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IMPACT OF TRADITIONAL AND NANO SIO_2 AGAINST *BEMISIA TABACI* AND *APHIS GOSSYBII* AND THEIR RESIDUES IN MINT AND THYME PLANTS.

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ABSTRACT

Silica provides expanded possibilities for use in horticultural crops. However, there are many crop pests on the lower surface of plant leaves. This is particularly challenging when silica is used because the material must have a direct contact with the insect to be effective. In this study, we evaluate the efficacy of two traditional forms for SiO2 and SNPs against two different pests Bemisia tabaci, Aphis gossybsii under laboratory conditions. LC50 concentrations were measured after 24, 48, 72 and 96 hours, respectively. The SiO2 residues of traditional and their Nano particles were studied in leaves of mint, thyme, stems and soil, as well as the effect of drying and boiling techniques in the pesticide removal were also studied.

The results indicated that *the cumulative mortality rate of white fly and cotton aphid increased with increased pesticide concentration and/or increased time. The highest value of mortality in the tested insects was recorded in 96 hours of traditional SiO2 application. In case of silica Nano particles (SNPs), the highest mortality rate of white fly values were observed at concentration of 2.50 µg/ml after 48 hours of treatment. While, the same mortality value was observed at 2.50 µg/ml after 24h of treatment for A. gossybsii. These results indicate that A. gossybsii was more susceptible to silica and Nano-silica particles SNPs than B. tabaci and that the Nano-silica particles SNPs are more effective than the traditional silica.*

Conclusively, *the results indicated that SiO2 of two forms plays the effective roles in integrated pest management and will be more effective when applied direct against white flies and aphis.*

Key words: Lethality, B. tabaci and A. gossybii, insects, SiO2, SNPs, mint, thyme

INTRODUCTION

To prevent loss of crop, green revolution provided the chemical pesticide to agriculture during 1930-1960 but indiscriminate use of synthetic pesticides like DDT, pyrethroids, methyl bromide, organophosphates and so forth generated environmental, health issue, resistance development in pest and detrimental impact on non-targeted organisms. *Bemisia tabaci* is a major pest of vegetable and ornamental crops of many regions and its control is based mainly on chemical insecticides. (Beard *et al*., 2003; Guillette and Iguchi, 2012; Mei *et al*., 2015 and Naqqash *et al*., 2016).

Bemisia tabaci is a serious economic pest of agronomic, horticultural and ornamental crops throughout warm regions of the world and have escalated over the past 25 years as new and more virulent biotypes have spread to all continents except Antarctica (Brown 1994; De Barro *et al*. 2005). Another researchers indicated that *B. tabaci* has tremendous potential to develop resistance to different insecticides. It is indicative that resistance to *B. tabaci* has been reported worldwide and to more than 40 active ingredients (De Barro *et al*., 2000; Horowitz *et al*., 2007).

Aphis gossypii Glover is a destructive pest of numerous crops throughout the world. Although the expansion of *Bacillus thuriengensis* (Bt) cotton cultivation has helped to control some insect pests, the damage from cotton aphids has not been mitigate. Severe attacks by *A. gossypii* may also reduce leaf area and biomass, besides branching and plant height, harming the crop and results in economic losses. (Sarwar *et al*. 2014).

Nano pesticide is small engineered structure which provides pesticide properties or formulation of active ingredient of pesticide in Nano form. In current decade, Nanotechnology showed large scope in different fields like medicine, electronics, catalysis, remediation and agriculture (Subramanyam *et al*. 2000 and Bulera *et al*. 2001).

The use of Nanotechnology in agriculture provided new tools in the form of Nano fertilizer, Nano pesticide and Nanosesnsor to conventional agricultural practices (Chhipa and Kaushik, 2015 and Chhipa and Joshi, 2016). Many researchers mentioned that nanoparticles have specific morphology, size, high surface area and high reactivity, which provide them high chemical, physical and optical properties. They also added these Nano structures have shown slow degradation and controlled release of active ingredient for long time. Nanoparticles can serve as “magic bullets”, containing herbicides, Nano-pesticide and fertilizers, which target specific cellular organelles in plant to release their content (Gonzales *et al*., 2015; Chhipa and Kaushik, 2015 and Chhipa and Joshi, 2016); (Gonzales *et al*., 2015); (Chhipa and Kaushik, 2015 and Chhipa and Joshi, 2016). Our study have been carried out to study insecticidal efficacies of traditional and Nano SiO_2 against two different pests, *Bemisia tabaci* and *Aphis gossybii* under laboratory conditions, and to determine the residues of traditional SiO_2 and its Nano particles SNPs in mint and thyme leaves, stems and soil.

Therefore, the present study may provide various research findings of usage of both chemical and Nano materials for pest management.

MATERIALS AND METHODS

1. *The insects used:*

Bemisia tabaci (Hemiptera: Aleyrodidae) and *Aphis gossypii* (Homoptera: Aphididae), were used in this study. *B. tabaci* infected leaves were collected from at least 10 different sampling spots at each site spots during the survey from agricultural greenhouses and open field crops adjacent to the educational administration building in El-kasasin, Ismailia Governorate, Egypt during the period from July to September 2018. After the insects collection, they were placed in a cool box within a few hours, and then transported to the conducted place for experiment, inside a glass greenhouse in the El-kasasin area. Also, *A. gossypii* used in this study was collected from two different hosts, kidney bean and cucumber plants.

Aphid colonies were maintained in separate in Petri dishes (7 cm diameter) and held at 24 ±2C, 65 ±10% relative humidity (RH) and 16 h of artificial light (approximately 7000 lux).

2. *Tested insecticide used*.

Commercial formulations of traditional silicon dioxide (SiO_2) at maximum label rate 200 g/L was used: The material has been used in regular and Nano form. The silica was obtained from MTM Company, Egypt. The SiO_2 Nanoparticles SNPs were prepared at the National Research Centre Lab., Cairo, Egypt.

3. *Experimental design*:

The experiment was conducted in a glass greenhouse at a private farm of El-kasasin, Ismailia Governorate, Egypt, during the period from July to September 2018, with two types of medicinal plants, namely *Mentha pulegium* (*M. pulegium*) and *Thymus vulgaris*, (*T. vulgaris*), as plant hosts.

Mint seedling were cultivated in plastic pots (150mminner diameter and 100 ml depth) with 500g soil, each pots contained 3 seedling plants The chemical fertilizers were added by 5g of urea (46.50%) dissolved in water and fertilized as irrigation four times throughout the experimental periods.

The experimental design consisted of 5 concentrations for traditional SiO_2 and 5 concentrations for Nano SiO_2 (SNPs) replicated three times. Each concentration contained 5 pots. Also 5 pots were used as control.

The concentration used for traditional silica were 1.56, 3.12, 6.12, 12.15 and 25.00 µg/ml, while the concentrations used for Nano Sio_2 were 0.156, 0.312, 1.25 and 2.50 µg/ml. The experiment was designed in randomized complete block design. The same experiment repeated with seedling of thyme. The experiment was irrigated every two days.

Collected leaves of plants tomatoes and pepper infected with white flies and cotton aphids on each leaf plant infected with both insects, and examined the upper and lower surface of the leaves of each plant.

The infected leaves of tomato or pepper were infected the untreated mint and thyme then leaved for several days to reach the infected near 75%. The samples of leave were collected from about 10 to 15 pots of each treated and untreated in polyethylene bags and separately in the laboratory using stereomicroscope after 24, 48, 72 and 96hrs of treatment; to study the dissipation of the tested materials forms.

The efficiency of traditional and Nano SiO_2 were tested against *B. tabaci* and *A. gossybii*, by calculating the lethal concentrations (LCs), 50% (LC_{50}). Toxicity index (T.1) were determined by using Sun`s equation (1950) as follows:

Toxicity index (T.I.) = LC_{50} of the compound (A) / LC_{50} of the compound (B) x100

Where: A: is the most effective compound. B: is the other tested compound.

The number of living insects was recorded and the reduction percentages were calculated according to Henderson and Teleton equations (1955).

$$\text{Corrected \%} = \left(1 - \frac{\text{n in Co before treatment} * \text{n in T after treatment}}{\text{n in Co after treatment} * \text{n in T before treatment}}\right) \times 100$$

Where : n = Insect population , T = treated , Co = control

4. Residues study:

200gm of fresh leaves, stems and soil were collected randomly from each treated and untreated plants for residue analysis . The soil samples were taken from each treatment and it was placed in cloth bags then brought to laboratory for analysis. Soil samples were pooled and sieved, and extraneous matter including stones and pebbles, was separated.

5. *Procedure of preparation and determination of silica.*

a. Plant sample preparation:

The plant material was dried at 105 ◦C and powdered to a particle size of less than 500 µm in diameter the method described by Neumann *et al*., (2011). The leaves samples were divided to two sub sample (each 100g) one sub sample for the determination of traditional SiO_2 and other for determination of Nano silica SNPs residue.

6. ***Determination of silica SiO_2:***

The determination was performed at 812 nm using a UV-1801 ultraviolet spectrophotometer instrument method according to (Hailong Yang *et al*., 2015). The determination of SiO_2 were determined by Standard curve of SiO_2 was prepared by (Thibaud Coradin *et al*., 2004).

7. *Preparation of silica Nano particles (SNPs):*

SNP_s were prepared and analysis in Consulting Unit, National Research Centre, Cairo, Egypt.

a. **Transmission Electron Microscope (TEM)** (Model: Model: 400 ELPC, SER. No: 04-01198, 200 volts, 50/60 HZ, 5 APMS, Oxford. CT, 06478, USA).

b. **Methodology and preparation:** Nanometer transformed by high speed homogenizer, PRO, Scientific Inc, Technical preparation conditions: High speed homogenization at 20000 rpm, for homogenization time20 min, Temperature at 25 C. Beam of electrons is transmitted through an ultra thin specimen and interacts as passes through the sample. An image is formed from the electrons transmitted through the specimen, magnified and focused by objective lens and appears on an imaging screen.

8. *Statistical analysis*:

Mortality data obtained from the assays were subjected to statistical analysis using the Probit method (Finney, 1964) using the R ® software. The LC_{50} value was determined, as well as their respective 95% confidence intervals values were determined by logistic regression based on 50-99 the concentration probit-mortality, with the program XLSTAT-PRO v.7.5 for Windows (XLSTAT 2004). Data from the controlled field test were statistically analyzed using analysis of variance (ANOVA) at 5% probability. Toxicity index (T.1) were determined by using Sun`s equation (1950).

RESULTS AND DISCUSSIONS

1. *Character of Nano Sio_2 (SNPs)*

Data in Figure (1) indicated that **Nano Sio_2 (SNPs)** reached up 85 nm indicated that this preparation in the range of Nano particle from 1 to 1,000 nm using Nanometer transformed by high speed homogenizer, PRO,

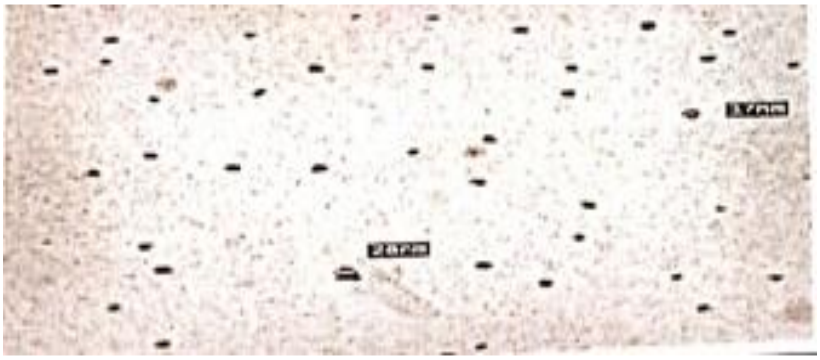

Figure (1): Transmission electron micrograph (TEM) of 85 nm diameter 99 % pure nanosilica.

Scientific Inc. According to International Organization for Standardization (ISO).

2. ***The median lethality dose (LC_{50}) of SiO_2 (SD) and its Nano-particles (SNPs).***

The toxicity of traditional SiO_2 and Nano particles SNPs agnist *B. tabaci* and *A. gossybsii* are shown in Tables 1 & 2 which Cumulative mortality of were increased as the compounds concentration and /or the time increased. The highest mortality value of *B. tabaci* and *A. gossybsii* was recorded at 96h after the SiO_2 from application. In case of SNPs highest mortality value of *B. tabaci* was recorded by concentration 2.50 ng/µl after 48h. But for *A. gossybsii* the same mortality value was observed with concentration 2.50 ng/µl after 24h.

The results lead to *A. gossybsii* was more susceptible for SiO_2 and SNPs than *Bemisia tabaci*. Also, SNPs were more effective than SiO_2. It could be concluded that the main cause of insect death is the dehydration caused by Nano-silica particles that affect or absorb the wax layer of insect cuticle and subsequently lead to loss of water from the body of the insect, and ultimately the death of insects due to drought and that was more or less similar to that description by (Ebeling 1971) and (Golob 1997). Also, results indicated NSPs were more effective on insect adults and this mortality could be attributed to the impairment of the digestive tract or to surface enlargement of the integument as a consequence of dehydration or blockage of spiracles and tracheas. Also, it refers to their enormously increased exposed surfaces which could interact with the insect cuticle. Damage occurs to the insects protective wax coat on the cuticle, both by sorption and abrasion. The use of nanomaterial products increased during

Table (1): Toxicity data of silicon dioxide in traditional and Nano form *B. tabaci*, at different time intervals.

Time in hours (h)	Traditional SiO_2				SNPs			
	LC_{50} (ppm) ± SE	Confidence limits at 95%	Slope	Toxicity index	LC_{50} (ppm) ± SE	Confidence limits at 95%	Slope	Toxicity index
24 h	9.22 ± 1.27	8.38-10-06	0.42	39.91	0.47 ± 0.0	0.42-0.52	0.26	38.29
48 h	4.32 ± 0.41	4.05-4.59	0.66	85.18	0.22 ±0.03	0.20-0.24	0.31	81.81
72h	3.98 ± 0.36	3.74-4.22	0.72	92.46	0.19 ± 0.02	0.18-0.20	0.34	94.73
96h	3.68 ± 0.32	3.47-3.89	0.80	100.00	0.18 ± 0.0	0.17-0.19	0.34	100.00

Table 2: Toxicity data of silicon dioxide in traditional and Nano form *A. gossybii*, at different time intervals.

Time in hours (h)	Traditional SiO2				SNPs			
	LC_{50} (ppm) ± SE	Confidence limits at 95%	Slope	Toxicity index	LC_{50}(ppm) ± SE	Confidence limits at 95%	Slope	Toxicity index
24 h	11.00 ± 2.38	10.08-1.92	0.25	26.54	0.29 ± 0.0	0.26-0.32	0.30	37.93
48 h	11.00 ± 2.38	10.32-1.68	0.25	26.54	0.15 ± 0.0	0.13-0.17	0.30	73.33
72h	2.97 ± 0.36	2.73-3.21	0.48	98.32	0.11 ± 0.02	0.097-.112	0.30	100.00
96h	2.92 ± 0.31	2.71-3.13	0.57	100.00	0.11 ± 0.0	0.097-.112	0.32	100.00

the last decade (Aitken *et al*., 2004) and is reported to interact with biological systems (Kreyling *et al*., 2002). Silica is one of the most abundant materials on earth. Silica Nanoparticles have several applications, including as pesticides in plant protection against agricultural pests (Barik *et al*., 2008; Gordon *et al*., 2009; (Ulrichs *et al*., 2006).

The results in Tables (1 and 2), clearly indicate that the silicon dioxide which has the particles size of 150 mm adhere poorly to insects and was found to be less lethal than those silica Nano particles (SNPs, 85 nm). Specific surface area of powdered materials is depending upon their particle size. Insects develop resistance to chemical insecticides using a variety of cuticular lipids to avoid death by desiccation (Ulrichs *et al*., 2006). However, (Neethirajan *et al*., 2009) reported that “surface charged, modified hydrophobic Nano silica by pass this defense by being absorbed into the cuticular lipids of the insects through physic sorption, causing the desiccation and death of the insect, which also supports our present findings where hydrophobic Nano silica was found more effective than hydrophilic and lipophilic Nano silica on mosquito vectors.

Generally, silica Nano particles SNPs would be effective to be applied for protection against those insect-pests under study and can be used at low concentrations considering the moisture content of commodities which affect the activity of silica. However, additional experiments are required to clarify silica Nano particles properties, their potential toxicity on different insect species, in various commodities and different environmental conditions. The concentration and time caused higher mortality rate of SNPs on *A. gossybii* and *B. tabaci* insects compared to silicon dioxide. After 72h 3.98 µg/ml in *B. tabaci* has shown higher effect recoded 92.46 % in silicon dioxide as compared to 0.19 µg/ml concentration was the most toxic recorded 94.73 % of SNPs. While in case of *A. gossybii* in (Table 2). After 72h 2.97 µg/ml in *A. gossybii* has shown higher effect recoded 98.32 % in silicon dioxide as compared to the 0.15 µg/ml concentration after 48h was the most toxic recorded 73.33 % of SNPs. Results of the present study indicated variable toxicity among different two types of silica on both the insects tested and the insecticidal effect of silicon dioxide and SNPs was in the order *A. gossybii* > *B. tabaci*.

3. *Residues of SiO_2 and its Nano particles (SNPs).*

a. Thyme leaves, stems and soil:

The obtained data Table (3) indicated that the initial deposits of SiO_2 and SNPs in thyme leaves were 1.125 and 0.140 µg/ml, respectively two hours after application. Samples of thyme leaves taken one day after spraying contained 0.588 and 0. 076 µg/ml with loss 52.96% and 45.71% of the initial amounts of SiO_2 and SNPs, respectively. Between days 1 and 14 after spraying a gradual decrease was observed in residues for SiO_2 and SNPs which reached 0.034 and 0.005 µg/ml with loss 96.98 and 97.88%, for SiO_2 and SNPs, respectively, 14 days after application. Data shown in the same Table demonstrated that the initial deposit found in and on stems, as determined two hours after application was 0.955 and 0.124 mg /kg for SiO_2 and SNPs, respectively. For SiO_2, the amount of residue was decreased sharply to 0.310 µg/ml with loss 67.54% within the first three days after spraying, at the same time the decrease reached 0.028 µg/ml with loss 77.45% for SNPs. This figure decreased gradually until reached 0.029, 0.002 µg/ml on leaves with loss 96.96 and 98.39%, for SiO_2 and SNPs,

respectively; of the initial residue, after 14 days of application. Also, the results in the same Table indicated that the initial deposits of SiO_2 and SNPs on thyme soil were 1.039 and 0.123 µg/ml, respectively two hours after application. Samples of thyme soil taken one day after spraying contained 0.514 and 0.062 µg/ml with loss 50.53% and 49.59% of the initial amounts of SiO_2 and SNPs, respectively. Between one day and 14 days after spraying a gradual decrease was observed in residues for SiO_2 and SNPs which reached 0.026, 0.003 µg/ml; respectively, 14 days after application; with loss 97.50 and 97.56%, for SiO_2 and SNPs, respectively.

***b. Mint leaves, stems and soil**:*

Data shown in Table (4) demonstrate the initial deposit and the residual behavior of SiO_2 and SNPs on mint leaves after spraying. The initial deposit found in and on leaves, as determined two hours after application was 1.011 and 0.127 mg/kg for SiO_2 and SNPs, respectively. within the first three days after spraying and it were decreased sharply to 0.176; 0.039 µg/ml with loss 82.59, 69.29%, for SiO_2 and SNPs; respectively. The results also, indicated decreased gradually until reached 0.045, 0.002 µg/ml of the residue in leaves with loss 95.55 and 98.42%, for SiO_2 and SNPs, respectively; after 14 days of application. The presented results in the same Table indicated that the initial deposits of SiO_2 and SNPs on mint stems were 0.823 and 0.097 µg/ml, respectively two hours after application. Samples of mint stems taken one day after spraying contained 0.547 and 0.048 µg/ml with loss 33.54 % and 50.53% of the initial amounts of SiO_2 and SNPs, respectively. Between one day and 14 days after spraying a gradual decrease was observed in residues for SiO_2 and SNPs which reached 0.017, 0.002 µg/ml; respectively, 14 days after application; with loss 97.93 and 97.94 %, for SiO_2 and SNPs, respectively.

Presented data in Table (4) indicated that the initial deposits of SiO_2 and SNPs in mint soil were 1.330 and 0.117 µg/ml, respectively. Samples of mint soil taken one day after spraying contained 0.527 and 0.056 µg/ml with loss 60.38 % and 52.14 % of the initial amounts of SiO_2 and SNPs, respectively. A gradual decrease was observed in residues for SiO_2 and SNPs, which reached 0.030, 0.003 µg/ml; respectively between days 1 and 14 day after spraying, at 14 days after application; with loss 97.74 and 97.43%, for SiO_2 and SNPs, respectively.

Degradation and dissipation residues of silicon dioxide from these two medicinal plants under study happened because the initial deposits and residues at different intervals of these pesticides are influenced by different factors: evaporation of the surface residue, which is dependent on

temperature condition, biological dilution which is dependent on the increase mass of plants, chemical or biochemical decomposition, metabolism and photolysis. Great interest to note that the same factors were studied by several investigators, (Christensen and Mather 2003) they reported that the decline of pesticides may due to biological, chemical or physical processes, or if still in the field, due to dilution by growth of the crop. Plant growth, particularly for fruits is also responsible to a great extent for decreasing the pesticide residue concentrations due to growth dilution effects (Walgenbach *et al*., 1991).

In addition, the rapid dissipation of originally applied pesticide is dependent on a variety of environmental factors such as sunlight and temperature. However, high temperature is reported to the major factor in reducing the pesticides from plant surfaces. Light plays an important role in the behavior of pesticide in the environment (Zepp and Cline, 1977).

CONCLUSION

The results suggest that SiO_2 and its Nano form SNPs, can make a valuable contribution to integrated pest management and will be most efficacious when directed against *B.tabaci* and *A. gossypii*. The possibility that these insecticides may exhibit lethal or sub lethal effects on the natural enemies of *B.tabaci* and *A. gossybii* cannot be overlooked and requires investigation. it is also suggested that different processing operations used can be effectively applied on mint and thyme to minimize the risk of pesticides on human health. There is a need to educate the consumers through media.

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تأثير السليكا والنانو سليكا ضد الذبابه البيضاء والمن ومتبقياتهما في نباتات النعناع و الزعتر.

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توفر السيليكا إمكانيات موسعة للاستخدام في المحاصيل البستانية. ومع ذلك ، توجد العديد من آفات المحاصيل على السطح السفلي لاوراق النباتات وهذا يعد تحديًا خاصًا عند استخدام السيليكا لأن المادة يجب أن يكون لها اتصال مباشر بالحشرة لتكون فعالة. في هذه الدراسة ، استخدمنا السيليكا ونانو سيليكا SiO2 لتقييم فعالية المبيدات الحشرية ضد افاتين مختلفتين الذبابه البيضاء ومن القطن تحت الظروف المعمليه ، تم قياس التركيزات النصف مميته بعد ٢٤ ، ٤٨ ، ٧٢ و ٩٦ ساعة علي التوالى. كما تمت دراسة بقايا التقليدية لثاني اكسيد السيليكون وجزيئاتها النانوية في أوراق النعناع والزعتر والسيقان والتربة.

أشارت نتائجنا إلى أن معدل الموت التراكمي للذبابه البيضاء والمن قد زاد مع زيادة تركيز المبيدات الحشرية أو زيادة الوقت. تم تسجيل أعلى قيمة للموت فى الحشرات المختبره في ٩٦ ساعة من المعاملهSiO2 بالسليكا و في حالة جزيئات السليكا النانونيه (SNPs) سجلت أعلى قيم لموت الذبابه البيضاء عند التركيز ٢,٥٠ نانوجرام / ميكرولتر بعد ٤٨ ساعة من المعامله. ولكن بالنسبة لحشرات المن ،

تشير النتائج الي ان نفس نسب الموت سجلت عند التركيز ٢,٥٠ ميكروجرام / ميكرولتر بعد ٢٤ ساعة من المعاملة. تشير النتائج إلى أنSiO2 صورتي ثاني اكسيد السيليكون تلعب دور هام في الادارة المتكاملة للافات، و سوف يكون لها تاثير عالي ضد حشرات الذبابة البيضاء و المن عند المعاملة المباشرة .

التوصية: اشارت النتائج إلى أن SiO2 من شكلين يلعب أدوار فعالة في الإدارة المتكاملة للآفات وسيكون أكثر فعالية عند تطبيقها مباشرة ضد الذباب الأبيض والأفيس.