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Acaricidal Activity of Formulated Plant Oil (Natrilo), The Biocide (*Bacillus thuringiensis*), Organophosphorus Pesticide (Pirimiphos-methyl) and their Binary Mixture Against the Acarid Mite *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae)

Cross Mark

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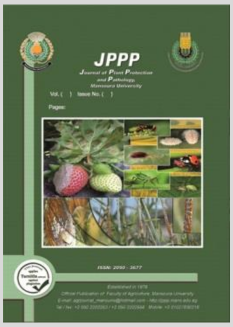

ABSTRACT

Laboratory experiments were conducted under controlled conditions to test the toxic activity of two biopesticides, the formulated plant oil, natrilo and *Bacillus thuringiensis* (Berliner) as well as one chemical insecticide, pirimiphos-methyl against adults and larvae of the acarid mite, *Tyrophagus putrescentiae* (Schrank). Furthermore, the joint action of the three mentioned agents was investigated against adults of *T. putrescentiae* to determine the contact toxicity and some other parameters of biology alongside the effect on mite sterility. The results showed that pirimiphos-methyl was the premier for contact toxicity 24 and 48 h after treatment against adults and larvae of mite species. The plant oil natrilo placed the second order, while the effect of bacteria did not appear until the second day after treatment. Concerning the effect of the tested materials on mite progeny parameters, the three materials demonstrated significant effects compared to untreated mite individuals. In addition, the tested materials had a positive effect on sterility percentage. The joint action of the tested compounds, the present results showed an additive and synergistic effect resulting in reaction of pirimiphos-methyl with natrilo and *B. thuringiensis*, respectively, while the combined effect of natrilo and *B. thuringiensis* gave antagonistic effect against the tested mite pest.

Keywords: *Tyrophagus putrescentiae*, pirimiphos-methyl, *Bacillus thuringiensis*, natrilo, progeny, sterility and joint action

INTRODUCTION

Wheat is one of the main strategist crops in Egypt, where the vast majority of the populations depend on it for their food and many other uses. Egypt needs about 10 million tons annually for filling the gap between the production and the consumption. The state and private sector are responsible filling that deficiency.

Wheat is subjected to attack from some stored pests such as insects, birds, rats and acarine mites. These pests cause many problems include a high reduction in quality, quantity, nutrition value as well as the poor germination. There are many ways to control these pests, the main method of which is the use of chemical pesticides which may cause the development of pest resistance, environmental pollution, death of non-target organisms and hazard residues in foodstuffs. To avoid these disadvantages, there was a need to find out alternatives that are safe, easy to obtain, cheap, highly effective, relatively quickly decomposing and have no harmful residues to man and environment.

Mites are considered one of the most abundant groups of pest arthropods (Howard *et al*., 2001; Athanassiou *et al*., 2005; Stejskal & Hubert, 2008 and Kilic, 2022). In addition to immediately harming the stored goods and lowering their nutritional content (Boczek, 1991 and Krantz, 1955), a large number of mite species in preserved food and its derivatives can also trigger allergic reactions in people (Kondreddi et al., 2006). Physical and chemical approaches for controlling storage mites appear to hold a lot of promise as substitutes for conventional methods of eliminating pests from stored cereals and their derivatives. According to Duek et al. (2001), the astigmatid mite *Tyrophagus putrescentiae* is most commonly detected in foods that have been preserved and have a high fat and protein content, such as wheat, flour, cheese, rye bread, herring meal, date fruits, dried milk, and other seeds.

Biopesticides is a term that is currently up for debate. Biopesticides include a wide range of compounds produced by living organisms, including microbial or fungal toxins, insect pheromones, and plant secondary materials, as well as complete species including microbes, fungi, entomophagous invertebrates, parasitoids, and predators (Saad, *et al*., 2021). Since organisms frequently act through toxic substances and it is impossible to distinguish between the pesticidal activity of an entire organism and substances produced by pests, some researchers exclude plant extracts or even all chemical agents from the category of bioinsecticides, while others classify these compounds as such (Glare, 2015). Regulations from the European Union state that plant extracts and food items are included in the category of bioinsecticides (Marchand, 2017).

Bacillus thuringiensis (Berliner), a gram positive rod-shaped bacterium that generates poisonous protein crystals during sporulation and, when consumed, transforms into an

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active diamond-shaped toxin, which is one of the well-studied insect-pathogenic bacterium (Arbogast, 1984). According to Mummigatti *et al.* (1994), the toxins cause osmotic imbalance, which causes pores in the midgut epithelial cell membrane, which causes the cells to expand and lyse.

There is a rising trend for use of natural pesticides which are derived from plant or micro-organisms, since they are safe than synthetic chemicals (Radhakrishnan & Prabhakaran, 2014). Several plant oils contain lipophilic compounds that have a direct contact and fumigant action (Rajendran, 2008) and acetylecholine-sterase (ACHE) inhibitory activities (Owokotomo *et al*., 2015). Efficacy of plant based oils has been used to suppress mite pest populations, as well as some insect spesies (Koul *et al*., 2008 and Ray *et al*., 2017).

As of right now, controlling arthropod infestations in stored goods primarily involves the use of fumigants, pyrethroids, and organophosphates (Attia *et al*., 2020). In order to manage or lower pest populations, biopesticides are natural products that incorporate live creatures such as plants, nematodes, minerals, bacteria, fungi, and viruses (Samada & Tambunan, 2020). The efficiency of biopesticides can be enhanced by the combined application of different biocontrol agents, rather than a single agent (Devi, 2019).

Wakil *et al*., (2023) reported that the combined treatment of entomopathogenic nematode; *Steinernema carpocapsae* and entomopathogenic fungus, *Beauveria bassiana* caused a higher lethality compared to single treatments against some of major stored product insects.

No microorganism specific to mites has been identified thus far (Poinar & Poinar, 1998). Nonetheless, Hoy & Ouyang, (1987) discovered that adult females of Pacific spider mite, *Tetranychus pacificus* (Prostigmata: Tetranychidae) and the phytoseiid predatory mite, *Metaseiulus accidentalis* (Mesostigmata: Phytoseiidae) were both poisonous to the B-exotoxin of *B. thuringiensis*. Therefore, the current study aimed to evaluate the acaricidal activity of two bioinsecticides (according to European Union Regulations), formulated plant oil natrilo and *Bacillus thuringiensis* and their joint action against the acarid mite, *Tyrophagus putrescentiae*.

MATERIALS AND METHODS

Rearing of *Tyrophagus putrescentiae* (Schrank)

Baker's yeast was kept at a consistent heat of 25 ± 5°C and 65 ± 5 R.H. mite individuals were cultivated in 3 cm diameter by 4 cm deep plastic units that were closed with glass lid. Each unit was filled to a maximum of 0.7 cm with a 9:1 dual combination of plaster and charcoal.

1) Chemical used:

a) Organophosphorus compound (OP):

- Common name: Pirimiphos-methyl.

b) Formulated plant oil:

- Natrilo
- Natural vegetable oil 93 % (w/v).
- Inert ingredients: 7 %.
- Stoller chemical L.T.D.-ENGLAND.

c) Biocide:

- *Bacillus thuringiensis* (B.t) var. kurstaki, 6.4 %.

2) Methods of bioassay of the tested compounds:

Toxicity:

According to Dike *et al*., (1953) un-sixed adults or larvae, Baker's yeast (0.2 g each), placed on filter paper (2 × 2 cm) were divided into groups each of 3 replicates. One group was treated by distilled 0.2 ml water as control. Treatments and control checked at two periods (24 and 48 hr) and maintained at the same conditions aforementioned. The treated groups exposed to series of concentrations carried on pieces of yeast). After 24 and 48 hours mortality were corrected according to Abbott's formula (Abbott, 1925).

Data were statistically analyzed according to Litchfield and Wilcoxon (1949).

Toxicity index (TI) of tested compounds were determined according to Sun (1950) as follows:

$$\textbf{TI} = \frac{\text{LC50 of the most effective compound}}{\text{LC50 of a tested compound}} \times \textbf{100.}$$

3) Biological effect of the tested compounds

As previously stated, the method of Dike et al. (1953) was used. Adults were exposed to LC_{50} in the identical circumstances as stated above. After 24 and 48 hours, mortality counts were taken and adjusted using Abbott's formula (Abbott, 1925). After three days, the survival animals were discarded It was noted how many eggs, larvae, and adults there were. The following formulas were used to determine the percent reduction (R%) of all phases, sterility, and percent of hatchability (%) when compared to the untreated check:

$$R\% = \frac{\text{C}-\text{T}}{\text{C}} \times \textbf{100.}$$

Where:

C = N. of control.
T = N. of treated.

$$\textbf{\% H.} = \frac{\text{Number of Larvae}}{\text{Number of eggs}} \times \textbf{100.}$$

$$\textbf{\% Sterility} = 100 - [\frac{\text{a} \times \text{b}}{\text{A} \times \text{B}} \times \textbf{100}].$$

Where:

a = No. of eggs laid/female in treated female.
b = f hatchability% of treated female.
A = No of eggs laid/female in untreated control.
B = hatchability% in untreated treatment.

4) Joint action of tested compounds against *T. putrescentiae*:

Mansour et al. (1966) described how to calculate the combined effect of the binary mixtures of pirimiphos-methyl + *Bacillus thuringiensis*; pirimiphos-methyl + natrilo and Bacillus thuringiensis + natrilo against *T. putrescentiae* (2–3 days old). At the LC_{50} value , the mixture's constituent parts were applied singly or in combination. Each toxicant's predicted LC_{50} was independently dissolved in acetone, either by itself or in a binary mixture. A petri dish of 9 cm in diameter was filled with one milliliter of each toxicant, either separately or in combination, and allowed to dry. Ten adult *T. putrescentiae* (2–3 days old) were put into a petri dish once the film was completely dried. Two days following therapy, the mortality % was recorded and adjusted using Abbott's formula (Abbott, 1925). Acetone alone was used to prepare for the untreated control. Three repetitions of each treatment and control were conducted. The total of the actual moralities of the dosages that were combined to form the mixture was the predicted mortality. The real mortality resulting from treating the combined LC_{50} was the mixture's observed mortality.

The joint action was evaluated by using the following equation:

$$\textbf{Co-toxicity factor (Co-F)} = \frac{\text{Observed \% mortality} - \text{Expected \% mortality}}{\text{Expected \% mortality}} \times \textbf{100.}$$

Statistical analysis

Three replicates of the investigated treatments were set up in a completely randomized block design (CRBD). The statistical software package MSTAT-C was used to do the analysis of variance (Freed, 1991). Duncan's multiple range tests (Duncan, 1955) was used to compare the means with a 0.05% probability.

RESULTS AND DISCUSSION

Toxicity effect:

Results in Table 1, summarized the activity of pirimiphos-methyl insecticide and the two biocides, natural oil, natrilo and the bacterium *B. thuringiensis* against adults and larvae of *T. putrescentiae* infesting wheat grains. Based on LC_{50}, pirimiphos-methyl had the strongest effect with LC_{50} value of 0.7 µg/g 24 h after exposure and followed by natrilo oil with 166.0 µg/g wheat. Meanwhile, the biocide *B. thuringiensis* did not achieve any effect on the tested mite pest. For larvae, results showed the distinct of pirimiphos-methyl compared with the two biocides, which did not exhibit any activity against the tested mite pest. After 48 h of exposure, the results obtained evoked the same trend of 24 h where the pirimiphos-methyl was the most effective agent compared with the two biocides with LC_{50} values of 0.4, 150 and 600 µg/g wheat grain, respectively.

Table 1. Toxicity of the materials against adults and larvae of *T. putrescentiae* 24 and 48 h after treatment on wheat grains.

Materials	Adults					Larvae				
	LC_{50} µg/g	C. L. Lower	C. L. Upper	S.V.	TI	LC_{50} µg/g	C. L. Lower	C. L. Upper	S.V.	TI
24 h										
pirimiphos-methyl	0.70	0.43	1.20	1.4	100	1.1	0.8	1.4	2.5	100
Natrilo	166.0	117.2	240.70	2.2	0.35	0.0	0.0	0.0	0.0	0.0
Bacillus thuringiensis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
48 h										
pirimiphos-methyl	0.4	0.32	0.85	1.2	100	0.8	0.6	1.1	2.1	100
Natrilo	150.0	102.80	235.5	1.7	0.27	200	142.9	280	2.5	0.4
Bacillus thuringiensis	600	461.5	780.0	2.5	0.07	900	642.9	1260	2.0	0.09

TI = Toxicity Index

Regarding the OP compound, the current agree with the findings of Zettler and Janes (1997) who reported that pirimiphos-methyl was the premier agent against the susceptible and malathion resistant strain of *T. castaneum* and more toxic than malathion itself. The efficiency of botanicl substances were confirmed by numerous researchers as stored product protectants (Pereira 1983; Arnason *et al*. 1989; Shaaya *et al*. 1991; El-Aidy and Helal, 1997 and Abo-Arab *et al*. 1998). Moreover, Guirguis *et al*. (1991) investigated the insecticidal activity of 12 citrus oils and pirimiphos-methyl to adults of *S. oryzae* using thin film method, they claimed pirimiphos-methyl was the strongest agent comparable to the other tested oils.

Effect on mite progeny and sterility %

Data in Table 2, included the effect of the investigated materials on some important parameters closed related with the effect on the growth development of *T. putrescentiae*. The present findings obviously showed significant differences between the untreated control and other three treatments. Accordingly, pirimiphos-methyl was the strongest compound which achieved the highest effect on all tested parameters compared to natrilo and *B. thuringiensis*. Except the effect on progeny% and sterility %, natrilo and *B. thuringiensis* had semilar effect on the other remainder parameters as shown in Table (2). Numbers of total eggs and emerged adults showed the best action of the tested materials either pirimiphos-methyl or the two biocides natrilo oil or *B. thuringiensis* against the tested mite pest. For example, there was significant inhibition in numbers of deposited eggs and emerged adults, where these numbers were 0.03, 17 and 19 eggs and 0.03, 4 and 6.33 emerged adults when mites were subjected to pirimiphos-methyl, natrilo oil and *B. thuringiensis*, compared to 40 eggs and 40 adults for control, respectively.

Table 2. Average numbers of hatched eggs, hatchability, progeny and sterility of *T. putrescentiae* treated with three different materials

Treatment	Average of						
	Total eggs	Egg/female	No. of hatching	% H.	% progeny	Adults	% sterility
Control	40.00^{c}	2.70^{c}	39.00^{c}	100.00^{c}	100.00^{c}	40.00^{c}	0.00^{a}
Pirimiphos-methyl	0.03^{a}	0.03^{a}	0.03^{a}	0.02^{a}	0.03^{a}	0.03^{a}	0.03^{a}
Natrilo	17.00^{b}	1.10^{b}	4.00^{b}	70.60^{b}	70.60^{b}	4.00^{b}	71.20^{c}
B. thuringiensis	19.00^{b}	1.30^{b}	6.33^{b}	100.00^{c}	100.00^{c}	6.33^{b}	52.00^{b}

Results in Table 3 markedly showed that the all tested materials significantly affected on the all tested tested parameters. For mean of progeny, pirimiphos-methyl was the most effective compound followed by natrilo oil and *B. thuringiensis,* where percent of sterility had the same trend. Finally, all tested compounds showed positive effect on all tested criteria compared to untreated control.

Table 3. Analysis of variance for the number of eggs laid, hatched eggs, % hatching, number of progeny and % sterility of the tested mite.

S.	DF	MS						
		Total eggs	Egg/F.	No. of hatching	% hatching	Adults	% progeny	% sterility
R	2	12.01	0.04	3.10	1.62	3.50	0.01	8.42
T	3	488.10	2.27	508.33	683.10	531.80	7410.33	3595.01
E	6	2.10	0.05	3.20	1.70	4.60	2.30	2.52

Joint action of binary mixtures of the test compounds against *T. putrescentiae*

R (replicate) T: (Treatment) E: (error)

Mansour *et al.* (1966) reported that the (Co-Factor) was estimated in order to determine the joint action. The present results were divided into three groups using this factor. Synergism is indicated by a positive factor of 20 or more, antagonism is indicated by a negative factor of (- 20 or more), while an intermediate value was thought to have an additive effect. Table 4 documents the investigated materials' anti-*T. putrescentiae* effects in binary combinations. The results obtained from the combination of *B. thuringiensis* with pirimiphos-methyl, showed an additive or synergistic impact. While, mixture of pirimiphos-methyl and natrilo demonstrated additive effect, while the joint action of natrilo and *B. thuringiensis* showed an antagonism effect (Table 4).

Table 4. Toxicity of pirimiphos-methyl, formulated plant oil (natrilo) and biocide (*Bacillus thuringiensis*) and their binary mixtures versus the tested mite.

Treatment	% mortality (24h)	Co-toxicity factor	Combined effect
Pirimiphos-methyl	50.0		
Natrilo	50.0		
B. thuringiensis	0.0		
Pirimiphos-methyl + Natrilo	93.3	-6.7	Additive
Pirimiphos-methyl + *B. thuringiensis*	100.0	0.0	Additive or synergism
Natrilo + *B. thuringiensis*	0.0	-100.0	Antagonism

What are synergism and antagonism?

When two chemical substances combine, an activating or inhibitory effect may arise. In the case of activation, the resulting from the mixture of the two substances is greater than the sum of their effect of each substance separately and vice versa in the case of an inhibitory effect, where the sum of the effect resulting from the mixture of the two substances is less than the sum of their effect individually. In conclusion, the present results clearly indicated that the joint action of both *B. thuringiensis* with natrilo had antagonistic effect against *T. putrescentiae*. Also, a combination of I primiphos-methyl with the other tested compounds showed additive and synergistic effects against *T. putrescentiae* adults.

In general, the present results are consistent with those of Abo-Arab *et al*. (2022), who investigated the effect of mineral oil KZ, pirimiphos-methyl, and the biocide ivomic on *T. putrescentiae* adults and larvae. They claimed that ivomic and KZ oil were the second most effective compounds after pirimiphos-methyl. Moreover, pirimiphos-methyl in conjunction with ivomic and KZ oil had a synergistic or additive impact on mite pest. Moreover, *Sophora flavescens* and *S. alopeuroides'* roots provided the quinoizidine alkaloid plant material matrine (Mao and Henderson 2007). According to Liu *et al*. (2007), hormones have a wide range of pharmacological and cytotoxic effects on *Sitophilus zeamais* and *Tribolium castaneum*. Hwang (2009) studied the effectiveness of a combination of matrine and neem oil against phytophagous mites, validating the toxic and biological properties of matrine against arthropods. In addition, Badawy *et al*., (2022) reported that the joint toxic effect of the tested acaricidal mixtures of abamectin, chlorfenapyr and pyridine with the formulated Agromectin, Challenger and Sanmite at LC_{50} values exhibited a potentialtion effect and the toxicity was increased significantly against eggs and adults compared to the individual pesticide. Additionally previous studies reported that formamidine acaricides, amitraz and chlordimeform effectively synergized the toxic action of certain pyrethroids (deltamethrin, permethrin, cypermethrin and phenothrin) and neonicotinoids (imidacloprid, thiamethoxam and dinotefuran) on some mites such as *Tetranychus urticae* Koch (El-Sayed & Knowles 1984). However, inappropriate pesticide combination would cause an antagonism.

Sayed *et al*., (2017) studied the combined effect of two synthetic insecticides, cypermethrin (25% E.C.) and clorpyrifos (48% E.C.) with five plant oils against the cotton leaf worm *Spodoptera littoralis* (Boisd). They confirmed that the most toxic mixture was compounds mixed with cypermethrin, especially that of cypermethrin and plant oil, *Allium sativum* L. (1:1).

CONCLUSION

In the current research clearly showed that all tested materials had positive effects on the all progeny parameters especially sterility % according the laid eggs and hatchability %. Furthermore, the results of joint action can by exploited to minimize the use amount of mixed material in the event of synergism to reduce the cost of control process as well as reduce the harmful side effect of the mixed materials. While, in the event of antagonism resulting from the mixing process, we suggest to prevent the combination of used materials and use each material separately.

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النشاط الأبادي للزيت النباتي المصنع ناتريلو، المبيد الحيوي باسيللس ثورينجينسس والمبيد الفوسفوري بريميفوس ميثيل وتأثيرهم المشترك ضد أكاروس تايروفاجس بيوترسكينتا (شرانك)

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الملخص

إجريت تجارب معملية تحت ظروف محددة وذلك لإختبار النشاط الإبادي لإثنين من المبيدات الحيوية أحدهما في صورة زيت نباتي مصنع وهو زيت الناتريلو (Natrilo) والأخر هو بكتريا باسيللس ثورينجينسس (B.T) بالإضافة لمبيد كيماوي هو البريميفوس ميثيل (Pirimiphos-methyl).تم بحث التأثير المشترك للعوامل الثلاثة المذكورة ضد بالغات ويرقات أحد أكاروسات المواد المخزونة وهو تايروفاجس بيوترسكينتا (Schrank) *Tyrophagus putrescentiae* بإستخدام طريقة الخلط مع البيئة وذلك لتقدير السمية بالملامسة، وبعض المعايير الخاصة بيولوجي الأكاروس المذكور بالإضافة للتأثير على الخصوبة.أظهرت النتائج أن المبيد الكيماوي Pirimiphos-methyl كان العامل الأشد تأثيراً في السمية بالملامسة بعد 24، 48 ساعة من المعاملة عند كل من الطورين الحشرة البالغة واليرقة وقد جاء الزيت المصنع ناتريلو natrilo oil في المرتبة الثانية بينما لم يظهر أي تأثير للبكتريا B.T المختبرة إلا بعد يومين من المعاملة.أظهرت نتائج المواد الثلاثة المدروسة تأثيراً معنوياً على المعايير البيولوجية مقارنةً بالتجربة الغير معاملة (الكنترول).بالإضافة لذلك أظهرت المواد المدروسة تأثيراً إيجابياً على عملية الخصوبة.أظهرت النتائج في تجربة التأثير المشترك Joint action بين المركبات الثلاثة تأثيراً إضافياً وتأثيراً تنشيطياً عند خلط المبيد الكيماوي مع كل من الزيت النباتي والبكتريا على الترتيب. بينما أظهر التأثير المشترك لمخلوط البكتريا (B.T) مع الزيت النباتي المصنع تأثيراً مثبطاً ضد الأفة المختبرة.