Evaluating the Efficiency of the Entomopathogenic Fungus *Beauveria bassiana* to Control the Brown Banded Cockroach *Supella longipalpa* F.

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Abstract

Entomopathogenic fungi can be ideal for the biocontrol of cockroaches since it is environment-friendly microbial pesticide. Susceptibility of second and fourth instar of nymphs and adults of the brown banded cockroach, *Supella longipalpa* (F.) (Blattodea: Blattellidae) to the entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. at two concentrations 1x 10^7 and 1x 10^8 spore/ml was evaluated. Fungus was tested by using two different methods: bait and direct contact. Mortality was monitored after 3, 5 and 7 days' post exposure. Direct contact of *B. bassiana* at concentration 1x 10^7 spore/ml produced mortality on adults 82.76% and for fourth and second instar of nymphs 82.76 and 93.10% after 7 days' post treatment, respectively. When *S. longipalpa* was exposed to bait with *B. bassiana* the mortality percentage was 37.93% for the adults, and caused 64.29 and 58.62% mortality to the second and fourth instar of nymphs, after 7 days from treatment, respectively. Nymphs and adults of *S. longipalpa* treated by direct contact with *B. bassiana* at 1x 10^8 spore/ml, produced mortality on adults, fourth and second instar of nymphs 78.57, 93.10 and100% after 7 days' post treatment, respectively. Method of bait the mortality for adults, fourth and second instar of nymphs were 51.72, 72.41 and 78.57% after 7 days' post treatment, respectively. Results showed differences in susceptibility between nymphs and adults of *S. longipalpa*. Adult and nymph instars (fourth and second) mortalities due to direct contact by *B. bassiana* suspension (1x 10^7 and 1x 10^8 spore/ml) produce high mortalities (53.51, 93.10, 82.76% and 78.57, 100, 93.10%) respectively.

Keywords: *Supella longipalpa*; entomopathogenic; Direct contact: Bait; *Beauveria bassiana*.

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Introduction

Cockroaches have for some time been known as vectors of nourishment harming and irresistible living beings [1-3]. The brown-banded cockroach, *Supella longipalpa* (F.) (Blattodea: Blattellidae) can be considered as an about cosmopolitan cockroach [1-4]. It is currently appropriated all through tropical and subtropical areas of the world [1]. This cockroach species conveys an assortment of microorganisms, as a vector for pathogenic microscopic organisms in urban situations and it has likewise been accounted for as a wellspring of allergens [3, 5, 6]. It is generally found in homes, condo, inns and healing facilities and less frequently in stores, eateries and kitchen [1, 2, 7]. Cockroaches are customarily controlled by applications of fluid details of pyrethroids, carbamates or organophosphates in or close-by plagued harborages [7]. This technique is hard to apply if there should be an occurrence of *S. longipalpa* infestations since it is important to treat all the distinctive regions of a room that the cockroaches may inhabit without making contamination issues, which make harm furniture and different focuses where this creepy crawly wants to paste its ootheca [1, 7. 8]. The different danger components connected with the utilization of concoction bug sprays, for example, the advancement of resistance, related resurgence in creepy crawlies, gathering of pesticide deposit in an evolved way of life, natural contamination, wellbeing dangers and high expenses have driven researchers and agriculturists to create alternative methodologies for irritation control [8, 9]. Entomopathogenic organisms are getting restored enthusiasm as biocontrol specialists in occurrences where synthetic pesticides exhibit a danger to human wellbeing [10]. Growths enter the host through the fingernail skin and a contact with the creepy crawly can bring about disease. Bountiful mycosis creates on the bodies of the creepy crawlies that have kicked the bucket because of parasitic disease. These mycotic corpses propagate the deadly parasitic contamination in the creepy crawly populace Hyphomycetes growths have been accounted for to contaminate cockroaches [11-13]. It ought to be anything but difficult to impel an entomopathogenic contagious disease misleadingly in cockroaches in light of the fact that the advancement of bountiful mycosis on the creepy crawlies is favored under the moist conditions winning in their environmental specialties. A quick spread of the disease in the creepy crawly populace is along these lines very likely. Beauveria bassiana, an entomogenous organism with a wide host range, happens more generally than *Metarhizium spp*. In the tropics [14]. Strains of *B. bassiana* (Bals.) Vuill. contrast in their host range [10]. The disease instrument of Beauveria in the host creepy crawlies has been accounted for [15-17]. The essential course of host intrusion is through the outer integument by means of the connection of the conidia to the fingernail skin, germination, trailed by infiltration into the fingernail skin. Once in the hemocoel, the mycelium ramifies all through the host, shaping yeast-like hyphal bodies or blastospores. Host passing is regularly because of a blend of the activity of a contagious poison, the physical check of blood course, supplement consumption. also, the intrusion of organs. The overall objective of the present study was to evaluate differential susceptibility of nymphs and adults of *S. longipalpa* to entomopathogenic fungi *B. bassiana* comparing between direct contact and bait treatment methods.

Materials and Methods

Insects: The cockroaches were collected from April to September / 2014. The cockroaches were reared in plastic boxes covered by plastic lids (30 x 25 x 20 cm) and maintained at 26 ± 2˚C, 50 ± 5% RH for a photoperiod of 12:12 (L: D) from October/2014 to February/2016. They were fed with dry crumble biscuits, bread and water. Pieces of facial tissue were provided as harborage. Cockroaches were anesthetized by chilling to facilitate handling. This *B. bassiana* (Bals.) Vuill. (the Chinese isolate B.C.) strain was cultured on Sabouraud

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dextrose agar + 1% yeast extract (SDAY 1%) medium, incubated at 25 ± 1°C and 12 h photophase. Conidia were harvested from 15 day old cultures. They were scraped with a sterile looper and collected into sterile plastic tubes (45 cm3) containing 5 ml of 0.01% (v/v) Tween 20 (Merck, México). A suspension of the conidia was vortexed for 5 min; the concentration of propagules was quantified by using a hemocytometer (Neubauer chamber) and the suspension was adjusted to 1 x 10^7 and 1x 10^8 spore/ml, its concentration was based on the results of a preliminary experiment. Virulence of the fungal strain of *B. bassiana*, towards nymphs and adults of *S. longipalpa* was evaluated by direct contact and bait groups of ten adults and ten second and forth instars nymph cockroaches were exposed to 2 ml of a suspension containing 1x10^7 and 1x10^8 spore/ml of *B. bassiana* on a dry filter paper disc (9 mm diam.). Each filter paper was placed into the bottom of a sterilized Petri dish (100 mm) for three replicates. Control was treated with discs of filter paper, with Tween 20, 0.01% (v/v). After 24 h, cockroaches were transferred to plastic cups (250 cm3). and incubated at 26 ± 2°C, 50 ± 5% RH, and 12 h photophase. Food and water were placed inside the containers and they were replaced and changed every two days. Mortality was monitored daily up to 7 days' post treatment. In another experimental series, the conidial suspension was applied on bait. Bait was prepared and mixed with bran wheat flour proportion w/v mean 1 gm/ ml, and 4 gm of this mixture was applied on the bottom of 35 mm sterilized Petri dishes. Groups of ten adults and ten second and fourth instars nymph's cockroaches were exposed to these baits for 72 h. Then, cockroaches were transferred to plastic cups (250 cm3) and bait assays were maintained under the conditions mentioned for the direct contact assays, as mentioned above. Mortality was monitored daily up to 7 days' post treatment (17).

Statistical Analysis

Analyses by ANOVA and comparison of mortality percentage means were done in a completely randomized design by Tukey's test ($P < 0.05$), using the SAS software [18], to study the effect of difference factors for different parameters. Least significant difference (L.S.D.) test was used to compare the significance between percentage of mortality in this study. Percentage mortalities were corrected according to Abbott's formula [19].

Results and Discussion

As evident from (Table 1) that the highest mortality of adults of *S. longipalpa* reached 53.51% after 7 days' post treatment of direct exposure to the fungus *B. bassiana* at concentration 1 x 10^7 spores / ml, at 26 ± 2˚C, 50 ± 5% RH for a photoperiod of 12:12 (L: D), and the lowest mortality reached 28.57% after 3 days' post treatment at the same concentration and there were significant differences between the mortality to different periods of exposing the adult by the direct method to fungus. Table (1) indicated higher mortality to the fourth instar of the nymphs reached 82.76% after 7 days' post treatment of direct exposure to the fungus concentration of 1x 10^7 spore / ml and reached its lowest mortality 55.17% after 3 days post treatment, but after 5 days' post treatment reached mortality 68.96 %, and shows there are significant differences between the mortality of the different periods post treatment to the fourth instar of the nymphs, illustrates the same table, the difference in mortality to the second instar nymphs of *S. longipalpa* and reached its lowest mortality 57.14 % after 3 days post treatment of exposure, and the highest mortality after 7 days and reached 93.10% and show significant differences were high between the mortality to the second instar of the nymphs of the different periods after direct exposure to the fungus. Table (1) shows the present significant differences between the mortality of various stages of the *S. longipalpa* for each period on alone post treatment to periods of 3, 5 and 7 days. Where we noted greater the period of post treatment for direct exposure then greater the mortality rates. We conclude that

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the second instar of nymphs are more sensitive to fungus *B. bassiana* from fourth instar of nymphs, While the adult stage is more resistant. Table (2) shows the highest mortality rate was the second instar of the nymphs and reached 64.29 % after 7 days for the treatment of baits mixed with bran wheat flour proportion weight / volume mean 1 gm/ ml of a suspension containing $1x10^7$ spore per milliliter of *B. bassiana*, and 4 gm of this mixture was applied, the lowest mortality rate was 53.57% after 3 days of exposure to bait for the same instar nymphs. As for the fourth instar of nymph highest mortality rate was58.62%, and the lowest mortality rate reached 31.03%, after 7and 3 days of exposure to the baits, respectively. When exposure, the adults to the baits treatment with fungus *B. bassiana* reached the highest mortality rate 37.93 % after 7 days of treatment, but when 3 days after feeding on baits the lowest percentage of mortality reached 20.69 %. The table (2) indicated the presence of a significant difference in the mortality rate for each stage of the *S. longipalpa* and to periods after exposure, also there are significant differences between the stages for each of the periods after exposure separately. It shows that the mortality rate in the treatment of direct exposure which were higher than the treatment of baits to all stages of the insect treated. Direct contact treatment when compared to bait treatment was more effective, the bait treatment was less efficient than direct contact. The study results do not correspond with the results [20], Using 3×10^7 conidia/mL of *B. bassiana* and *T. harzianum* as inoculated bait against adults of S. longipalpa in Iraq resulted 86.67% and 36.67% mortality at seven days' post exposure, respectively. Our results of treatment with direct contact did not agree with results recorded by [21] which *M. anisopliae* Iran 437C had a high virulence against *S. longipalpa* adult and nymph mortality were 52.5 and 45% treated with 6.6×10^5 conidia/cm^2, at concentrations of *M. anisopliae* 1.65×10^7 and 6.6×10^7 conidia/cm^2, adult and nymph mortality were 100 %, after 7 days of exposure, respectively. Also [22] indicated that mortality percentages of nymphs and adults of S. longipalpa were 82.5% and 85% at seven days' post treatment at similar doses (proportion of 10% ≈ 6.6 × 10 conidia/cm^2) and survival times were 4.1 and 3.9 days, respectively, it seems that molting and ecdysis in the tracheal system of nymphs decreases the probability of spiracle blocking and decrease the rate of nymphs' mortality. results recorded by [23] indicated when the *B. germanica* treated with *B. bassiana* at concentration 1×10^9 conidia/ml exposed to direct contact the mortality rate of nymphs and adults were 16.7 and 50 % after 5 days after inoculation, also mortality rate was 40 and 80 % after 20 days after inoculation, respectively, but when the *B. germanica* treated with *B. bassiana* at concentration 1×10^9 conidia/ml exposed to bait, the mortality levels of nymphs and adults treated with bait reach values 50 and 40% after 20 days, respectively. In direct contact treatment contact cockroaches could get higher percentage of conidia. Table (3) shows the highest mortality of adults of *S. longipalpa* reached 78.57% after 7 days' post treatment of direct exposure to the fungus *B. bassiana* concentration of 1×10^8 spores / ml, at 26 ± 2˚C, 50 ± 5% RH for a photoperiod of 12:12 (L: D), and the lowest mortality reached 46.43% after 3 days post treatment at the same concentration and note that there were significant differences between the mortality to different periods of exposing the adult by the direct method to *B. bassiana*. Intersegment regions of the thorax and abdomen, mouthparts and legs as favorable areas for conidia adherence are more difficult to clean and lead to conidia penetration and adult contamination [24]. Table (3) is evident to higher mortality to the fourth instar of the nymphs reached 93.10 % after 7 days' post treatment of direct exposure to the fungus concentration of $1x\ 10^8$ spore / ml and reached its low and the same method of bait. It shows that the mortality rate in the treatment of direct exposure was higher than the treatment of baits west mortality 62.07 % after 3 days post treatment, but after 5 days post treatment reached mortality 79.31 %, and shows there are significant differences between the mortality of the different periods post treatment to the fourth instar of the nymphs, illustrates the same table, the difference in mortality to the second instar nymphs of *S. longipalpa* and reached its lowest mortality 64.29

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% after 3 days post treatment of exposure, and the highest mortality after 7 days and reached 100 % which show significant differences were high between the mortality to the second instar of the nymphs of the different periods after direct exposure to the *B. bassiana*. Ecdysis has been reported to be an important factor in insect resistance to fungal infection, particularly when the time interval between successive molting is short [25]. Table (3) shows the presence of significant differences between the mortality of various stages of the *S. longipalpa* for each period on alone post treatment to periods of 3, 5 and 7 days. Where we noted that greater the period of post treatment for direct exposure was then greater the mortality rates. conclude that the second instar of nymphs are more sensitive to fungus *B. bassiana* from fourth instar of nymphs. While the adult stage is more resistant. It appears in each of Table (1 and 3) that there are no significant differences in rate mortality for each of second and fourth nymphs' instar for all periods after exposure. Otherwise, it has been reported elsewhere that Cuticular lipids have a profound effect on fungal spore germination and differentiation: they can be toxic, fungistatic, or occasionally, for some pathogenic species, stimulatory [26]. When comparing the mortality rate in each of Table (1 and 3) for all stages of the *S. longipalpa*, we find there is a clear difference in the table (3) where it has the highest mortality rate due to use a higher concentration of the *B. bassiana* and the same method of direct exposure. Table (4) shows the highest mortality rate was the second instar of the nymphs and reached 78.57 % after 7 days for the treatment of baits mixed with bran wheat flour proportion weight / volume mean 1 gm/ 1ml of a suspension containing $1x10^8$ spore/ml of *B. bassiana*, and 4 gm of this mixture was applied, the lowest mortality rate was 57.14% after 3 days of exposure to bait for the same instar nymphs. As for the fourth instar of nymphs' highest mortality rate was 72.41%, and the lowest mortality rate reached 48.28%, after 7and 3 days of exposure to the baits, respectively. When exposure the adults to the baits treatment with fungus *B. bassiana* it reached the highest mortality rate 51.72 % after 7 days of treatment, but when 3 days after feeding on baits the lowest percentage of mortality reached 31.03 %. It is known that certain secondary metabolites of entomopathogenic fungi, including destruxins, cyclosporine, and beauverolides, possess functions in impairing the immune response of the host insect, causing death [27]. [28] suggested that the secondary metabolites produced by *B. bassiana* disable several immune mechanisms of *Eurygaster integriceps*, thus helping the fungus to overcome and kill its host, as the enzymatic defense in insects, detoxification enzymes play significant roles in eliminating exotic. Table (4) indicates the presence of a significant difference in the mortality rate for each stage of the *S. longipalpa* and to periods after exposure and there are also significant differences between the stages for each of the periods after exposure separately. There are no significant differences in rate mortality for each of second and fourth nymphs' instar for 3 and7 days after exposure Table (4). When comparing the mortality rate in each of Table (2 and 4) for all stages of the *S. longipalpa*, we find there is a clear difference in table (4) where it has the highest mortality rate due to use a higher concentration of the *B. bassiana* to all stages of the insect treated. Direct contact treatment when compared to bait treatment was more effective, the bait treatment was less efficient than direct contact.

Conclusion

Spiracle blocking by fungal conidia could be another reason contributing to higher mortality rates in nymphs. Although the cuticle of insects constitutes an important physical barrier for protection against penetration of entomopathogenic fungal conidia, high mortality rates were observed when nymphs were exposed to suspension of fungus. That the period of ecdysis for nymphs of *S. longipalpa* take a long period for turning from instar to instar spending about a month, for that do not affect the process of molting in a reduced incidence of the fungus because of the mortality shortly. This differential susceptibility at various life

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stages can be attributed to interaction between the insect integument being penetrated by the fungus and ecdysis of nymph's stages. These studies showed differences in mortality between nymphs and adults in the same species. We can conclude that *B. bassiana* (the Chinese isolate B.C.) strains showed potential as a biological control agent of nymphs and adults *S. longipalpa* by direct contact treatment. Seem anti-toxins enzymes secreted by the insect against Mycotoxins are few and inactive in the nymphs, especially the second instar reversing what exists in the nymphs fourth instar and adults, where the older the insect in a lifetime increased these enzymes quantitative and activity for this reason, we note the high mortality rate in the nymphs second instar and a decline in adults.

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Table (1) Adults and nymphal mortalities of the Brown-Banded, *Supella longipalpa* for different periods post treated by direct contact with spore suspension concentration 1 x 10^7 spore/ml of fungus *Beauveria bassiana.*

The stage	Mortality / Days			L.S.D. value
	3 days	5 days	7 days	
Adults	28.57	35.71	53.51	9.702*
Nymph second instar	57.14	79.31	93.10	8.612*
Nymph fourth instar	55.17	68.96	82.76	8.694*
L.S.D. value	8.315*	10.339*	10.963*	

* (P<0.05)

Table (2) Adults and nymphal mortalities of the Brown-Banded, *Supella longipalpa* for different periods after exposure by the conidial suspension was applied on bait concentration 1 x 10^7 spore/ml of fungus *Beauveria bassiana*.

The stage	Mortality /Days			L.S.D. value
	3 days	5 days	7 days	
Adults	20.69	31.03	37.93	9.215*
Nymph second instar	53.57	57.14	64.29	6.784*
Nymph fourth instar	31.03	44.82	58.62	8.966*
L.S.D. value	8.139*	7.502*	8.940*	

* (P<0.05)

Table (3) Adults and nymphal mortalities of the Brown-Banded, *Supella longipalpa* for different periods post treated by direct contact with spore suspension concentration 1 x 10^8 spore/ml of fungus *Beauveria bassiana*.

The stage	Mortality / Days			L.S.D. value
	3 days	5 days	7 days	
Adults	46.43	64.29	78.57	8.967*
Nymph second instar	64.29	85.72	100	8.31*
Nymph fourth instar	62.07	79.31	93.10	8.457*
L.S.D. value	8.562*	7.869*	7.240*	

* (P<0.05)

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Table (4) Adults and nymphal mortalities of the Brown-Banded, *Supella longipalpa* for different periods after exposure by the conidial suspension was applied on bait concentration 1 x 10^8 spore/ml of fungus *Beauveria bassiana*.

The stage	Mortality / Days			L.S.D. value
	3 days	5 days	7 days	
Adults	31.03	41.38	51.72	7.094*
Nymph second instar	57.14	64.29	78.57	8.144*
Nymph fourth instar	48.28	58.62	72.41	8.695*
L.S.D. value	7.635*	8.933*	7.625*	

* ($P<0.05$)

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