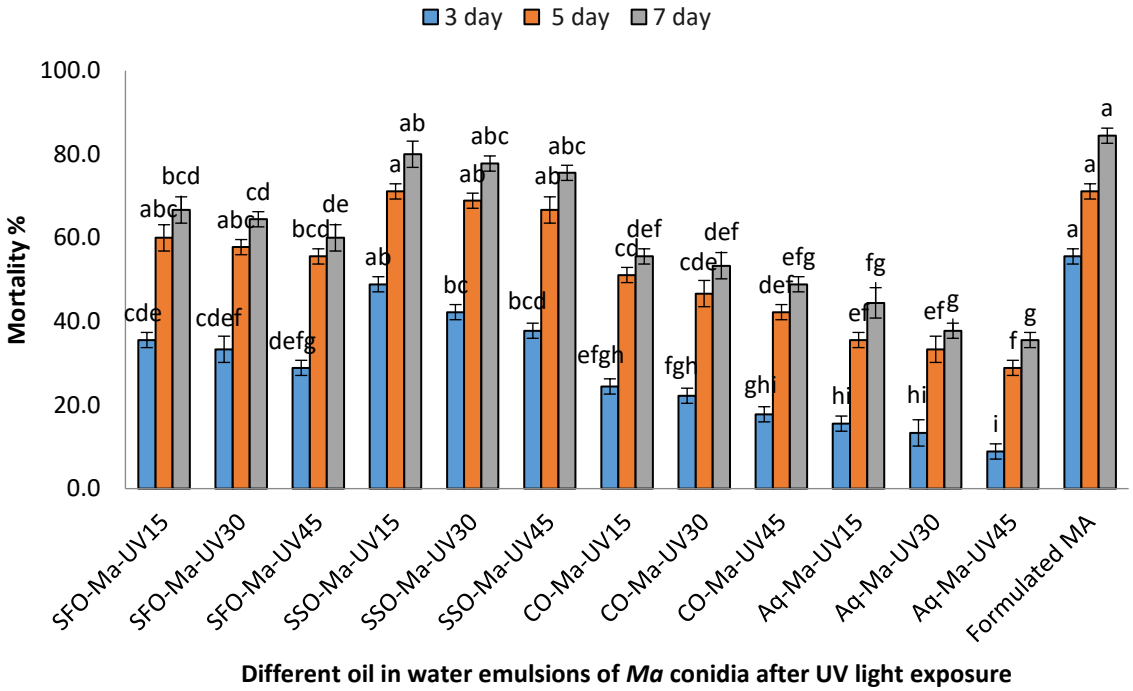


Figure 3 Adult mortality (Mean±SE) of *B. zonata* at different intervals when oil in water emulsions of Ma conidia was applied after UV light exposure
(Bars with the same letter indicates their means are not significantly different at α=0.05).

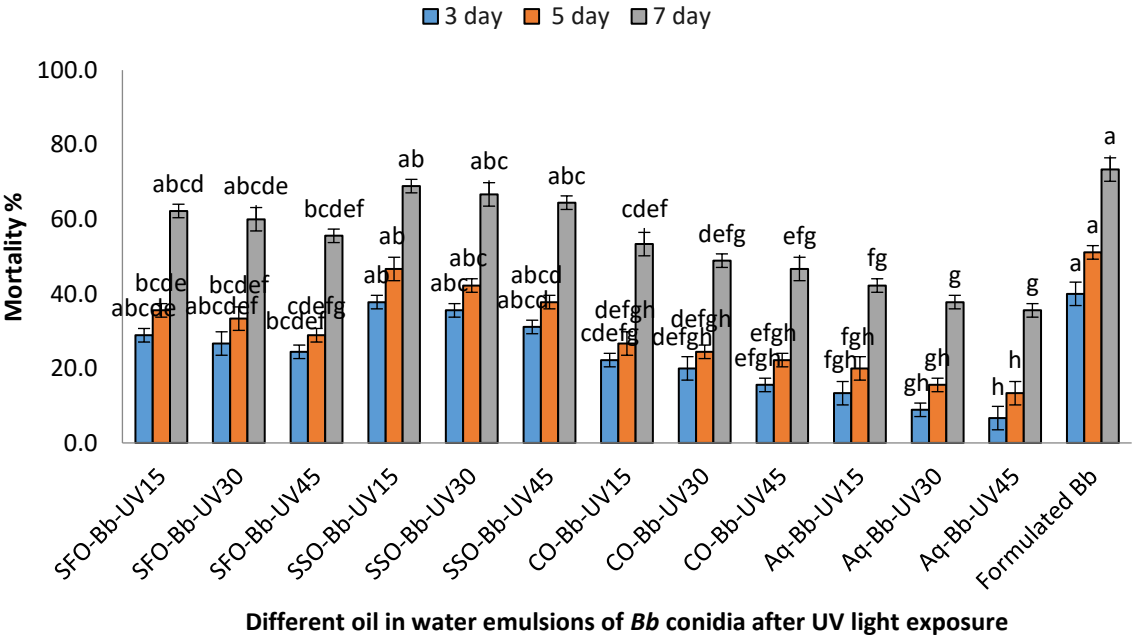


Figure 4. Adult mortality (Mean±SE) of *B. zonata* at different intervals when oil in water emulsions of Bb conidia was applied after UV light exposure
(Bars with the same letter indicates their means are not significantly different at α=0.05

**Pupal bioassay for *B. zonata***

After the evaluation of the viability, bioassay on pupa of *B. zonata* was carried out to assess the pathogenicity of formulation of EPF through contact method. 3 day old pupae of *B. zonata* were treated with *B. bassiana* and *M. anisopliae.* Virulence of conidia was assessed against a batch of 15 *B. zonata* pupae. The treatments used against pupae were; Sunflower, Sesame and Corn oil with UV light for three time durations 15, 30 and 45 minutes (SFO-UV15, SFO-UV30, SFO-UV45, SSO-UV15, SSO-UV30, SSO-UV45, CO-UV15, CO-UV30, CO-UV45, AQ-UV15, AQ-UV30, AQ-UV45 and Formulated product (Control as no exposure of UV Light), there are total 13 treatments including a formulated product as a control. The mortality of the pupae was evaluated at 3, 5, and 7 days after treatment as an adult emergence value.

There were significant differences for adult emergence percentage from pupae of *B. zonata* at all intervals after 3 days ($F_{12, 26}$ = 21.21, α=0.05, P=0.000), after 5 days ($F_{12, 26}$ = 40.47, α=0.05, P=0.000) and after 7 days ($F_{12, 26}$ = 18.82, α=0.05, P=0.000). Adult emergence percentage increase in the sequence of: Formulated Ma < SSO-Ma-UV15 < SSO-Ma-UV30 < SSO-Ma-UV45 < SFO-Ma-UV15 < SFO-Ma-UV30 < SFO-Ma-UV45 < CO-Ma-UV15 < CO-Ma-UV30 < CO-Ma-UV45 < AQ-Ma-UV15 < AQ-Ma-UV30 < AQ-Ma-UV45. In case of *M. anisopliae* the lowest percentage adult emergence after treatments was 22.2% after 3 days, 31.1% after 5 days and 37.8% after 7 days for formulated product (Control as no exposure of UV Light) of *M. anisopliae*. In case of oil protection used on conidia the highest protection was given by sesame oil, when exposed with UV light for 45 minutes, so the lowest adult emergence was achieved when conidia protected with sesame oil, the adult emergence was 31.1% after 3 days, 40.00% after 5 days and 48.9% after 7 days. And highest was in case of AQ-Ma-UV45 treatment after 45 minutes which has only water and fungus and not any oil, 62.2% after 3 days, 77.8% after 5 days and 80.00% after 7 days (Fig. 5).

There were significant differences for adult emergence percentage from pupae of *B. zonata* at all intervals after 3 days ($F_{12, 26}$ = 15.49, α=0.05, P=0.000), after 5 days ($F_{12, 26}$ = 14.52, α=0.05, P=0.000) and after 7 days ($F_{12, 26}$ = 28.99, α=0.05, P=0.000). Adult emergence percentage increase in the sequence of: Formulated Bb < SSO-Bb-UV15 < SSO-Bb-UV30 < SSO-Bb-UV45 < SFO-Bb-UV15 < SFO-Bb-UV30 < SFO-Bb-UV45 < CO-Bb-UV15 < CO-Bb-UV30 < CO-Bb-UV45 < AQ-Bb-UV15 < AQ-Bb-UV30 < AQ-Bb-UV45. In case of *B. bassiana* the lowest percentage adult emergence after treatments was 17.8% after 3 days, 24.4% after 5 days and 31.1% after 7 days for formulated product (Control as no exposure of UV Light) of *B. bassiana*. In case of oil protection used on conidia the highest protection was given by sesame oil, when exposed with UV light for 45 minutes, so the lowest adult emergence was achieved when conidia protected with sesame oil, the adult emergence was 26.7% after 3 days, 33.3% after 5 days and 40.00% after 7 days. And highest was in case of AQ-Ma-UV45 treatment after 45 minutes which has only water and fungus and not any oil, 53.3% after 3 days, 62.2% after 5 days and 77.8% after 7 days. The results show that the pupal mortality for *M. anisopliae* was more than *B. bassiana* (Fig. 6).

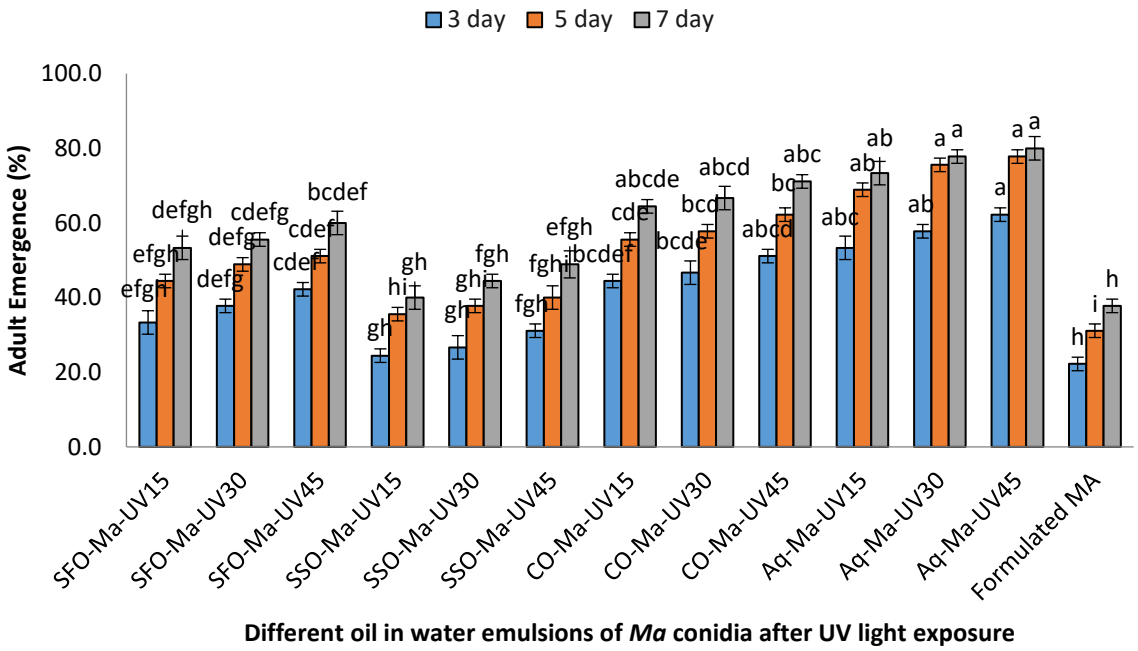


Figure 5. Adult emergences from pupae (Mean±SE) of *B. zonata* at different intervals when oil in water emulsions of Ma conidia was applied after UV light exposure
(Bars with the same letter indicates their means are not significantly different at α=0.05).

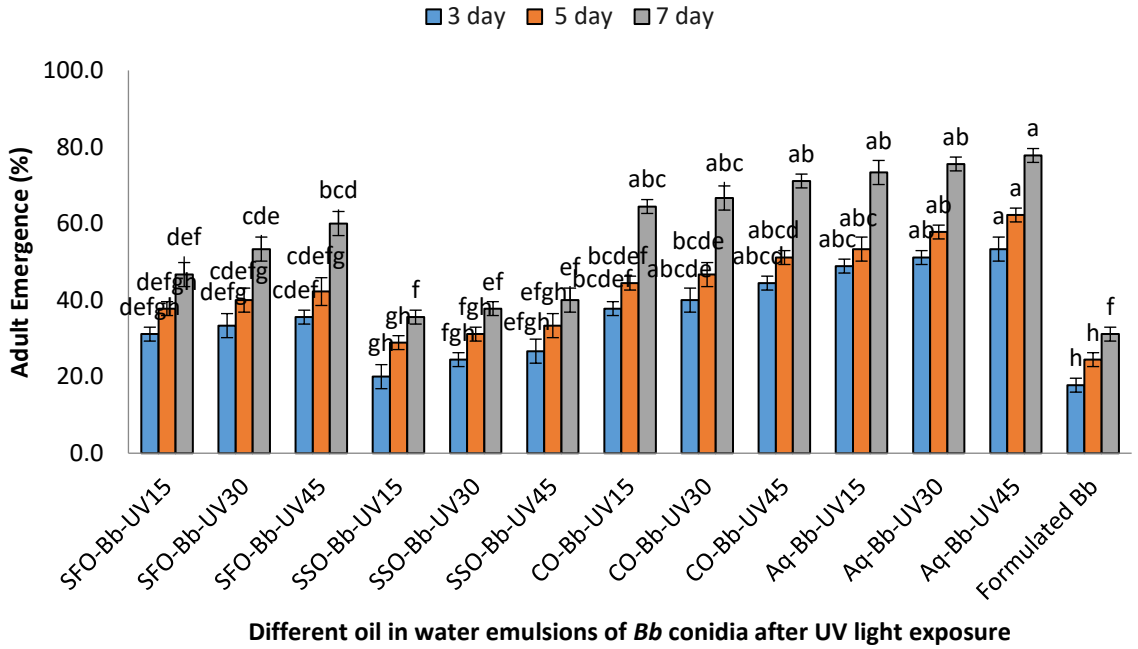


Figure 6. Adult emergence from pupae (Mean±SE) of *B. zonata* at different intervals when oil in water emulsions of Bb conidia was applied after UV light exposure
(Bars with the same letter indicates their means are not significantly different at α=0.05).

## DISCUSSION

In this study the formulation (oil in water emulsion) of two Entomopathogenic fungi *M. anisopliae* and *B. bassiana* were exposed with different three time interval (15, 30 and 45 minutes) of UV light then evaluate the conidial viability and virulence against pupae and adult of *B. zonata.* Oil-based formulation shield conidia from UV-induced damage by creating a protective film around the conidia. Fernandes *et al.,* 2015 concluded that sesame oil was particularly

effective due to its UV-absorbing properties, supporting this study's findings that sesame oil (SSO) performed well over sunflower oil (SFO) and corn oil (CO). It is oil-based carriers enhanced fungal spore survival under UV stress.
Edgington explained Exposing *B. bassiana* conidia to UV light or natural sunshine quickly inactivated them. However, adding protective chemicals lowered the rate of inactivation (Edgington *et al*., 2000). Previous investigations have shown that conidia in oil-in-water emulsion have higher relative germination when exposed to UV-B than conidia in aqueous suspension (Bernardo *et al*., 2020; Corval *et al*., 2021). These studies are in conformation to our study that vegetable oil formulations give more protection to conidia of *M. anisopliae* and *B. bassiana* than aqueous formulation with water only.
Oil in water emulsion helps conidia adhere and disperse on insect cuticles. It protects conidia from desiccation in low humidity conditions and mitigates the harmful effects of abiotic factors such heat stress and UV radiation. Converting *M. anisopliae* and *B. bassiana* conidia into an emulsion improves shelf life, increases insect host mortality rates, and protects conidia from damage during dilution. Techniques for preparing oil emulsions can boost the effectiveness and harmfulness of fungus (Gurpreet and Singh, 2018). Tween 80 has been used in laboratory bioassays to aid in the suspension of hydrophobic conidia (Qaderi and Safaie, 2023). According to Fernandes *et al*. (2015), employing the examined vegetable oils does not permanently damage the viability of *B. bassiana* conidia. Non-ionic surfactants, such as phenolic and alcoholic hydroxyls, carbonyl oxygens of esters and amides, ether oxygens, and comparable sulfur-containing configurations, are commonly used as surface-active agents (Muñoz *et al*., 2022).
According to our research sesame oil was the most effective vegetable oil that gave protection to conidia and more control of fruit fly. Sunflower oil was the second in list while corn oil was third. Less protection of conidia and less control of fruit fly was achieved in aqueous formulation which contains no oil. These results are in conformation to previous studies which tells that different vegetables and mineral oils, including soybean oil have been shown to provide significant Ultraviolet radiation protection in studies of EPF formulation (Behle *et al*., 2009; Posadas *et al*., 2012), as well as paraffinic oils and also peanut oil (Birnbaum *et al*., 2021; Fernandes *et al*., 2015; Bernardo *et al*., 2020), rapeseed oil and sesame oils (Kaiser *et al*., 2019), sunflower oils and corn oils (Posadas *et al*., 2012).
This study implies that adding *M. anisopliae* and *B. bassiana* to oils can be effective in enhancing their potency. The shelf life, heat tolerance, and fruit fly virulence were greatly improved in the oil-based formulation. Sesame oil has greater thermal stability that can be due to its composition containing unsaturated fatty acids and natural antioxidant like sesamin, sesamolin and sesamol. Due to greatest protection from thermal stress makes it an excellent carrier. Presence of antioxidant in the formulation can protect fungal protein from oxidation (Mohamed Ahmed et al., 2020). Oleic acid is also an important component of fatty acid in the composition of sesame oil. These components contribute to prevent protein oxidation and thus minimize quality loss (Yasothai, 2014). This study reveals that sesame oil was best liquid carrier among those tested liquid carriers. Sesame oil has retained efficacy of the formulation up to 30 day of the preparation. So, sesame oil was recommended for preparation of oil in water emulsion (formulation) of entomopathogenic fungi.

## CONCLUSION

This study concluded that oil in water emulsion (formulation) of entomopathogenic fungi *M. anisopliae* and *B. bassiana* has performed better than aqueous suspension. Among test oil in water emulsion (formulation) of EPF sesame oil has performed superior than other oils. Sesame oil is recommended as best carrier for preparation of formulation of EPF.

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Not applicable.

## AUTHOR CONTRIBUTIONS

Mirza Abdul Qayyum concealed the idea. Muhammad Zeeshan Shahid conducted the research trial and collected data. Saira Akhtar performed statistical analysis. Umer Sharif wrote this manuscript of the paper. Mirza Abdul Qayyum, Hasan Taha, Owais Hameed and Muhammad Ishtiaq reviewed the manuscript.

## COMPETING OF INTEREST

The authors declare that the research was carried without any commercial or financial relationships that could be construed as a potential conflict of interest.

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