

Laboratory Evaluation of a Botanical Natural Product (*AkseBio2*) against the Pear Psylla *Cacopsylla pyri*

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A botanical natural product, *AkseBio2*, was evaluated under laboratory conditions for its oviposition deterrent, ovicidal and larvicidal (nymphicidal) effects against the pear psylla *Cacopsylla pyri* (L.) (Hemiptera: Psyllidae). The product exhibited a strong oviposition deterrent effect for winterform and summerform females and caused a reduction in the total number of eggs laid in both choice and no-choice assays. Significant mortalities in freshly laid eggs (0–48 h) and various nymphal stages of the pest were recorded in toxicity assays. At a concentration of 0.1% (formulation), the highest biological activity of the product was recorded against the young (1st and 2nd) nymphal stages (up to 87.4% mortality) in comparison with the other biological stages of the pest. It was less active against the older (3rd–5th) nymphs, causing 62.1% mortality at the same concentration. In assays with non-target organisms, a significant negative effect was not observed. There were no significant changes on treated plants up to 7 days after treatment in any trial, nor was there any phytotoxicity on plant tissue as a result of *AkseBio2* treatments. The results suggest that the product can be used in psylla control instead of synthetic insecticides and may serve as an integrated pest management (IPM) component in pear orchards.

KEY WORDS: *AkseBio2*; botanical products; *Cacopsylla pyri*; oviposition deterrent; ovicidal; larvicidal; Turkey.

INTRODUCTION

Two psyllid species, namely, *Cacopsylla pyri* (L.) and *Cacopsylla pyricola* (Foerster) (Hemiptera: Psyllidae), have been reported to cause damage on pear trees in pear-growing regions of Turkey, but *C. pyri* is considered to be the only species of economic importance (5,8). The damage caused by this pest over the last 10 years in Antalya province (southwest Turkey) has increased in both intensity and extent. After the serious outbreaks of pear psylla in that region in 1992, 1993 and 1994, many orchards were sprayed against this pest with various groups (including organophosphates and pyrethroids) of insecticides on an average of eight or nine times each year (personal observations). A previous study by the author showed that pear psylla was worst in places that had been very heavily sprayed in the past, probably due to a reduction in natural enemies (5). Regional growers reported that treatments with many kinds of insecticides had failed to keep the pest in check, with the exception of amitraz. Many orchards have been sprayed repeatedly, often with high doses of this insecticide. For this reason – and considering that timing and application were also faulty in many instances – it is not surprising that resistance to amitraz has been found in some populations (10).

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Currently, the usual practice against pear psylla in Antalya is six to eight insecticidal treatments per year. The recent increase in chemical control in pear orchards is causing a high level of damage by this pest. Various chemical control programs have been tried by farmers, but usually the density of pear psylla in treated orchards is similar to that in untreated orchards and has even been found to reach higher levels in treated orchards late in the growing season (5). Because of the use of broad-spectrum insecticides against this species and other pests in pear orchards, the effectiveness of predators and parasitoids has greatly diminished. Due to failure of conventional chemical control against pear psylla, management strategies have been developed to enhance natural control of this pest. Since the alternative control tactics substitute for chemicals, natural enemies are able to increase in pear orchards.

Recently, pear psylla management has become increasingly difficult because of the psylla's ability to develop resistance to pesticides (3,4,9,10), and the problems with psylla control in pear orchards treated with broad-spectrum insecticides in Antalya induced us to try an alternative approach. The purpose of this study was to evaluate a botanical natural product which could be used in psylla control instead of synthetic pesticides.

MATERIALS AND METHODS

The product used in the study, *AkseBio2*, is a botanical, completely natural and water-suspensible, which was developed by Prof. Dr. Oktay Yegen (Akdeniz University, Antalya, Turkey); it is not yet available commercially. No chemicals are used in the manufacturing process (Table 1).

The oviposition deterrent, ovicidal and larvicidal (nymphicidal) effects of *AkseBio2* on *C. pyri*, the only pear psylla species in the study area (Dr. Aynur Önuçar, personal communication), were studied in the laboratory. Winterform and summerform adults of the pest were collected from an abandoned pear orchard in Korkuteli near Antalya. Adults not used immediately for the experiments were transferred to field-collected pear shoots (winterform) and pear seedlings (summerform) and placed in screened cages at room temperature ($22\pm 1^{\circ}\text{C}$) and a long-day photoperiod (16L:8D). The product was applied as an aqueous (tap water) mixture with a handgun sprayer, and used in all experiments at a concentration of 1 ml l^{-1} water. There were five replicates for each laboratory assay with pear psylla, and experiments were repeated twice.

Oviposition assays The product was evaluated for its ability to inhibit pear psylla oviposition in both choice and no-choice tests. Choice tests were conducted to determine ovipositional preference of winterform and summerform females. For the tests involving winterform females, two field-collected dormant shoots, one of which was sprayed with the test material and the other with tap water until runoff, were placed in each cage with cut ends dipped into a flask containing water. Ten gravid winterform females were introduced into each cage. The number of eggs laid on each treated shoot was recorded daily over a period of 7 days.

To study the deterrent effect of the product against summerform females, one-year-old pear seedlings (*Pyrus communis* cv. 'Ankara', which is the common pear variety in the study area) grown in a controlled climate chamber were used. Each seedling was trimmed so that only two sprouts remained, and then sprayed. One sprout of each seedling was treated with the test material, the other with tap water. Each treated seedling was placed in

a screened cage along with ten gravid summerform females. The number of eggs laid on both sprouts was determined daily during a 7-day period.

In no-choice tests the product was screened for its ability to deter oviposition when no alternatively treated shoot or sprout was available. The same procedure was followed as in the choice tests, except that both dormant shoots (winterform) and sprouts on each seedling (summerform) were sprayed only with the test material. Water-treated ones were included in separate cages as control. For each test five cages were used, and observations continued until no live females were found.

Ovicidal assays were conducted to determine the effect of the product on eggs laid by winterform and summerform pear psylla females. For assays with the winterform females, two dormant shoots were placed in each cage with their cut ends dipped into the flask containing water, and exposed to ten gravid winterform females. The insects were carefully removed after 48 h and the number of eggs on each shoot was recorded. One shoot was sprayed with the test material, the other with tap water until runoff. At daily intervals all eggs, live or dead, were carefully counted to determine the ovicidal effect of the test material until the end of the test period (7 days).

For assays with the summerform females, pear seedlings were trimmed as described above and then placed individually in screened cages. Ten gravid summerform females were introduced into each cage and the number of eggs on each sprout was determined after 48 h. One sprout of each seedling was treated with the test material, the other with tap water. The effect on eggs laid by summerform females was determined by counting treated eggs, live or dead, on sprayed sprouts until the end of the test period. For each test, five cages were used.

Larvicidal (nymphicidal) assays The effect of the test material on different nymphal stages of pear psylla was also examined. Thirty young (1st and 2nd stages) or older (3rd–5th stages) nymphs were transferred onto a pear seedling with a sprout (including 8–10 fully expanded leaves), using a fine camel's hair brush, and then treated with the test material. There were five replicates and experiments were repeated twice. The controls were treated with tap water. In order to determine the effect of the product on different nymphal stages of the pest on sprayed sprouts, all nymphs, live or dead, were counted daily over a period of 7 days.

Toxicity assays with non-target organisms were conducted to determine the effect of the product on the most common predators of the pest in the study area, *Anthocoris nemoralis* (Fabricius) (Hemiptera: Anthocoridae) and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). Both species were obtained from cultures maintained in the Plant Protection Department, Faculty of Agriculture, Akdeniz University. Adults of *A. nemoralis* >1 week old and first instar nymphs/larvae of both species were transferred individually into plastic petri dishes (4.5 cm diam) with blotting paper discs at their bases, and then treated with the test material at a concentration of 1 ml l⁻¹ water. The dishes were closed with their lids, each with a gauze window for air circulation. There were 15 replicates for each group and experiments were repeated twice. A similar number of adults or nymphs/larvae was treated with tap water as controls. Pear psylla eggs and young nymphs were offered as food to both species. Observations on mortality were taken daily for 7 successive days. Mortality data were compared with those in controls to calculate percentages of mortalities.

Data analysis All mortalities were converted to Abbott's corrected mortalities (1), with each treatment mortality being adjusted by factoring out mortalities in the controls. Data related to summerform and winterform oviposition, from choice and no-choice assays, were analyzed by the Statistical Analysis System (ANOVA). Duncan's Multiple Range Test was used to test for differences among accession means and the controls. Oviposition deterrent index (ODI) values (%) were calculated by using the formula $ODI (\%) = (B - A) / (A + B) \times 100$, where A is the number of eggs laid on the treated portion and B is the number of eggs laid on the untreated portion (control), as defined by Lundgren (7).

RESULTS AND DISCUSSION

Oviposition deterrency In choice tests, application of *AkseBio2* hindered oviposition by the winterform females during the 7-day period, where the ODI reached 100%. The product significantly reduced the summerform oviposition, showing 97.4% oviposition deterrency. Oviposition rates of winterform or summerform females were significantly greater on water-treated shoots or sprouts than on those treated with the product (Table 2). In no-choice tests, oviposition rates by winterform and summerform females were significantly reduced by application of the test material in comparison with rates on water-treated controls in separate cages. The ODI values of *AkseBio2* treatments for winterform and summerform females in no-choice assays were 96.7% and 94.6%, respectively (Table 2).

TABLE 1. Standard specifications of *AkseBio2* used in the study^z

Formulation type	EC 7 (Liquid)
Ingredients (70 ml a.i. l ⁻¹)	Aromatic (thyme, <i>Thymbra spicata</i> var. <i>spicata</i> L.; oregano, <i>Origanum syriacum</i> var. <i>bevanii</i> (Holmes); and anise, <i>Pimpinella anisum</i> L. essential oils) ^y and edible (sesame oil and maize oil) plant extracts; essential oil components (carvacrol, thymol and anethole); a natural emulgator; and a fluorescent bacterium, <i>Pseudomonas fluorescens</i> , TR 97
Appearance	Yellow to light brown
Smell	Thyme-like odor
Suspensibility	Completely suspensible in water
Toxicity to non-target organisms	Low to moderate, depending on species and life stage (see Table 3)
Shelf life	Two years, when stored under proper conditions and without opening the lid
Recommended storage conditions	Store in a cool, dark and dry place

^zSome of them were determined after a 4-year study.

^yThe plant materials used as the source of essential oils originated in Antalya.

Ovicidal effect The product showed varying ovicidal activity against freshly laid eggs (0–48 h) of both forms. At the 7-day period, it resulted in 72.3% and 78.1% mortality against eggs laying winterform and summerform females, respectively (Table 3).

Larvicidal (nymphicidal) effect The product also demonstrated varying biological activity against pear psylla nymphs. The young (1st and 2nd) nymphal stages were more sensitive to *AkseBio2* than the older (3rd–5th stages), with 87.4% and 62.1% mortality, respectively (Table 3).

TABLE 2. Effect of 0.1% *AkseBio2* on the oviposition by winterform (Wf) and summerform (Sf) *Cacopsylla pyri* females in choice (between shoots/sprouts treated with test material and water) and no-choice assays

Material	Eggs per female during the 7-day period (mean±S.E.)		Oviposition deterrent index (ODI, %)	
	Wf	Sf	Wf	Sf
<i>Choice tests</i>				
<i>AkseBio2</i>	0.0±0.0 Aa ^{z,y}	3.2±0.6 Ab	100	97.4
Water	137.8±8.3 Ba	247.4±7.1 Bb	-	-
<i>No-choice test</i>				
<i>AkseBio2</i>	2.6±0.6 Aa	7.2±1.2 Ab	96.7	94.6
Control ^x	153.2±8.5 Ba	261.2±8.6 Bb	-	-

^z Within columns, means with the same capital letter do not differ significantly (DMRT, $P < 0.05$).

^y Within rows, means with the same lower case letter do not differ significantly (DMRT, $P < 0.05$).

^x Water-treated controls were included in separate cages.

TABLE 3. Effect of 0.1% *AkseBio2* on the mortality of eggs laid by winterform (Wf) and summerform (Sf) *Cacopsylla pyri* females, different nymphal stages of pear psylla, and non-target organisms (*Anthocoris nemoralis* and *Chrysoperla carnea*) during the 7-day experiment period^z

Material	Egg mortality (%)		Nymph mortality (%)		Non-target mortality (%)		
	Laid by Wf	Laid by Sf	1st & 2nd stage	3rd–5th stage	<i>A. nemoralis</i> adults	First-instar larvae	
						<i>A. nemoralis</i>	<i>C. carnea</i>
<i>AkseBio2</i>	72.3	78.1	87.4	62.1	10.0	16.7	23.3
Water	2.8	3.2	7.8	4.3	3.3	6.7	10.0

^z Eggs 0–48 h old of both forms of pear psylla; *A. nemoralis* adults >1 week old; and first-instar larvae of both species were used in the tests.

When different life stages of pear psylla are compared on the basis of their mortality values, the order of susceptibility to the product in descending order was 1st and 2nd nymphal stages, eggs laid by summerform females, eggs laid by winterform females, and 3rd–5th nymphal stages.

Effect on non-target organisms There was no significant negative effect on the non-target organisms used in the assays. The order of sensitivity to the product was as follows: *C. carnea* first instars > *A. nemoralis* first instars > *A. nemoralis* adults. Based on the mortalities, young stages (first instars) were more susceptible to the product than adult stage. However, the same life stage (first instars) of both species had varying sensitivities (Table 3). Thus, mortality was species- and life-stage-dependent.

No phytotoxicity was observed during the study and there was no difference in appearance of *AkseBio2*-treated plants (even in young pear foliage) when compared with the controls.

The mechanism(s) involved in the ovicidal and nymphicidal actions of *AkseBio2* in this study are unclear and further study is required to gain more insight into the effects of the product. Since oils have long been known as oviposition deterrents (6,7,13), oviposition deterrence of the product could be attributed to an oily mixture. Observations of winter- and summerforms suggested that adult psylla had ambulatory difficulties on oily surfaces and preferred untreated portions (personal observation). However, few compounds other than oils have been evaluated for their ability to delay winterform and summerform pear psylla oviposition (11). Although phytotoxicity caused by the application of oils after the dormant period has been an obstacle to their use in spring and summer, the results of this

study showed that the product could be used safely in spring and summer applications.

The active ingredients in *AkseBio2* have no known toxicities to humans, and the plants from which the extracts are obtained have been used as pharmaceuticals and flavorings for centuries without any reported illnesses or side effects resulting from their use. They are therefore considered less harmful to humans than most conventional insecticides. Furthermore, studies have shown that they are readily biodegradable (2) and less detrimental to non-target organisms than pesticides (12).

In conclusion, applying *AkseBio2* – as well as other compounds – to pear leaves may be of value in pear psylla management programs by decreasing oviposition and feeding. In addition, the results obtained in this study may be useful in the search for novel, more selective, and biodegradable insecticidal natural compounds.

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