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Open Access Journal of Mycology & Mycological Sciences
ISSN: 2689-7822

Biocidal Effect of Essential Oil of Verbena Officinalis on the Black Bean Aphis Fabae

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Research Article

Volume 6 Issue 1

Received Date: September 28, 2023

Published Date: December 29, 2023

DOI: 10.23880/oajmms-16000180

Abstract

The polyphagia and the great capacity of reproduction of the aphids, arouse the use of several control methods, among others, essential oils which constitute a good alternative to the fight chemical and one of the most explored pathways in the biological regulation of pests. The aim of the study is to estimate the insecticidal power of a bio product formulated from an oil bitter *Verbena officinalis*, on the reduction of fertility and population density of different biological forms of the black bean aphid Aphis fabae. The temporal effectiveness of the formulated bio product was estimated by evaluating the rates of residual populations Methods: The essential oil is extracted by hydrodistilation from *Verbena officinalis* leaves. After formulation, two doses were recommended (D=0.4g/l and DD=1g/l), and two treatment methods were used (foliar application and root absorption) on broad bean plants infested by Aphis fabae with the aim of highlight the insecticidal capacity of the essential oil of *Verbena officinalis*. The results showed that the two treatment modes have a very marked effect on the density of Aphis fabae where the dose parameter has little influence on the aphicidal activity of the essential oil of *Verbena officinalis*. On the other hand, the treatment by root absorption acts more on the residual populations and the fecundity of the black bean aphid compared to foliar application, the Biocidal potential of which is accentuated with increasing doses. The formulation has optimized the effectiveness of the essential oil of *Verbena officinalis*, which resulted in its high toxicity, the most effective root application of which.

Keywords: Aphis Fabae; Bio formulation; *Verbena Officinalis*; Essential Oil; Toxicity

Introduction

The broad bean is the culture that has been doing for a long time part of the Algerian agrarian systems in different agro-ecological zones of the country. She is the most important among the food legumes with an area of 34617.1 hectares. Its average production national annual is 338062.8 quintals, and an average yield of 8.56 quintals/ha [1,2]. In plus abiotic constraints (cold, frost, heat and salinity), the beans are exposed the harmful effects of weeds, fungal and viral diseases, nematodes and finally insects [3].

Among the bean-dependent insects, the aphids occupy a very special place [4]. They are considered among the insects the most important pests inducing significant economic losses due on the one hand to their direct damage from food intake causing the weakening of the plant, flower abortion, leaf roll and leaf fall reducing area photosynthetic and the desiccation of shoots [5]. On the other hand, their indirect damage causes the establishment of a fungus genus *Fumago* growing on honeydew excreted by aphids inducing the formation sooty mold and plant virus transmission [6].

Chemical control of aphids poses often problems due to the fact that these insects usually attach to the underside of leaves and that they are difficult to reach by treatments Sauvion N [7] therefore, the use very spread of these pesticides has resulted in the emergence of forms of resistance among treated insects [8]. The search for means limitation of the use of these insecticides dangerous takes more and more of importance. To this end, many works recent studies have focused on the search for natural substances with powers insecticides and respectful of human health and the environment. Whether in the developed or developing countries, essential oils currently hold an important place in the control systems representing an alternative solution for the crop protection [9].

However, to improve the performance active ingredients, guarantee the stability and increase the effectiveness of these phytopesticides in particular by allowing a reduction in use doses thus limiting their impact on the fauna and flora, the use of the formulation of essential oils as natural molecules of ecological and economic interest to insecticidal properties turns out to be a step crucial and very promising and is no longer to demonstrate in research phytopharmaceuticals [10]. It is in this context that we propose to ourselves by this work to assess the potential aphicides of a bioproduct formulated from the oil essential of *Verbena officinalis* (1753) regarding the black aphid of *Aphis fabae* Scop. (1763), and for this we tried to meet the following hypotheses: Is what the method of foliar application and root absorption influence the activity aphicide of the bioproduct? Does the concentration of the active principle of the formulation impacts the aphicidal activity of bioproduct? The performance of the bioproduct is visible on the reduction of infestations or on the disruption of the potential biotic of *Aphis fabae*?

Materials and Methods

The work began in the laboratory with the extraction of the essential oil of *Verbena officinalis* then in the field at the experimental station of the Faculty of Nature and Life Sciences of the University of Ghardaia in order to test the different treatments and evaluate their biocidal power on the black bean *aphid Aphis fabae*.

Biologique material

Material vegetal spontane

The leaves of *Verbena officinalis* were collected during the spring season between 29/02/2022 *Verbena officinalis* sampled was identified by reference to the specimen.

In a greenhouse, a sowing of seeds pre-germinated from the bean *Vicia fabae*. Was carried out in cells filled with peat. After emergence and at the 4-leaf stage, 198 seedlings were transplanted into plastic bags of 1300 ml capacity, filled with a mixture of peat and earth then placed on a black plastic mulching to avoid any weed development. The containers have openings for drainage at their base allowing evacuation of the amount of water surplus. Spacing is 50cm between the rows and 25 cm between the seedlings. Culture is conducted without any insecticide treatment and without fertilization. Throughout the trial period, a temperature fluctuating between 24 and 37°C with a relative air humidity of 75 to 89% have been registered. Seedlings were irrigated regularly according to the cultural needs.

Biological animal

Aphis fabae adults have been obtained from the colonies established on broad bean crops grown under cover greenhouse with a view to artificial infestation of seedlings bean to be processed.

Study methods

Distillation of essential oils

The distillation of oil essential was carried out by the hydrodistillation technique on a Clevenger-type apparatus according to method recommended in the European Pharmacopoeia [12]. At during each test, 100 g of dried leaves have been processed for a period of 45. Oil essential was stored at 4°C in darkness.

Activity bioassays

Insecticide from essential oils of bitter *Verbena officinalis*

In order to optimize the activity Verbena officinalis essential oil, we opted for a liquid formulation, with reference to Barr [13] which states that much attention has been focused on the wetting because of their stability of storage, good miscibility with water and of practical application. The formulation consists of mixing a surfactant and a co- plant-based surfactant with oil essential of the Verbena officinalis tree as an active ingredient [14].

Establishment of treatments

In order to assess the effectiveness of bioproduct formulated on the *black aphid* of the bean for the two modes of administration of treatments (foliar application and absorption root), two dilutions were respectively prepared, namely a dose complete (D) containing 0.5g of the formulation mother (10% essential oil + 90% bioadjuvants) diluted in 1L of running water and a double dose (DD) composed of 1g of the parent formulation (10% essential oil

+ 90% bioadjuvants) also diluted in 1 L of water.

The control being a blank formulation (without active ingredient), the doses of which are at the same concentrations than those of the treaties.

Experimental apparatus

The test is carried out in block factorial. The experimental device is composed of 40 experimental units relating to the eight treatments of the two factors (foliar application with two treatments D and DD, absorption root with two treatments D and DD, control foliar with two treatments D and DD and the root control with two treatments D and DD) performed in five repetitions. Each treatment contains 20 bean plants, for a total of 800 seedlings. Foliar application was carried out by a manual sprayer while the application root was carried out per container of 200ml to deliver the same amount of treatment to all bean plants. The monitoring of *Aphis fabae* populations has been driven over a period of 11 days Technical sampling

The method consists of taking randomly three rods 20 cm long each block before the application of treatments then renews the samples at a 24h time interval after their application with the aim of monitoring the incidence of the latter on the evolution of the availability black aphid and their density. Furthermore the different biological forms will be estimated i.e. the number of young larvae (L1 and L2), older larvae (L3 and L4), pupae as well as winged and flightless adults.

Density estimation aphid populations bean black

Using a binocular loupe (G×40), the different biological forms of *Aphis fabae* have been identified and counted after each sample.

The density is estimated by the ratio of the number of aphids per rod/ the length of the rod, and is expressed in number of individuals per cm.

Estimate of residual populations of black bean aphid

The effect of the treatments applied has was estimated by comparing the populations residual (P.R.) according to the DUNNETT test [15].

P.R. = Number of mobile shapes (NFM) per treatment x 100/ Number of mobile forms (NFM) by control (water).

With: P.R. <30% toxic molecule, 30% < P.R <60% molecule average toxic, P.R > 60% neutral molecule or weakly toxic.

Estimate of adult fertility black bean aphid Fertility is estimated by the ratio of the number of larvae to the number adults, according to the formula

FEC = NL/NF

With:

NL: number of larvae

NF: number of females

Statistical analyzes of data

The statistical analysis concerned evaluation of the insecticidal activity of a bioproduct formulated with essential oil of *Verbena officinalis* on the different forms biological and biotic potential of *Aphis fabae*. Variance analyzes are done on homogeneous averages adopted on the based on a coefficient of variance (C.V. <15%).

The significance of comparisons of averages was confirmed by a test of pairwise comparison (ANOVA test and the Newman-Keuls). Contributions significant and marginally significant retained are respectively at the threshold of a probability of 5% and 8%, the calculations were produced by the XLSTAT software verse [16,17].

		L1-L2	L3-L4	Apterous	Winged	Nymphs
		F=36,746, p<0,0001	F=27,527, p<0,0001	F=18,104, p<0,0001	F=9,653, p<0,0001	TF=17,337, <0,0001
Foliar application	TF	21,97±1,41a	17,73±1,31b	11,00±1,09a	7,39±0,59a	3,61±0,47a
	DF	15,80±3,23b	12,36±1,96bc	9,36±1,24b	6,14±0,86b	2,78±0,59b
	DDF	15,16±1,94 b	11,43±1,15 c	8,20±0,86 b	5,65±0,77 b	2,40±0,22 b
Root absorption	TF	46,11±1,53 a	31,43±1,70 a	20,31±1,17 a	11,22±0,36 a	6,63±0,40 a
	DF	18,85±2,81 b	13,15±1,58bc	11,55±1,23 b	6,88±0,71 b	2,33±0,34b
	DDF	15,03±3,40 b	12,45±2,03bc	11,67±1,05b	6,39±0,71b	4,12±0,40 b

Table 1: The number of young larvae (L1 and L2), older larvae (L3 and L4), pupae as well as winged and flightless adults.
Tf: Foliar control, **Df:** Foliar dose, **DDf:** Double foliar dose, **Tr:** Root witness, **Dr:** Root dose**, DDr:** Double root dose

Temporal variation of population's residual Aphis fabae under the effect of formulated bioproduct

Results relating to variations of residual populations of Aphis fabae under the effect of the different doses formulated bioproduct provided by foliar application and root absorption, clearly show greater toxicity *Verbena officinalis* essential oil administered by root absorption compared to that assigned by foliar spraying and this for the entire follow-up period.

However, the toxicity of foliar application remains average for the two doses tested (Dose) and (Double Dose) with rates of respective residual populations: (50% ≤ PR ≤60%) and (40% ≤ PR ≤ 60%) and that of the 2nd until the 7th day after treatment where the loss of toxicity is displayed (PR ≥ 60%). On the other hand, for root absorption, the double dose (DDR) shows itself moderately toxic during the first two days (40%≤ PR≤50%), highly toxic from 3rd to 6th (20%≤ PR≤ 30%) then becomes moderately toxic until the end of follow-up while the dose remains more or less toxic throughout the follow-up period.

Residual populations of *Aphis fabae* do not show any significant difference in terms of the effect of doses of the bioproduct applied by route leaf (p>5%). On the other hand, under the effect of application by root absorption, the formulated bioproduct expresses an activity very significant aphicide which increases gradually depending on the concentration of the active ingredient.

The Newman-Keuls Post-Hoc test designates the presence of 2 homogeneous groups relating to bearings effectiveness of the bioproduct formulated with of *Verbena officinalis* essential oil. The first landing is signaled through the application of the dose (0.5g/l) revealing the population rate largest residual, affiliated with the group homogeneous (a). The second stage is remarkable for the application of the double dose (1g/l) showing population rate lowest residual, affiliated to the group homogeneous

Variation in the fecundity of *Aphis fabae* under the effect of the formulated bioproduct

To highlight the effect bioproduct disruptor formulated with bitter orange essential oil *Verbena officinalis* on black aphid offspring of the bean, we estimated the potential biotic by fertility assessment. By comparison of adult fertility exposed to different treatments fertility of adult controls, we find that the two modes of treatment (foliar and root) have a certain effect on the fecundity of the black aphid with an efficiency progressive in time and a supremacy of action of the essential oil formulated administered by root absorption and its double dose compared to the tested dose About the mode of administration factor formulated bioproduct.

The analysis of variance considers that the foliar route does not affect the fecundity of *Aphis fabae* (p>5%). However, the root uptake pathway, significantly pontificates biotic potential black bean aphid. The test results of Newman-Keuls reported show the presence of 2 homogeneous groups of efficiency (a and b), of which the highest fertility marked is allocated to the witness thus forming the homogeneous group (a), hence the homogeneous group (b) contains simultaneously the dose and the double dose whose fertility is less important.

Discussion

The results relating to the potentialities oil-based formulated bioproduct aphicides essential oil of bitter orange showed effectiveness on infestation rates of different biological forms as well as on the biotic potential of adult forms. By reference to the processing methods (foliar application and root absorption) and at the concentrations of the bioproduct (dose and double dose), population fluctuations of *Aphis fabae* evolve in the same trajectory with strong toxicity by compared to those of the witnesses. Both Modes of treatment showed a decrease remarkable population density of the black bean aphid. Likewise, the results relating to population fluctuations residual under the effect of the formulation of *Verbena officinalis* essential oil, a similarity of action on the reduction of populations residual biological forms of the aphid black of the bean was observed. The double dose root absorption leads to toxicity higher compared to the root dose and at foliar application rates. The suppressive effect of the essential oil formulated of *Verbena officinalis* joins the numerous studies who made an inventory of the quality of the components of essential oils. So our results are confirmed by those found by Belhadi R, et al. [18] who showed that the toxicity of *Verbena officinalis* essential oil formulation is dose-dependent indicating that the greater the dose increases the more the formulation presents a greater biocidal effect which results in reduction in population density aphids. Haubruge, et al. [19] showed than linalyl acetate (56.80%) whose concentration is the highest would be the active ingredient which plays a determining role in the biocidal activity of the essential oil of Sour orange. Isman [20] assumes that the oils essentials act directly on the cuticle soft-bodied arthropods since several essential oils seem more effective against this genus of arthropods and less effective with shelled insects hard such as beetles and Adult Hymenoptera [21]. The toxicity by contact with essential oils can be very high with LC50s of 9,5µg/cm^2.

Results

The temporal fluctuation of densities observed in populations of *Aphis fabae* under the effect of the bioproduct formulated with of *Verbena officinalis* essential oil shows a significant aphicidal effect including the reduction number signaled under the effect of the different treatments stands out clearly from the reduction reported in different controls. The aphicidal activity is influenced by the mode of supply of the bioproduct, and by consequently it seems to obey a gradient positive Foliar application<Absorption.

From ANOVA test results, we notice that the dose factor registers an effect significant on the reduction of densities of *Aphis fabae* for the treatment foliar (p<5%). The test of Newman's Post-Hoc multiple comparison- Keuls designates the presence of 2 groups homogeneous relative to the levels of activity aphicide of the bioproduct.

The activity aphicide remains less consequent by comparison to recorded aphicide activity by foliar application. The same test indicates that the reported global densities under the effect of the bioproduct (homogeneous group b) very significantly different from the densities global recorded in the control.

In order to estimate the impact of oil-based bioproduct essential of the *Verbena officinalis* tree on the different biological forms of *Aphis fabae*, we advanced the comparison test by pair for each life stage in depending on the mode of application factor and of the dose factor. The very significant differences registered between biological forms L1- L2, L3-L4, Nymph, wingless adults and adults from the results of the study, it appears that the foliar treatment of the bio product would have an effect of direct toxicity by contact on the aphid and indirect toxicity by system whereas the root treatment would only act by system. However, the essential oil chemo types formulated that have been absorbed by the plant are carried by the sap to the apical part where the sucking biting insect feeds, which could affect the quality of this sap in its composition and therefore conditions the taking systemic food by the insect. In general, essential oils are known as neurotoxins with acute effects interfering with transmitter's octopaminergic arthropods [22]. The work of Obeng-Ofori, et al. [23] have shown that certain components of the oils essential in contact with insects, act by blocking the synthesis of the hormone juvenile, they inhibit acetyl-cholinesterase by occupying the hydrophobic site of this enzyme which is very active. Toxic effects essential oils depend on the species insect, plant and time exposure [24]. The formulated essential oil of *Verbena officinalis* is effective against all life stages of insects, without mention of supremacy of stage-specific toxicity. Stamopoulos, et al. [25] pointed out that the susceptibility of insects to the actions of essential oils vary with age larval stages where the oldest larvae resist better and can tolerate strong essential oil concentrations, This would probably be explained by the size of the larvae rather than by difference in the mode action of the oil. According to Ngamo, et al. [26], an essential oil does not exert necessarily the same activity at different life cycle stages of the insect, such as there is a wide variation in the susceptibilities of insect species for a same essential oil. The work carried out by Ketoh [27], on the biological activity of different oils essential oils have shown that essentials of Ocimum basilicum (*Lamiaceae*) and Cymbopogon schoenanthus (*Poaceae*) presented an action on all stages of development of Callosobruchus maculatus (*Coleoptera: Chrysomelidae*). The results of the time evolution of the fecundity of *Aphis fabae* populations under the influence of the doses of the essential oil formulated with *Verbena officinalis* administered by foliar application and root absorption have shown that the root absorption mode at doses tested shows a suppressive effect by report to the witness. The reduction in fertility under the effect of treatments is probably due to an evolutionary strategy of the species in the face of stress caused by the application of the bioproduct. Indeed, according to the subject allocation theory energy, females devote their energy in the degradation of matter from active ingredient to the detriment of development ovarian. This hypothesis is supported by the works carried out by several authors, who demonstrated the action of oils essential on the demographic parameter of some pests. The reduction in fertility of females results from the reduction of the adult longevity [28]. According to Kellouche and Soltani [29], the reduction in fertility is not only related to the laying period or survival of adult females, but it can be also the result of a disturbance of the vitellogenesis process. The oils essential oils and their constituents exert insecticidal effects and reduce or disrupt the insect growth at different stages of their lives [30-32]. They have effects antiappetants, affecting growth, moulting, fertility and the development of insects and mites [33]. These products therefore have their place as tools plant protection in agricultural environments either by greenhouse or open field, by application topical [34-36] or ground [37].

Conclusion

This work was carried out within the framework of estimation of the toxicity of the essential oil formulated from *Verbena officinalis* by different modes of administration on the aphid population black bean *Aphis fabae* (1763). The results of this study seem to be interesting and confirm the power insecticide of the formulated essential oil of *Verbena officinalis* brought by different modes application on the black bean aphid. However, the toxicity of the treatments differs according to the mode of application of the bioproduct and dose concentration

The double dose administered by foliar route is more toxic on the density of the black aphid of the bean while that of the application root is more effective on residual populations of biological forms studied and fertility. Additionally, the assessment residual populations a showed early toxicity as early as 72 h applicable for all treatments applied.

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