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TOXICITY AND BIOCHEMICAL EFFECTS OF SOME SAFE ALTERNATIVE MATERIALS AGAINST *PTEROCHLOROIDES PERSICAE* CHLOD. INDIVIDUALS (ORDER HOMOPTERA : FAM. APHIDIDAE) ON PEACH TREES

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Abstract

The present work was to study the efficacy of some safe alternative materials against *Pterochloroides persicae* Chlod. (Ord. Homoptera, Fam. Aphididae), also the biochemical effects of such materials on the aphid. All tested alternatives reduced *P. persicae* population. The use of entomopathogenic fungi and yeast as a biocontrol agent, natural (vegetable) oil, K.Z. oil, potassium sulfate + detergent against *P. persicae*. All reduced significantly the number of *P. persicae* on trees compared to the untreated ones. The mixture of potassium sulfate + detergent treated trees revealed the lowest number of aphid individuals followed by *Metarhizium anisopliae* L., natural oil, KZ oil and yeast mixture, respectively. After 2 days of spray, the percent reduction of aphid population for potassium sulfate + detergent treatment was 89.68 %, whereas, it was 82.78, 82.61, 82.31 and 72.77 % for natural oil, *M. anisopliae*, KZ oil and yeast mixture, respectively. After 5 days of spray, the efficacy of tested materials was slightly decreased in comparison with the second days after treatment, further research and evaluation on large scale field trials.

The activities of glutamic pyruvic transaminase (GPT), and glutamic oxaloacetic transaminase (GOT) enzymes increased in treated aphids with all tested materials. On the other hand, the *M. anisopliae* and KZ oil treated aphid individuals exhibited remarkable decrease in their total protein level, whereas the protein level increased in yeast mixture, potassium sulfate and natural oil treated insects.

INTRODUCTION

The peach aphid, *P. persicae* Chlod. (Order Homoptera; Family Aphididae) is one of the most important pests which attack orchard trees in North Sinai and cause a severe damage. However, chemical control is the primarily method to manage this pest population. Although its population densities changes from year to year, it considered one of the most economic pests to peach trees in Egypt. An efficient IPM program has to depend on the using some safe alternative materials against *P. persicae*. Using entomopathogenic fungi such as *Verticillium lecanii, Metarhizium anisopliae* and *Beauveria bassiana* against aphid has been investigated by Wick (1990), Santiago (1991), Birk-

mose (1994) and Butt *et al.* (1995). Also, using of bacteria *B. thuringiensis* or its δ–endotoxin revealed promising result against aphid insect (*i.e.*, Dik *et al.*, 1992; Walters and English, 1995; Lopez-Meza and Ibarra, 1996).

On the other hand, the biochemical tests for specific agent could help in the knowledge of resistance mechanism and can provide a powerful mean of monitoring the selective control treatments and appraising putative tactics for countering resistance that will be appeared (Wick, 1990).

Proteins are present in all viable cells. They are essential to the processes of cell division and as enzymes, and hormones. They control many chemical reactions in the metabolism of cells of insect (Moises, 1978).

The amino-transferases especially transaminases enzymes (GOT and GPT) are two of the components of oxidative metabolism of protein which in certain insects is utilized during the initial periods of flights (Bursell, 1963). They also act as a catalytic agent in the metabolism of carbohydrate (Katunuma *et al.*, 1968).

The present work aimed to study the effect of tested materials on the pest's (GOT, GPT and total proteins).

MATERIAL AND METHODS

I. Tested materials

In this study, the five alternative materials namely, the fungus, *Metarhizium anisopliae*, yeast mixture, potassium sulphate + detergent, natural oil (vegetable oil), natural oil + yeast and KZ oil were tested against *P. persicae* infesting peach trees at North Sinai Governorate, Egypt, during 1999 season. The experimental area was about one feddan. This area was divided into 24 plots (8 trees each) and every tested compound was applied at the recommended dose in four plots selected randomly. Also, four untreated plots served as untreated control.

The concentration of tested materials were as follows :

- *Metarhizium anisopliae* at 400 ml/100 liter water.
- Brewer's yeast (1.5 kg) + wheat flour (1 kg) + molasses (1kg)/100 liter water.
- Potassium sulfate (1 kg) + Detergent (1.5 liter)/100 liter water.
- Natural oil (vegetable oil), 250 ml/100 liter water.
- Natural oil (250 ml) + Brewer's yeast (0.5 kg)/100 liter water.

- KZ oil at the rate of 1.5 liter/100 liter water, Kafr El-Zayat Company.

The fungus *M. anisopliae* extract was prepared according to Smith and Anion (1993) as follows :

- 230 ml of potatoes extract + 770 ml water + 20 gm glucose + 20 gm agar were blended and sterilized.
- The mixture was poured in Petri dishes (20 ml/Petri dish).
- The media were inoculated with fungus spores isolated from fungi dead insects.
- The inoculated dishes were incubated for 15 days on 25°C for germination.
- 15 ml of 0.01 % Tween-80 solution was poured on the surface of each dish as a detergent agent for helping harvesting.
- Harvested spores solution were put in sterilized bottle (Smith and Anion, 1993).

Preparation of yeast extract

- 1.5 kg of Brewer's yeast (produces by Alexandria Company) were mixed with 4 liters warmed water.
- Wheat flour (1 kg) and molasses (1 kg) were mixed thoroughly with yeast extract.
- Before application, 96 liter water were added to the mixture.

All treatments was applied during August 1999 using high volume (land spray gun). All tested materials are water suspended.

Before spraying, the population of aphid was determined on 30 square inch chosen at random from stems and branches of 10 trees at the rate of 3 square inch per tree at the lower, middle and upper levels of each tree. The mortality among aphid insects was evaluated at 2 and 5 days after spraying. The number of survivals was considered as a criterion for the tested materials performance. The reduction in the population density of aphids was calculated according to Henderson and Tilton (1955).

II. Biochemical studies of the tested alternative materials on aphids

The transaminases enzymes (GOT and GPT) are one of the components of oxidative metabolism of protein which in certain insects is utilized during the initial periods of flights (Bursell, 1963), they also acts as a catalytic agent in the metabolism of carbohydrate (Katunuma *et al.*, 1968). Therefore, biochemical analysis were done to determine the effect of these alternative materials on the vital processes.

1. Sample preparation for biochemical assay

For biochemical analysis, alive aphid individuals were collected after 2 and 5 days from treated and untreated plots, placed in clean glass vials and kept in deep freezer at -20°C. Then, the aphid individuals were homogenized in distilled water (10 individuals/ ml) using a Teflon homogenizer surrounded with a jacket of crushed ice for minutes. Homogenates were centrifuged at 3500 rpm for 10 minutes at 5°C and the supernatants were used directly to determine the activity of glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT) and total protein.

2. Transaminase enzymes (GPT & GOT)

Glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were determined colourimetrically according to the method of Reitman and Frankle (1957). GOT transfer the amino group aspartate to α-ketoglutarate producing a new amino acid (L-glutamate) and a new keto acid (oxaloacetic acid). GPT transfer the amino group from D, L alanine to α-ketoglutarate (α-ketoglutaric acid), resulting in a new amino acid (L-glutamate) and a new acid (pyruvic acid). Pyruvic or oxaloacetate reacts with 2,4- dinitrophenylhydrazine in alkaline medium, forming pyruvate or oxaloacetate hydrazone which in alkaline medium form a brown colour, which can be measured spectrophotometrically. The reaction mixture consisted of 1 ml of a mixture of phosphate buffer (pH 7.4), 0.2 m mole α-ketoglutaric and 200 m mole D, L alanine or L-aspartate. 0.2 ml of the aphid homogenate was added to this mixture. Incubated for exactly 30 minutes. One ml of 0.001 mole 2,4- dinitrophenylhydrazine was added. After at least 30 minutes. Then, after 10 ml of 0.4 N NaOH was added. The optical density of the produced brown colour is measured after 5 minutes, using spectrophotometer at 505 nm. The enzyme activity is expressed as μM pyruvate/aphid individual/minute.

3. Preparation of pyruvate standard curve

Serial dilutions of a standard stock solution of sodium pyruvate (22 mg/100 ml) were pipetted into test tubes (1 ml for each tube). After adding 0.2 ml of distilled water, the 2,4-dinitrophenylhydrazine reagent and the alkali were added as described before. The changes in optical density were plotted against pyruvate concentrations. Thus, a standard curve for determination of transaminase can be constructed. Pyruvate instead of oxaloacetate was selected for the standard curve because it is known that oxaloacetate is partially converted to pyruvate (Reitman and Frankle, 1957).

4. Total protein

The protein concentration was determined following the method of Biuret (Gornall *et al.*, 1949) as following :

- 20 individuals from check and treated aphids were homogenized in 2 ml distilled water, then centrifuged at 3000 rpm for 10 minutes to separate the protein content.
- 20 ml of supernatant + 1 ml Biuret reagent (Biosystems kit, Barcelona, Spain) were mixed thoroughly. After 10 min., at room temperature, the optical density of the produced violet-pink colour is measured spectrophotometrically at 545 nm.
- Standard solution of protein was prepared by dissolving 6 gm protein (Ovalbulmin)/ 100 ml distilled water.

RESULTS AND DISCUSSION

I. The relative efficiency against P. persicae

Fig. 1 shows that all tested compounds reduced significantly the number of *P. persicae* on peach trees compared to the untreated one. After 2 days of spray, the mixture of potassium sulfate + detergent revealed the lowest number of aphid individuals followed by *M. anisopliae*, natural oil, KZ oil and yeast extract, respectively. The percent reduction of aphid individuals for potassium sulfate + detergent was 89.68 %, whereas it was 82.78, 82.61, 82.31 and 72.77 % for natural oil, *M. anisopliae*, KZ oil and yeast mixture, respectively. After 5 days of spray, the efficacy of tested materials was slightly decreased in comparison with the second days after treatment.

It could be concluded that using of natural oil and petroleum oil (KZ oil) may be cause their effect by preventing oxygen from entry to insect haemolymph. Consequently, oxygen shortage induce cytochrom reduction and this causing rapid consumption of O_2 in the cells. As for the fungus *M. anisopliae*, since the insect cuticle contains chitin fibrils within protein matrix together with lipids and waxes and small quantities of phenol, inorganics and pigments, so penetrates insect cuticle causing their death via adhering on curicle surface and remain to permit germination and subsequent invasion, through nonspecific hydrophobic forces. Thereafter, germination specific glycoproteins and cuticle enzymes such as proteases, chitinases and lipases play an important role in penetration (Charley, 1984; Charley and St. Leger, 1991). So, the insect death may be due to exhausting of insect haemolymph biochemical content, or it could be due to the lethal amounts of destructive materials produced by *M. anisopliae* (Gordon *et al.*, 1978). The important role of cuticle-degrading proteases in fungal pathogenesis of in-

sects is confirmed by the results obtained by Butt *et al.* (1995). They found that difference in susceptibility of aphid to *M. anisopliae* is dependent on the reflection response of the pathogen of cuticular cues. More conidia adhered to cuticle and germinated readily on the surface of live aphids.

II. Biochemical effects of the tested alternative materials on aphids

1. Transaminase enzymes (GPT & GOT)

At days after treatment, data in Fig. 2 indicated that the activity of glutamic pyruvic transaminase (GPT) significantly increased in treated aphid individuals in comparison with the untreated one. The highest activity of GPT was obtained with natural oil + yeast mixture treatment, followed by potassium sulfate + detergent, natural oil, KZ oil, *M. anisopliae*, yeast, control, respectively. After 5 days of treatment, there was a depression of GPT activity in potassium sulfate, natural oil + yeast extract and KZ oil, but the enzyme activity in fungi, yeast and control treatments increased.

Taking into account, the activity of GOT, data in Fig. 3 indicated that all treatments increased the enzyme activity after 2 days of treatments in comparison with the control treatment. There was a depression in enzyme activity in all treatments after 5 days of treatment except in *M. anisopliae*, yeast mixture and control. Natural oil revealed the lowest enzyme activity followed by KZ oil, potassium sulfate and natural oil + yeast extract, respectively. It could be stated that variance of enzyme activiies may be due to variance of mechanism of effect of each material.

2. Total protein

Data in Fig. 4 at 2 days of treatment, indicate that both *M. anisopliae* and KZ oil decreased the protein level in the treated aphid individuals, whereas the protein level was increased in yeast extract, potassium sulfate, natural oil and control. Yeast extract treatment revealed the highest level of total protein followed by potassium sulfate, natural oil, natural oil + yeast extract and control. On the other hand, total protein level in the 5*th* day after treatment was highest than that obtained at the 2 days after spray except in aphids tested with natural oil, whereas it was greatly decreased.

It could be concluded that all tested alternatives would reduce *P. persicae* population. The use of such entomopathogenic fungi and yeast as biocontrol agent, natural oil, KZ oil, potassium sulfate + detergent against *P. persicae*, therefore appears promising, but require further research and evaluation on large-scale field trials. The mechanism contributed as the cause of killing in aphid population by fungi could be resulted in

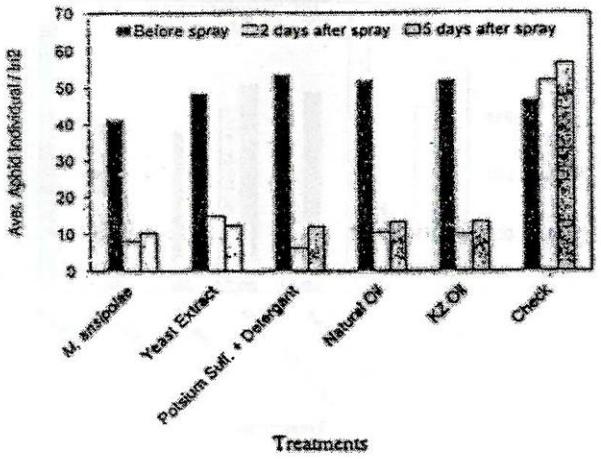

Fig. 1. Average of *P. persicae* individuals on peach trees before and after spray in the different treatments at North Sinai Gov., Egypt, 1999 season.

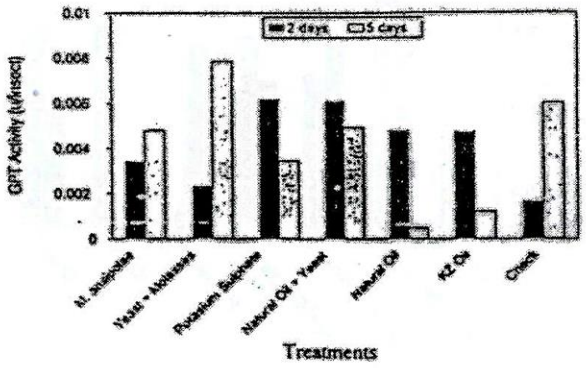

Fig. 2. Glutamic pyruvic transaminase (GPT) activity of *P. persicae* individuals on peach trees after spray in the different treatments at North Sinai Gov., Egypt, 1999 season.

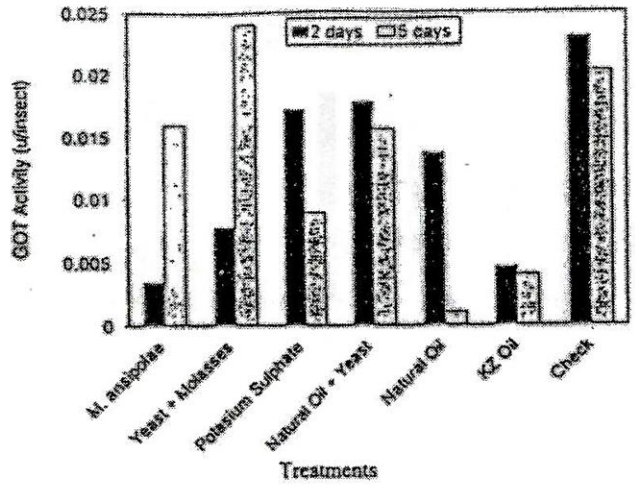

Fig. 3. Glutamic oxaloacetic transaminase (GOT) activity of *P. persicae* individuals on peach trees after spray in the different treatments at North Sinai Governorate, Egypt, 1999 season.

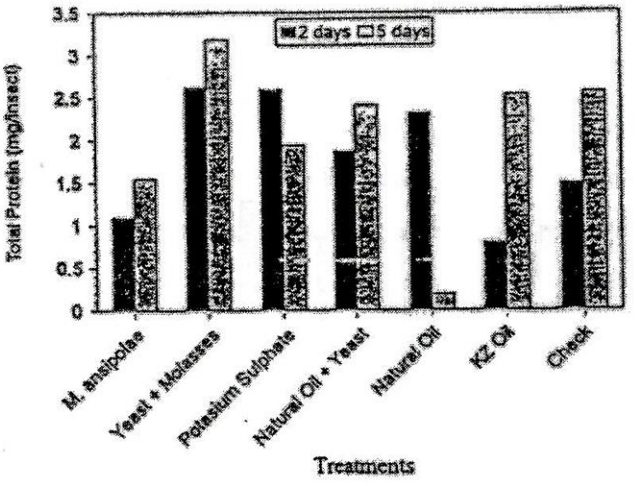

Fig. 4. Total protein of *P. persicae* individuals on peach trees after spray in the different treatments at North Sinai Governorate, Egypt, 1999 season.

exhausting of haemolymph contents and the lethal effect of destruxins which produced by *M. anisopliae*. As for the biochemical effects may be due to that the fungus use certain amino acids in insect haemolymph as a sole source of carbon and energy. Consequently, this will result in considerable nitrogen liberation. So that the amino acid used as a carbon source provide an excess of nitrogen, which is then excreted into the medium, usually as ammonia or used in protein synthesis. In the case of using a carbohydrate or an organic acid as a carbon and energy source in performance to an amino acid. In such forms, the induced formation of enzymes metabolizing the amino acid is prevented as long as the preferred carbon and energy source is present (catabolite repression). But, when nitrogen is in excess, the predominant mode of amino-group formation in fungi is by the reductive amination of α-ketoglutarate by free NH_3. Under these conditions, less glutamine is required and glutamine synthase formation is repressed. On the other hand, when nitrogen is limited, NH_3 is more efficiently utilized by the energy-dependent glutamine synthesis reaction and the amide group of glutamine is used in the reductive amination of α-ketoglutarate catalyzed by glutamate synthase. Under these conditions, formation of glutamine synthase is induced.

The effect of KZ oil, natural oil and detergent can be attributed to the suffocation effect result of preventing O_2 of entering the tracheal system, also their ability to dissolve the outer layer of aphids cuticle which in sequence increase the water loss of the insect body.

The present work is in full coincidence with work published by Wick (1990) and Birkmose (1994). They used the fungus *Verticillium lecanii* to control *Aphis gossypii* and pathogenic fungi against *Brevicoryne brassicae*, respectively. Similar results were obtained by Santiago (1991) and Butt *et al.* (1995), who used the *Metarhizium anisopliae* to control *Br. brassicae* and *A. gossypii*, respectively.

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التأثيرات البيوكيميائية السامة لبعض البدائل الآمنة ضد من البرقوق العسلى على أشجار الخوخ *Pterochloroides persicae*

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فى هذا البحث تمت دراسة تأثير بعض المواد البديلة والآمنة (الفطر الممرض، خميرة البيرة، الزيت الطبيعى، زيت KZ ومخلوط محسن الإنتشار وسلفات البوتاسيوم) وكذلك دراسة التأثيرات البيوكيميائية لهذه المواد ضد حشرة من البرقوق العسلى التى تصيب سيقان وأفرع أشجار الخوخ فى شمال سيناء وقد أدى إستخدام هذه المواد إلى :

١- حدوث خفض معنوى فى تعداد الحشرات على الأشجار المعاملة مقارنة بالأشجار غير المعاملة حيث تراوحت نسبة الإبادة للمواد المختلفة مابين ٧٦,٠٠ ٪ إلى ٨٥,٦٩ ٪ وقد أعطى إستخدام مخلوط محسن الإنتشار مع سلفات البوتاسيوم أعلا إبادة وأن كفاءة هذه المواد تنخفض مع مرور الوقت حيث كانت نسبة الإبادة أعلا بعد ٤٨ ساعة منها بعد ٥ أيام من المعاملة.

٢- أظهرت التقديرات البيوكيميائية أن نشاط أنزيمى جلوتاميك بيروفيك ترانس أمينيز، جلوتاميك أوكسالوأسيتيك ترانس أمينيز، والبروتين الكلى فى جسم الحشرة قد تأثرت تأثيرا معنويا بإستخدام هذه المواد فى الحشرات المعاملة مقارنة بالحشرات غير المعاملة حيث زاد نشاط الانزيمين.

٣- أدى إستخدام زيت K.Z. ومستخلص الفطر إلى نقص نسبة البروتين الكلى فى الحشرات المعاملة فى حين أدى إستخدام الزيت الطبيعى ومستخلص الخميرة ومخلوط سلفات البوتاسيوم مع محسن الإنتشار إلى زيادة نسبة البروتين الكلى فى الحشرات المعاملة.