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Impact of Temperature and Ultra-Violet Radiation on the effectiveness of *Beauveria bassiana* inverted emulsion formulation to Spodoptera *littoralis*

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Abstract
Background: Temperature and UV light can have a highly detrimental effect on entomopathogenic fungi, potentially resulting in conidia destruction. Formulations can aid in shielding these fungus structures from high temperature and radiation. The goal of this investigation was to prepare *Beauveria bassiana* oil-based formulation and assess the impact of different storage temperature and ultraviolet radiation on their potential pathogenicity on *Spodoptera littoralis*. the findings demonstrated that addition of Sun flower to fungal spore increase their infectivity to *Spodoptera littoralis*, formulations stored at refrigerator recorded higher mortality percent when compared to formulations stored at 37°C and inverted emulsion formulation enhance tolerance of fungal spore to UV radiation for 4 hours .

Keywords: Entomopathogenic fungus, *Beauveria bassiana*, Formulations, Conidia, *Spodoptera littoralis*, temperature, UV radiation

1. Introduction

The Egyptian leafworm of cotton (CLW), scientifically known as Spodoptera littoralis (Boisduval) under the family Noctuidae, consider among the most common and costly insect pests. It is viewed as a damaging pest in Egypt that harms ornamentals, other crops, and vegetables in addition to cotton plants [1, 2]. Besides to the immediate harm, it also minimizes the area used for photosynthetic processes and lowers the market value of decorative plants and vegetables. [3]. Entomopathogens fungi as Metarhizium anisopliae and Beauveria bassiana have harmful effects on a variety of agricultural pests. [4, 5]. It is regard as environmentally friendly and is an essential part of integrated pest control. for controlling insects like Hemiptera, Coleoptera, and Lepidoptera. Some of the benefits of using microbial products in agriculture include their specificity and selectivity, reduced risk of target insects developing resistance, prolonged effectiveness in pest management, minimal harm to the environment and reduced the preparation and registration costs, [6]. The proper application of the entomopathogenic fungi in the field is restricted by extreme heat and ultraviolet light, which lower the vitality of conidia in the field [7].
For entomopathogenic fungi, temperature is crucial as it modifies processes associated with enzymes, poisons, spore growth, germ tube formation, penetration, colonization, and reproductive. The efficiency of EFPs is significantly influenced by temperature, the high temperatures having a negative impact on germination and conidial survival. [8, 9]. For instance, it was observed that at 26 °C rather than 30 °C, Beauveria bassiana was more efficient against Rhyzopertha. dominica and Sitophilus. oryzae. [10]. conversely, radiation may alter the initial phases of the germinative tube's growth and conidia growth [11]. Reactive oxygen molecules (ROM) from UV radiation mainly target living organisms' DNA by deoxyribose oxidation and strand breakage, which generate single oxygen atoms (O2) and peroxides (H2O2) [12]. UV-C (245 nm) is highly destructive radiation. They are efficient at killing Microorganisms at low dosages [13] after three-hour exposure to direct sunlight; *Beauveria bassiana* has lost its capacity to infect [14]. One potential strategy to preserve conidia in a field, decrease expenses as well as maintain management is to utilize the formulated products which offer advantages including shielding conidia from light, extending their duration of storage, and making preservation and transportation easier, [15] Approximately 25% of the commercial biopesticides on the market today are oil dispersion-formulated products, and the remaining 75% are only technical products without any additional ingredients or treatment to ensure better performance during distribution, storage, and utilization, field persistence, and successful pest management [16,17].

Invert emulsions (water within oil type) have the moisture essential to conidial development within utilization throughout the dry, warm preservation circumstances; they are seen to be among the most likely successful formulations

[18]. As the oil based fluid formulations are primarily made of natural ingredients that are utilized as additives for food or in the production of skin care products, they have no negative harmful impacts on treated crops or plant-based products or adverse environmental side effects. Furthermore, it is simply to isolate the fungal species that are utilized in these emulsions from nature formulations [19]. B. bassiana's invert emulsion formulation has a milky-like. form, contains non-toxic chemicals that can encourage conidial proliferation and insect host penetration, and can supply the formed fungal conidia with the water needed for germination after application. [20]. Therefore, this study aimed to prepare *B. bassiana* inverted emulsion formulation and evaluate the influence of different temperatures and ultraviolet radiation on the pathogenicity of formulated conidia to *Spodoptera littoralis*.

2. Materials and Methods

2.1. breeding of Egyptian leaf worm of cotton, *Spodoptera littoralis*

Egg clusters from cotton leaf worm (Spodoptera littoralis) have been collected from the Anshas region's Egyptian Atomic Energy Authority's Nuclear Research Center. Freshly born larvae were maintained In muslin cloth-covered glass bottles, and fed on sterile, new castor bean (Ricinus communis L.) leaves.., and kept at a laboratory condition with 27±1.0ºC and 70.0±5.0% Relative moisture in a photoperiod of 12:12.. L: D as mentioned by [21, 22]. While the larvae came to their second instar, they were utilized in a following investigation.

2.2. Cultural conditions

Beauveria bassiana (AUMC 9896), an entomopathogenic fungus isolate, was recognized in the Mycological Center of the Assiut University, Faculty of Science after being isolated in in Production Unit of Bio-Insecticides, Egypt's Agricultural Research Center, Plant Protection Research Institute, **[10]**. isolate was grown on Czapek medium for fifteen days at a temperature of 25±1°C.

2.3. Production of the inverted emulsion (a formulation of water within oil)

Two states made up the inverted emulsion formulation: (1) the aqueous state which included a blend of glycerin (4.00% w⁄w), sterile distilled water (forty-five percent w⁄ w), as well as the water-soluble emulsifier (span 60: six percent w⁄ w); and (2) the oil state, which consisted of a blend of sunflower oil (forty-three percent w⁄ w) and oily soluble emulsifier Tween 20 (2.00% w⁄ w). The components of each step are made independently by combining them according to weight, and the two states are then quickly combined using a mixer (20–25,000 rpm for one and a half minutes). In order to achieve higher stability and preserve the homogeneity of the resulting emulsion, rapid mixing is required. The final inverted emulsion contains a predetermined proportion of each state (50%, w/w) to ensure that there is a sufficient amount of water present in the emulsion. [20].

2.4 The addition of *B. bassiana* conidia to an inverted emulsion formulation

The conidia were gathered by carefully scratching the 14–15-day-old culture's surface with a sterile glass slide, followed by hanging it in sterile, de-ionized water. Following that, the suspension of conidial has been standardized at 4.00×10^7 conidia ⁄ milliliter, resulting in a final conidial concentration of 1.00×10^7 conidia/milliliter in the produced formulation.. Standardized conidial suspension was added to the emulsion during production of component by first combining them with sterile de-ionized water that made up 45% of the emulsion's overall volume. The produced emulsion was stored within 500 ml dark-colored screw-capped glass containers and maintained at different temperatures (4°C and37°C) for the complete period of bioassays

2.5. The virulence of *B. bassiana* inverted emulsion formulation toward *S. littoralis* larvae

To evaluate the infectiousness of ***B. bassiana*** toward 2nd instars larvae of ***S. littoralis***; Four concentrations were produce (100, 75, 50**,** and 25%) from IE formulation using distilled water. Fresh caster- leaves and plastic containers were splashed with all of the concentrations and the distilled water utilized as an inverse control. The leaves that are treated were placed into the contaminated containers after drying in the air and provided with ten larvae of *S. littoralis* in their second instar. Each treatment consisted of four replicates. All containers were wrapped with the muslin fabric for aeration [23] then maintained in an incubator at 27.0±1.00ºC and 70.00±5.00% Relative moisture. Every day, the death rate and the overall larval mortality was calculated at the final stage of the larval instar at zero time (IE without storage) and during storage period. Abbott's technique was used to adjust the death percentages. [24].

2.6. Ultraviolet Radiation Test

To assess UV radiation's effects on effectiveness of *B. bassiana* inverted emulsion formulation toward *S. littoralis* larvae in their second instar. In the ultraviolet cabinet, four concentrations of IE formulation (100, 75, 50, and 25%) were subjected to UV radiation with a wavelength of 254 nm at various intervals of 2, 4, 6, and 8 hours and IE without UV exposure was used as a control [25]. The exposed suspensions were placed 30cm away from the UV source. For a minimum of one hour during and following UV treatment, the irradiated fungal formulation was maintained in the dark to avoid light reactivation **[26].** The pathogenicity of irradiated formula to *S.*

littoralis larvae was determined as previously described.

2.7. Analytical statistics

The mortality data for all treatments were analyzed by 2-way analysis of variance (**ANOVA**) followed by comparison of the averages using Tukey's HSD technique (SPSS Statistics) ($P < 0.05$).

3. Results

Obtained results reveal efficiency of *B.bassiana's* inverted emulsion formulation stored at different storage temperature on the mean total percentage of second-instar S. littoralis larval mortality. The average total percentage of larval mortality of S littoralis larvae in their second instar exposed to various concentrations of ***B bassiana's*** inverted emulsion formulation maintained under refrigerator conditions (4°C) is displayed in **Table (1)**.

Table (1): Impact of *B.bassiana's* inverted emulsion formulation on the mean total percentage larval mortality of S. littoralis larvae in their second instar under refrigerator conditions (4°C)

Storage time(Weeks)	Total larval mortality ±SE					The mean of thetotal percentage larval mortality
	Control	25 %	50 %	75%	100%	
Zero time	05.0±2.50	55.0±2.89	62.5±2.50	75.0±2.89	90.0±0.00	**57.50 a***
1st week	05.0±2.50	47.5±2.50	50.0±0.00	60.0±0.00	72.5±2.50	**47.00 b**
2nd week	05.0±2.50	42.5±2.50	47.5±2.50	57.5±2.50	65.0±2.89	**43.50 bc**
3rd week	05.0±2.50	37.5±2.50	47.5±2.50	55.0±2.89	62.5±2.50	**41.50 c**
4th week	05.0±2.50	32.5±2.50	40.0±2.89	47.5±2.50	57.5±2.50	**36.50 d**
Two month (8 week)	05.0±2.50	30.0±0.00	37.5±2.50	42.5±2.50	52.5±2.50	**33.50 de**
three month (12 week)	05.0±2.50	27.5±2.50	35.0±2.88	40.0±0.00	47.5±2.50	**31.00 ef**
Four month (16 week)	05.0±2.50	25.0±2.89	32.5±2.50	37.5±2.50	42.5±2.50	**28.50 f**
The mean of the total percentage larval mortality	**5.000 E***	**37.188 D**	**44.063 C**	**51.875 B**	**61.250 A**	

***The similar letter (capital letters) after the average in each column and each raw (small letters) indicate that there is no substantial variation at (p below 0.05).**

comparison to 5% in control treatment, the overall mean of total percentage larval mortality vertically increased dramatically as the concentration of the formulated conidia climbed to 37.19, 44.06, 51.87, and 61.25% at the concentrations of 25, 50, 75, and 100%, respectively.. In contrast, the overall mean of total percentage larval mortality horizontally decreased significantly with increasing the storage period as it decreased to 47, 43.5, 41.5, 36.5, 33.5, 31 and 28.5% at the first, second, third, fourth, eighth, twelve, and sixteenth week of the storage periods, as opposed to 57.5% in the zero-time treatment, respectively. The infectivity of IE formulation persisted only for 4th month. while, at 37°C, the infectivity of WP formulation persisted only for 3 weeks as shown in **Table (2)** and the average total percentage larval mortality horizontally dropped noticeably as the storage period was extended, going from 57.5% in the zero-time treatment to 39.5, 32.5, and 24.5% in the first, second, and third weeks of storage period, respectively.

Our data also assessed impact of UV radiation on infectivity of *B. bassiana's* inverted emulsion formulation to ***S. littoralis*** larvae in their second instar. The ultraviolet radiation in **Table (3)** had little impact on the infectivity of *B.bassiana's* inverted emulsion after 2 and 4 hr after exposure to UV –C light. The mean of the total larval mortality were 70 and 63.13% respectively, while it was 72.5% in the control treatment. As the period of exposure was extended to 6 and 8hr , UV–C light had substantial effect on the infectivity of *B.bassiana's* inverted emulsion as

Table (2): Impact of *B. bassiana's* inverted emulsion formulation on the mean total percentage larval mortality of S. littoralis larvae in their second instar at (37°C)

Storage time	Total larval mortality ±SE	The mean of

(Weeks)	Control	25 %	50 %	75%	100%	the total percentage larval mortality
Zero time	05.0±2.50	55.0±2.89	62.5±2.50	75.0±2.89	90.0±0.00	**57.50 a***
1st week	05.0±2.50	42.5±2.50	45.0±2.89	50.0±0.00	55.0±2.89	**39.50 b**
2nd week	05.0±2.50	27.5±2.50	37.5±2.50	45.0±2.89	47.5±2.50	**32.50 c**
3rd week	05.0±2.50	22.5±2.50	25.0±2.89	32.5±2.50	37.5±2.50	**24.50 d**
The mean of the total percentage larval mortality	**05.00 D***	**36.88 C**	**42.50 C**	**50.63 B**	**57.50 A**	

***The similar letter (capital letters) after the average in each column and each raw (small letters) indicate that there is no substantial variation at (p below 0.05).**

Table (3): Influence of *B. bassiana* inverted emulsion formulations exposed to UV radiation on the cumulative larval mortality percentage of the *S.littoralis*

Treatments Time of Exposure (hours)	The accumulative larval mortality% ±SE				The overall mean of the larval mortality %
	25 %	50%	75 %	100 %	
Zero time (Control without irradiation)	57.5±2.50	65.0±2.89	77.5±2.50	90.0±0.00	**72.50 a***
After 2 hours	50.0±2.50	70.0±0.00	72.5±2.50	87.5±2.50	**70.00 a**
After 4 hours	47.5±0.00	60.0±2.50	67.5±2.89	77.5±2.89	**63.13 b**
After 6 hours	30.0±2.89	37.5±2.89	45.0±0.00	67.5±2.50	**45.00 c**
After 8 hours	22.5±2.50	27.5±2.50	32.5±2.50	40.0±0.00	**30.63 d**
The overall mean of the larval mortality %	**37.50 D***	**48.75 C**	**54.38 B**	**68.13 A**	

***The similar letter (capital letters) after the average in each column and each raw (small letters) indicate that there is no substantial variation at (p below 0.05).**

The mean of the total percentage larval mortality were fallen down to 45 and 30.63 % respectively.

4-Disscussion

Our research's conclusions demonstrate how inverted emulsion formulation can enhance the entomopathogenic fungus spores' ability to infect *Spodoptera littoralis* larvae in their second instar during storage. Larval deaths in each treatment was concentration and time dependent, meaning that it rose as the concentration of formulated conidia and exposure duration raised.. This observation was in agreement with Umaru and Simarani **[27]** found that, *Aspergillus flavus* and *M. anisopliae* formulated in oil were efficient biopesticide for the control of *Enteroccocus pallens* and death rates of a bugs were also noticed to be in line to the concentrations of conidia . Kaisera **[28]** demonstrated that *B. bassiana* blastospores co-formulated with emulsified colza oil result in 70.8 ± 5.0%the pollen beetle mortality on day 20 of treatment and the mortality rate was significantly higher than each treatment individually. This rise in effectiveness of oil supplementation is linked to the lipophilic characteristics of oil, which make them very excellent spray transporters and spray stickers because they stick tightly to the hydrophobic surface areas of insect cuticles and fungi conidia.. Furthermore, oils diffuse quickly via insect cuticles, which can carry conidia into hidden areas of the insect body, particularly intersegmental areas where water amount are suitable to invasion and growth. [29] Additionally, It is known that oil damages the insect-protecting layer of epicuticular wax cuticles and interfere with the extraction of cuticle components causing damage to cuticle of the insect as well as encourage fungal penetration [30]. Batta[19] showed that inverted emulsion formulations are thought to be the most effective oil based formulations Because they provide adequate water for the growth of conidia within spraying at dry and warm preservation circumstances. The major abiotic factor influencing the shelf-life of the prepared formulations is storage temperature Connick [31] by keeping them at a modest metabolic activity level, Elzein [32]. With respect to conditions of storage, the formulation of *B. bassiana* stored at refrigerator (4°C) conditions showed high virulence to 2nd instar larvae of S. littoralis when compared to 37°C stored formulations. This could be because

biological activity will be at its lowest level in freezing and refrigerated conditions. Therefore, the living organisms may be performing very little metabolic. Life activities can be maintained for longer than the time at room temperature and 37 °C because there won't be any shifts or variations in the ambient conditions, **krishnaveni [33]**. Additionally, Gindro and Pezet [34] found that when the storage temperature rose there was a noticeable reduction in the level of energy, O2 intake, and aggression. Moreover it was shown that conidia kept at higher temperatures had undergone a greater change from ATP to ADP and AMP. The formulation of fungal spores has been explored as a potential means of partially shielding fungus from ultraviolet light. Our current study showed that UV- C rays had minimal impact on infectivity of fungal formulation of ***B. bassiana*** toward **S. *littoralis***. We found incorporation of 43% (v/v) of sun flower oils increased the UV tolerance of ***B. bassiana*** conidia as inverted emulsion formulation of ***B. bassiana*** maintained their infectivity even after 4hrs of UV exposure but the larval mortality percent began to decline sharply 6 and 8 hours after exposure. This result confirms the result obtained by **Alves** [**35**] who noticed that, as compared to traditional water based formulations, oil based formulations are capable of greatly raise resistance of conidia to UV light and promote conidia germination. Posadas [**25**] revealed after being subjected to UV-B radiation (235.7 nm) over four hours, formulated isolate with 5% (v/v) sunflower remained fatal. Devi [**36**] also noted that oils have been utilized as UV barriers since they eliminate UV radiation, protecting conidia from UV exposure. According to Jia [**37**] showed *B. bassiana* grown in emulsifable oil of soybean, groundnut and triton X-100 exhibit little resistance toward UV radiation and have enhanced the ability of *B. bassiana* to inhibit instar locusts in their fifth instar .

4. Conclusion

Insect population regulation is largely dependent on entomopathogenic fungal species. When infected by entomopathogenic fungi, insect pests do not quickly develop resistance [38]. In order to evade insect immune reactions, these fungal species infect their hosts by breaking through the cuticle, entering the hemolymph, releasing toxins, and spreading by using the nutrients in the hemocoel [39]. Moreover, when applying fungal entomopathogens on a wide scale, appropriate formulations with the necessary carriers are crucial for infectiousness to the host. [40]. Research findings indicate that oil-based formulations improved conidia adherence, provided protection against heat stress and UV radiation, raised the infectious capacity of entomopathogenic fungi on *S. littoralis*, and An increase in conidia concentrations was shown to correlate with a rise in S. littoralis larval mortality.

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