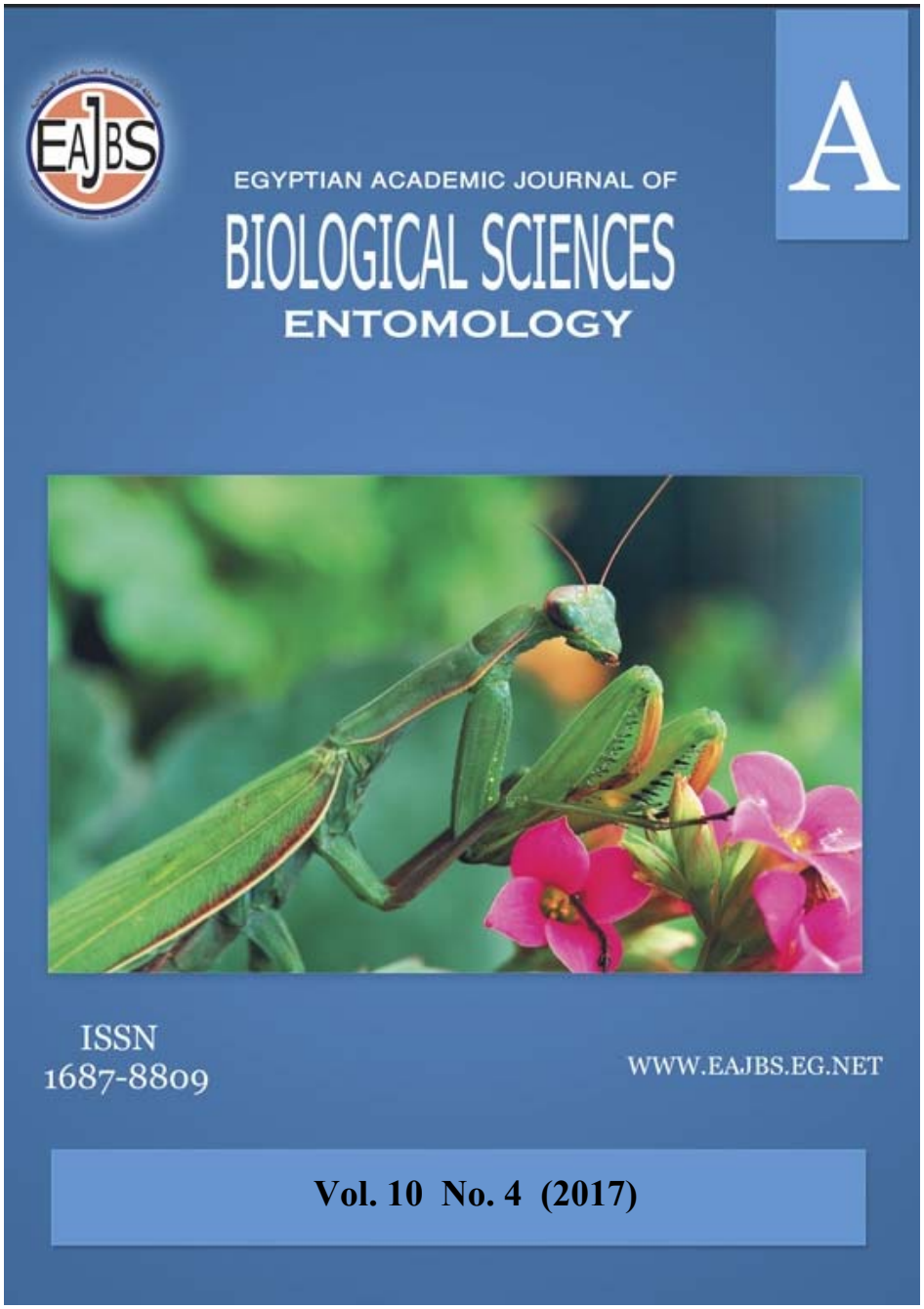

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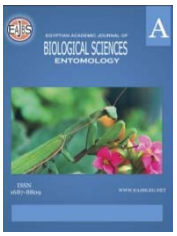

Evaluation the efficiency of mixing some oils with entomopathogenic fungus *Beauveria bassiana* against *Aphis craccivora*

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ABSTRACT

The entomopathogenic fungus, *Beauveria bassiana*, is a commonly used mycoinsecticide. Meanwhile, *B. bassiana* is highly susceptible to the damaging effects of solar radiation (specially UV) which in turn reducing its insecticidal efficacy. This report presents initial results from laboratory studies aimed at assessing the potential of oil formulations to enhance efficacy of *B. bassiana* against aphid pests. Four oil formulations are used under investigation in this study; orange, star, sunflower, and soybean oil. Aphid population reduction % were determined 3, 5, 7, and 10 days after treatment using Abbott's formula. The results revealed that oil formulations have significantly enhanced the aphicidal efficacy of *B. bassiana* by about 1.6 folds compared to aqueous preparations. This improvement may be contributed to the increased adhesion of the fungus on the insect's cuticle and homogenous distribution of fungal spores on the leaf surface as well as providing a protecting layer against UV-light.

In conclusion, oil formulations (horticulture /mineral oils) are one of the most promising technologies for enhancing efficacy of the entomopathogenic *B. bassiana* against aphids. mixing orange oil with *B. bassiana* has exerted a considerable mortality percentage against *A. craccivora.*

INTRODUCTION

It is well know that cowpea aphid, *Aphis craccivora*, infest a wide range of crops. It causes serious damage, either by sucking plant juices or indirectly as vectors of viral diseases (Atiri *et al*., 1984). The honeydew excreted by aphids on the leaves traps dust particles and is a substrate for the development of several species of black fungi. Such damage reduces the normal growth of plants (Takeda *et al*., 1982). Affect the production of flowers and fruits, consequently reducing quality and quantity of final yield (Tantawy, 1985). *A. craccivora* also prefer to feed on new foliage in the tops of plants, where they may be exposed to ambient environmental conditions that are unfavorable for fungal infection. Aphids also have exceptionally smooth, waxy cuticles, characteristics that tend to promote dry conditions on the cuticle surface (Wraight *et al*., 2016).

In Egypt, chemical insecticides are commonly used to control aphids.

Meanwhile, it is well known that chemical insecticides have a bad impact on the ecosystem as well as on humans and animals health. Resistance development against chemical control is also of increasing concern. As a consequence of these attributes, successful aphid control with microbial control agents, entomopathogenic fungi, may be the most practical for controlling sucking insect pests like aphid because they have a contact mode of action; infecting through the cuticle rather than requiring ingestion (Tanada & Kaya, 1993; Leland & Behle, 2005). *Beauveria bassiana* is a well-known entomopathogenic fungus which considered the important factor of insect population reduction (Hirose *et al*., 2001).

However, Hafiz *et al*., 2014 has demonstrated that *B. bassiana* has markedly increased the mortality percentage against aphids. A direct evidence was recently provided that the entomopathogenic is highly susceptible to the damaging effects of solar radiation (Leland & Behle, 2005). Moreover, some of the most important environmental factors reducing mycoinsecticide efficacy are inadequate moisture for infection and sporulation; temperature effects on survival, infection and disease development; and degradation of spores by solar radiation, which may in part be mitigated by formulation (Burges, 1998). Development of efficient formulation and delivery systems associated with oils is one of the most promising technologies for enhancing efficacy of fungal pathogens against aphids. Oil formulations can improve infection at low humidity (Bateman *et al*., 1993), thermal stress tolerance (McClatchie *et al*., 1994; Hedgecock *et al*., 1995; Hong *et al*. 2000; and Leland and Behle 2005), survival under solar radiation (Alves, Bateman, & Leather, 1998), and efficiency of application to large areas with ultra-low volume application (Burges, 1998).

Therefore, this study was designed to address the potential use of oil formulations in improving the insecticidal efficacy of *B. bassiana* against aphid (*A. craccivora)* in vitro. Different oil suspensions namely; orange, mineral oil (star oil), sunflower, and soybean oil were tested.

MATERIALS AND METHODS

Experimental insect

Field collected aphids, *A. craccivora*, were released in cage (75x50x50 cm) containing potted faba bean plant at 25±2ºC with a 14 h daily photoperiod. Then, they were used for assessment of bioefficacy of mixing of different oils with *B. bassiana* under laboratory conditions.

Preparation of fungal inocula

B. bassiana conidia were obtained in the form of commercial product, a clay-based wettable powder (Bio-Power®, T. Stanes Limited, India). Five formulations were prepared as shown in Table (1). pH of all formulations were measured using a pH meter.

Table 1: Experimental grouping and their formulations

S	Name	Symbol	Formulation
1	*B. bassiana*	Bb	2gm *B. bassiana* powder/1000 ml D.W
2	*B. bassiana*+ orange oil	Bb+Org	4ml orange oil (prev Am 6%)+ 2gm *B. bassiana* /1000 ml D.W
3	*B. bassiana*+ star oil	Bb+Str	4ml mineral oil (star oil 98%)+ 2gm *B. bassiana* /1000 ml D.W
4	*B. bassiana*+ sunflower oil	Bb+SunF	4ml sunflower oil+ 0.5ml triton X+ 2gm *B. bassiana* /1000 ml D.W
5	*B. bassiana*+ soybean oil	Bb+Soy	4ml soybean oil+ 0.5ml triton X+ 2gm *B. bassiana* /1000 ml D.W

Bioassay protocol

The bioassay protocol was performed with little modification according to (Wraight, Filotas, & Sanderson, 2016). Briefly, bioassay chambers comprised 10 cm diameter Petri dishes with plant leaves. A total fourty-eight dishes with 10 adult aphids each were used in this study. Twenty-four dishes were divided into six groups (4 dishes each). Group I, II, III, IV, V, and VI were sprayed at rate 0.02 µl suspension/mm2 of leaf surface with water only, Bb, Bb+Org, Bb+Str, Bb+SunF, or Bb+Soy, respectively. The other twenty-four dishes were also divided into six groups treated with water only and same prepared formulations mentioned above but by dipping. Then, all dishes were covered with ventilated lids and incubated at 25 ±2ºC and 65% ±5 RH with 14 h daily photoperiod. Aphid mortalities were recorded 3, 5, 7, and 10 days post-treatment.

Statistical analysis

Aphids mortalities were analyzed with corrections for estimated water formulation as control mortalities using Abbott's formula (Abbott, 1925). Analyses of variance (ANOVA) were conducted using the SPSS version 21.0 for windows (SPSS Inc,Chicago, IL) followed by Tukey's HSD test as a post hoc. Significance was considered when $P<0.05$.

RESULTS AND DISCUSSION

In the current study, various *B. bassiana* oil formulations have been tested in against aphid under laboratory conditions. Our data illustrated in Fig.1 represent the aphicidal efficacy of *B. bassiana* formulations by direct spray method. The percentage of reduction of *A. craccivora* was calculated after 3, 5, 7, and 10 days after treatment. The obtained data revealed that the highest percentage of reduction was noticed after 3 days (30.77%) caused by Bb+Soy and the lowest percentage of reduction was 20.51% caused by Bb.

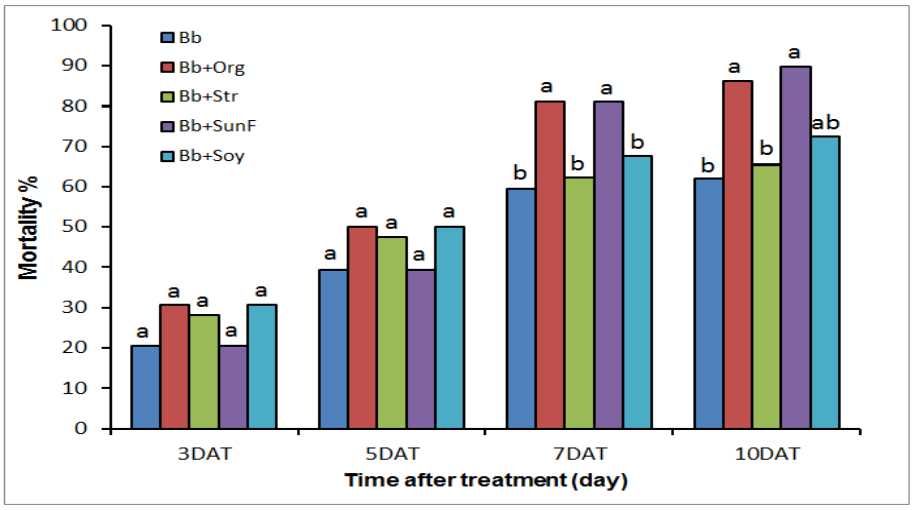

Fig.1: Aphicidal efficacy of different *Beauveria bassiana* formulations by spray method:
Aphid mortalities were evaluated 3, 5, 7, and 10 days after treatment (DAT) by spraying with *B. bassiana* formulated in different carriers; water (Bb), orange oil (Bb+Org), star oil (Bb+Str), sunflower oil (Bb+SunF), and soybean oil (Bb+Soy). Mortality data were adjusted for carrier-control mortality using Abbott's correction.
Values at each time-point have same letters are not significantly different.
(Tukey's HSD, $P< 0.05$).

While, after 5 days the highest percentage of reduction was 50.00% caused by Bb+soy and the lowest percentage of reduction was 39.47% caused by Bb. After 7 days, the highest percentage of reduction was 81.08% observed in Bb+Org and Bb+SunF and the lowest percentage of reduction was 59.46% caused by *B. bassiana*. In the same field, Ramanujam *et al*., 2017 mentioned that *B. bassiana* gave 60% mortality. After 10 days, the highest percentage of reduction was 89.66% caused by Bb+SunF and the lowest percentage of reduction was 62.07% caused by Bb.

As shown in Fig.2 represented the aphicidal efficacy of *B. bassiana* formulations by dipping method. The percentage of reduction of *A. craccivora* was calculated after 3, 5, 7, and 10 days after treatment. The obtained data revealed that the highest percentage of reduction was noticed after 3 days (41.03%) caused by Bb+Soy and the lowest percentage of reduction was 23.08% caused by Bb. While, after 5 days the highest percentage of reduction was 50.00% caused by Bb+soy and the lowest percentage of reduction was 39.47% caused by Bb. After 7 days, the highest percentage of reduction was 86.49% observed in Bb+Str and the lowest percentage of reduction was 51.35% caused by Bb finally. After 10 days, the highest percentage of reduction was 86.21%caused by Bb+Str and the lowest percentage of reduction was 51.72% caused by *B. bassiana*.

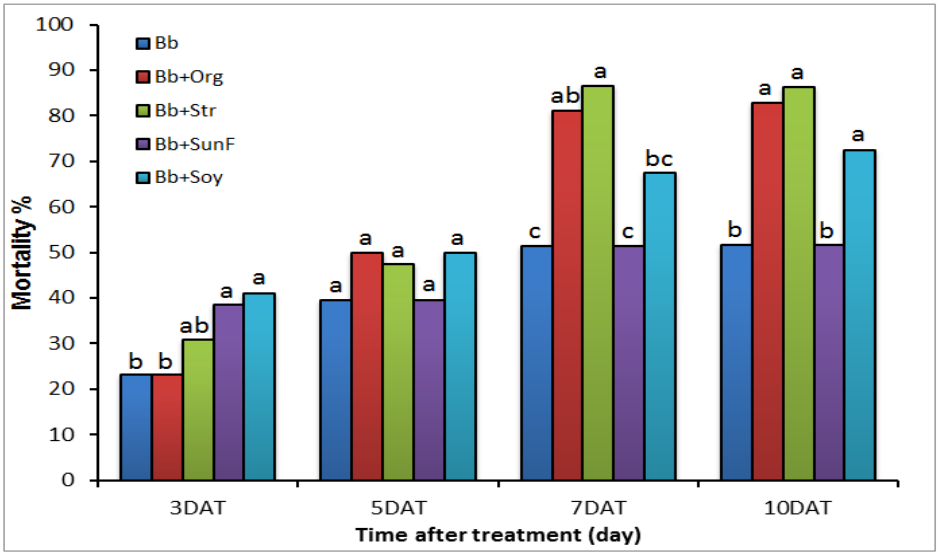

Fig. 2: Aphicidal efficacy of different *Beauveria bassiana* formulations by dipping method:
Aphid mortalities were evaluated 3, 5, 7, and 10 days after treatment (DAT) by dipping with *B. bassiana* formulated in different carriers; water (Bb), orange oil (Bb+Org), star oil (Bb+Str), sunflower oil (Bb+SunF), and soybean oil (Bb+Soy). Mortality data were adjusted for carrier-control mortality using Abbott's correction.
Values at each time-point have same letters are not significantly different.
(Tukey's HSD, $P< 0.05$).

All above-mentioned data indicating that oil formulation of *B. bassiana* have increased the mortality rate of aphids than commercial product used. Our results are in complete agreement with the previous studies which demonstrated the improved insecticidal efficacy of different oil formulation from entomopathogenic fungi against some pests (Osborne & Landa, 1992; Malsam *et al*., 2002; and Wraight *et al*., 2016).

B. bassiana proved to be effective in the control of aphid. Good control may be achieved by adding oils at low concentrations to the entomopathogen. In several studies, insect control by entomopathogenic fungi could be enhanced by the addition

of oils (Inglis *et al*. 1996; Fargures *et al*., 1997; and Wraight *et al*., 2016). Accumulating studies have explained the reasons regarding the improved efficacy of oil formulations in comparison to commercial product used. Firstly, the combination with oils improves the efficacy of biocontrol agents by promoting adhesion of fungi on the insect's cuticle (David-Henriet, Pye, & Butt, 1998) and by protecting them from both, UV-light and rapid desiccation (Bateman *et al*., 1994). It acts also by reduction of evaporation which help increasing the probability of spores finding favorable conditions for germination on the insects (Pas *et al*., 1998). It has been reported that oil formulations enhance efficacy of spores especially at low relative humidity, so entomopathogenic fungi can be used under climatic conditions, which are less suitable for these antagonists (Bateman *et al*., 1993; Kooyman & Godonou, 1997). Malsam *et al*., 2002 have concluded that the application of spore suspensions without oil to the leaves resulted in large droplets next to dry areas, accordingly some individuals were not affected. The addition of oil guaranteed a homogenous distribution of spores on the leaf surface. An improved distribution of spores resulted in an enormous increase in efficacy.

Moreover, it is well known that weak alkaline medium is the most favorable environment for fungal growth (Baker & Scher, 1987). In the present study, the pH of *B. bassiana* solution was 8.2 and *B. bassiana* formulation with some tested oils (Bb+Org, Bb+Str, Bb+SunF, and Bb+Soy) were (7.3, 7.3, 7.2, and 7.4, respectively) suggesting that oil formulations have increased the efficacy of *B. bassiana* via enhancing its growth. This finding supported that the data obtained by (Baker & Scher, 1987).

CONCLUSION

The current study demonstrated that using orange, star, sunflower, and soybean oil formulations have improved the insecticidal activity of *B. bassiana* more than commercial product. Based on this data and previous studies, oil formulation is one of the most promising technologies for enhancing efficacy of fungal pathogens against aphids.

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ARABIC SUMMARY

تأثير إضافة بعض الزيوت على فاعلية فطر البيوفاريا ضد مَن البقوليات

سامية منذر ابو زيد

قسم بحوث أفات الخضر والنباتات الطبية و العطرية و الزينة – معهد بحوث وقاية النباتات – مركز البحوث الزراعية

إن فطر البيوفاريا شائع الاستخدام فى مكافحة المن و لكنه يتاثر بأشعة الشمس و التى تؤثر فى كفاءتة فى خفض تعداد الأفه و لهذا فان هذه الدراسة المعملية تهدف الى زيادة فاعلية فطر البيوفاريا فى خفض تعداد المن عن طريق اضافة بعض الزيوت البستانيه. فى هذه الدراسة تم اضافة اربعة من الزيوت المختلفة الى فطر البيوفاريا و هى زيت الاورانج ، زيت معدنى خفيف(ستار اويل)، زيت عباد الشمس و زيت الفول الصويا. و تم حساب نسبة الخفض فى التعداد عن طريق معادلة أبوت بعد ٣، ٥، ٧ و١٠ ايام من المعاملة .و تشير هذة الدراسة الى ان اضافة الزيوت اعطت نسب معنوية فى خفض تعداد المن بمعدل يصل الى ١.٦ضعف مقارنة بالمستحضر المائي فقط .وهذا التحسن يمكن ان يعزى الى الزيادة فى قدرة التصاق الفطر بسطح الحشرة و ايضا عن طريق التوزيع المتجانس لجراثيم الفطر على اسطح اوراق النباتات و الذى يساعد على اصابة اكبر عدد ممكن من افراد المن .كما و جد ان اضافة الزيت يعمل كطبقة واقية للفطر ضد اشعة الشمس و العوامل الجوية الاخرى.

التوصية: تعتبر إضافة الزيوت (نباتيه-معدنيه خفيفه) من الطرق التكنولوجية الحديثة الواعدة لزيادة كفاءة فطر البيوفاريا ضد المن. و يوصى باضافة زيت الاورانج حيث انه اعطى تحسن مقبول فى نسبة الموت.