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Short communication. Insecticidal activity of different extracts of *Rhamnus dispermus* (Rhamnaceae) against peach trunk aphid, *Pterochloroides persicae* (Homoptera: Lachnidae)

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Abstract

Bark of *Rhamnus dispermus* Ehrenb (Rhamnaceae) was collected from Ash-Shoubak, Jordan, and extracted with hexane, chloroform, acetone and ethanol, respectively. The aim of this study was to test the insecticidal activity of these extracts against the peach trunk aphid (PTA), *Pterochloroides persicae* (Homoptera: Lachnidae), since it has been shown that *Rhamnus* spp. may contain compounds that can act as botanical pesticides. Three concentrations (100, 1000, and 10,000 ppm) of each dry extract were obtained by dissolving the dry extract in 0.01% solution of dimethyl sulfoxide (DMSO). Results showed that, at the highest concentration (10,000 ppm), all the extracts caused mortality in the PTA adults after 24 h of exposure. Both the acetone and ethanol extracts showed higher mortality (69 and 71%, respectively) than the hexane and chloroform extracts (40 and 56%, respectively) after 72 h of exposure at the highest concentration. In comparison, the synthetic control, Imidacloprid, killed 93% of the PTA adults. Nevertheless, extracts from *R. dispermus* provided valuable mortality rates for the PTA and can be used as botanical insecticides as part of the integrated pest management programs of this insect pest.

Additional key words: anthraquinones, botanical insecticide, flavonoids, IPM.

Resumen

Comunicación corta. Actividad insecticida de diferentes extractos de *Rhamnus dispermus* (Rhamnaceae) contra el pulgón negro de la madera, *Pterochloroides persicae* (Homoptera: Lachnidae)

Dado que *Rhamnus* spp. puede contener compuestos que actúen como pesticidas botánicos, se recolectó corteza de *Rhamnus dispermus* Ehrenb (Rhamnaceae) en Ash-Shoubak, Jordania, y se prepararon extractos de hexano, cloroformo, acetona y etanol, para analizar su actividad insecticida contra el pulgón negro de la madera (PTA), *Pterochloroides persicae* (Homoptera: Lachnidae). Se prepararon tres concentraciones de cada extracto seco (100, 1000, y 10.000 ppm) disolviendo cada extracto seco en soluciones dimetilsulfóxido (DMSO) al 0,01%. En la concentración máxima (10.000 ppm), todos los extractos produjeron mortalidad a los adultos de PTA después de 24 h de exposición. A las 72 h de exposición, los extractos de acetona y etanol a 10.000 ppm produjeron una mayor mortalidad (69 y 71%, respectivamente) que los extractos de hexano y cloroformo (40 y 56%, respectivamente). En comparación, el control sintético Imidacloprid produjo la muerte del 93% de los adultos de PTA. Sin embargo, los extractos de *R. dispermus* producen una mortalidad apreciable en los PTA y pueden ser utilizados como insecticidas botánicos como parte de los programas de manejo integrado de esta plaga.

Palabras clave adicionales: antraquinonas, flavonoides, insecticida botánico, IPM.

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Abbreviations used: df (degrees of freedom), DMSO (dimethyl sulfoxide), IPM (integrated pest management), LSD (least significant difference), ppm (parts per million), PTA (potato trunk aphid).

Aphids (Insecta: Homoptera: Aphidoidea) are a large group of insects. They are serious pests of a wide range of agricultural crops in that they can cause severe damage directly, by depriving the plant of its essential nutrients, or indirectly, by transmitting viruses (Blackman and Eastop, 2000). Most aphid species are host-specific and known to feed on a restricted range of host plants (Eastop, 1986). Peach trunk aphid (PTA), *Pterochloroides persicae* (Cholodkovsky) (Homoptera: Lachnidae), is also known as clouded peach bark aphid and cloudy-winged peach aphid (Stoetzel and Miller, 1998). Its main hosts include *Prunus* spp., such as the almond, apricot and peach trees; however, *P. persicae* has also been recorded to attack other plants including *Citrus* and *Malus*. *P. persicae* is found living on large branches and trunks of its host (Blackman and Eastop, 1994). Large populations of *P. persicae* occurring on the bark can cause fruit not to develop or premature fruit drop; this species produces large amounts of honeydew and is tended by ants (Stoetzel, 1994). *P. persicae* is not listed as transmitting a virus (Chan *et al*., 1991).

Control of this destructive insect poses serious problems. The greatest obstacle in the massive use of pesticides is their loss of efficacy caused by resistance development in insects (Georghiou and Mellon, 1983; Denholm *et al*., 1999). It is necessary to search for alternative strategies in pest control in order to circumvent existing resistance and minimize the danger of new resistance. Also, environmental aspects, like persistence of active compounds in soil, ground water and lakes, as well as effects on non-targets, have to be considered more consciously. The so-called “botanicals” (active substances or mixtures of substances) extracted from plants are desirable preparations that exhibit new modes of action and impair processes that are rather specific for the pests to be combated. Völlinger and Schmutterer (2002) elucidated that application of mixtures of active substances slows resistance development considerably.

Rhamnus dispermus is a medicinal plant that is mainly found in the southern parts of Jordan (Zohary, 1972). This plant contains both aglycones and glycosides of phenolic compounds such as anthraquinones and flavonoids (Abu Darwish, 2000). The aglycones of anthraquinones and flavonoids are soluble in chloroform and slightly soluble in hexane (Thomson, 1978). On the other hand, the glycosides of anthraquinones and flavonoids are soluble in polar solvents such as acetone and ethanol (Muzychkena, 1998). Abu-Darwish (2000) isolated and identified 11 compounds from barks of *R. dispermus* collected in Jordan: four anthraquinone aglycones (emodin, physcion, alaternin and 6-O-methoxyalaternin), four anthraquinone glycosides (franguilin B, glucofranguilin B, emodin-8-O-β-D-glucoside and alaternin-6-O-8-apiofuranosyloxy-8-O-L-rhamnoside), two flavonoid aglycones (kaempferol and quercetin), and one flavonoid glycoside (kaempferol-3-O- β- 8-glucopyranosyl-7-O- β-glucopyranoside).

This study aimed to test the insecticidal activity of *R. dispermus* against the PTA, since it has been shown that *Rhamnus* spp. may contain compounds that act as botanical pesticides, such as emodin (Trial and Dimond, 1979, Singh *et al*., 1992, Tsahar, 2001; Izhaki, 2002)

Bark of *R. dispermus* was collected from Ash-Shoubak, Jordan. Its botanical identification was confirmed by Dr. Talal Abu Rjae, at the University of Jordan, who keeps voucher specimens in his laboratory. The bark was air-dried at room temperature and finely ground. One hundred fifty grams of the crushed barks were extracted exhaustively in a Soxhlet extractor with hexane (purity 99.5%) and chloroform (purity 99.5%) to extract phenolic aglycones, and with acetone (purity 99.5%) and ethanol (purity 99.8%) to extract phenolic glycosides, respectively (Sullins *et al*., 1996). The extracts obtained were evaporated to dryness. The extraction yields were approximately 150–200 mg. Solvents were purchased from Fluka (Buchs, Switzerland). Each extract was kept in a small screw-capped glass tube over silica gel until used for insect bioassays. Bioassays were carried out within 2 weeks of extraction. Three concentrations (100, 1000, and 10,000 ppm) of each dry extract were prepared by dissolving the dry extract in 0.01% (v/v) dimethyl sulfoxide (DMSO) solution.

Two control treatments were used in each experiment. The negative control was 0.01% DMSO in water, since the 0.01% DMSO solution showed no significant mortality effect against the PTA (data not shown) compared with water. Imidacloprid (Confidor® 200SC, Bayer Crop Science, Jordan), a chemical insecticide, was used as a positive control treatment at the recommended field application rate of 0.25 mL L^{-1}.

The insecticidal activity of the above-mentioned extracts was tested against the PTA. Insects were collected in 2006 from the stone fruits orchard in Ash-Shoubak University College. A colony of aphids was established from a single apterous virginoparae adult in a glasshouse (at 25 ± 5°C) in Ash-Shoubak University College, on 2-year-old peach seedlings, *Prunus persica* (L.). Offspring were used to infest more peach seedlings. Subsequent colonies were reared in a glasshouse at 25 ± 5°C, and in a cycle of 16 h light:8 h dark (L16:D8), inside fine net cages to protect them from parasites.

A cut-shoot bioassay, adapted from the method developed by Desprez-Loustau (1990), was used. Colonies of PTA were established on 10-cm-long excised twigs under laboratory conditions (with temperature ranging from 20 to 25°C and relative humidity of 45 to 70%) from adult apterous virginoparae collected from the greenhouse. From the laboratory colonies, approximately 20 apterous parthenogenetic adults of PTA were taken to be placed on a damaged portion of an excised twig confined within a sleeve cage. The twigs with caged aphids were placed in plastic vials half-filled with water and placed on a test-tube rack. After one day, any newborn nymphs or adults failing to settle on the excised twigs were removed.

The excised twigs with aphids attached to them were dipped in the required solution for about 10 s, with five replicates at each concentration. Observations of mortality were carried out after 24 h and 72 h, at the same time each day, using a magnifying lens (×4). PTA adults that failed to settle on the excised twigs were considered as dead. The number of dead aphids and those failing to settle on each twig were counted along with the numbers left in the plastic vial attached to that plant. The percentage of PTA mortality was calculated by taking the number of dead adults and those failing to settle on the twig as a percentage of the total number of aphids before starting the tests. The experiment was repeated three times.

Arcsine-transformed percentages for each concentration and evaluation time were subjected to a one-way ANOVA, followed by a least significant difference (LSD) test at 95% confidence level (SAS Institute, 1995).

Within 24 h, the chloroform, acetone, ethanol, and hexane extracts of *R. dispermus*' bark powder, at a concentration of 10,000 ppm, produced significantly greater mortality ($F = 57.1$; $df = 5,12$; $P < 0.05$) in the PTA apterous adults with regard to the negative control (Table 1). During the same period, the synthetic control (Imidacloprid) produced 93% insect mortality, which was significantly greater than that of the *R. dispermus* extracts (Table 1). After 72 h, the acetone and ethanol extracts, but not the hexane and chloroform ones, resulted in significantly greater mortality ($F = 48.9$; $df = 5,12$; $P < 0.05$) in PTA adults than the negative control (Table 1). Within the same period, Imidacloprid caused 100% mortality in the PTA adults. This result was significantly higher than that obtained in adults exposed to the extracts (Table 1).

At an extract concentration of 1000 ppm, mortality of the PTA adults increased between 24 h and 72 h, but the treatments showed the same scenario in the two periods (Table 1). PTA mortality caused by the chloroform extract at a concentration of 1000 ppm, in contrast to the acetone, ethanol and hexane extracts used at the same concentration, was significantly higher when compared with the negative control (Table 1). The PTA mortality caused by the chloroform extract, however, was not as significant as that caused by Imidacloprid at this concentration.

Mortality of PTA apterous adults caused by Imidacloprid was also significantly greater with regard to the extracts at a concentration of 100 ppm and the negative control, at both 24 h and 72 h (Table 1). No significant differences were observed in the mortality rates of PTA adults between the chloroform, acetone, ethanol, and hexane extracts at this concentration, but all of them produced greater mortality of PTA apterous adults than the negative control (Table 1).

Anthraquinones, such as emodin, have numerous biological activities (Izhaki, 2002). Some of them exhibit a wide spectrum of ecological impacts by mediating biotic and abiotic interactions of plants with their environment (Izhaki, 2002). Abu-Darwish (2000) reported that emodin, in vegetative organs, may protect plants against herbivores, pathogens, competitors, and extrinsic abiotic factors. In the present study, all the *R. dispermus*' extracts tested (hexane, chloroform, acetone, and ethanol) showed significant insecticidal activity after 24 h against the PTA when used at the highest concentration (10,000 ppm). This activity could be the result of the aforementioned anthraquinone and flavonoid compounds found in this plant. In larvae of the gypsy moth, *Lymantra dispar*, the anthraquinone aglycone emodin caused pronounced mortality in 2–3 d (Trial and Dimond, 1979). Malicky *et al.* (1970) indicated that *Rhamnus cathartica* is attacked by very few insect species due to the presence of emodin and other anthraquinones. Emodin, the most abundant and active anthraquinone in *Cassia nigricans*, exhibited approximately 85% mortality on mosquito larvae *Anopheles gambiaea* and adults of the sweet potato whitefly, *Bemisia tabaci*, in 24 h (Georges *et al.*, 2008). In addition to anthraquinones, the PTA ingested a number of flavonoid compounds that were reported to have insecticidal activity against insects. Studies have shown that

Table 1. Percentage mortality (±SE) of apterous adults of peach trunk aphid (PTA), *Pterochloroides persicae*, subjected to different extracts from the bark of *Rhamnus dispermus* at concentrations of 10,000 ppm, 1000 ppm and 100 ppm

Extract	10,000 ppm		1,000 ppm		100 ppm	
	24 h	72 h	24 h	72 h	24 h	72 h
Hexane	$35.7^{b} \pm 5.9$	$40.0^{c} \pm 7.5$	$28.7^{c} \pm 4.3$	$42.0^{b} \pm 5.9$	$17.7^{b} \pm 4.7$	$33.3^{b} \pm 3.8$
Chloroform	$42.0^{b} \pm 9.0$	$55.7^{c} \pm 5.9$	$31.0^{b} \pm 2.0$	$43.3^{b} \pm 4.7$	$20.0^{b} \pm 4.0$	$35.7^{b} \pm 4.3$
Acetone	$35.7^{b} \pm 5.9$	$68.7^{b} \pm 8.1$	$20.0^{c} \pm 4.0$	$44.3^{b} \pm 11.3$	$20.0^{b} \pm 4.0$	$38.0^{b} \pm 5.9$
Ethanol	$51.3^{b} \pm 4.3$	$71.0^{b} \pm 5.9$	$20.0^{c} \pm 4.0$	$35.7^{b} \pm 5.9$	$22.3^{b} \pm 2.3$	$44.3^{b} \pm 5.9$
Imidacloprid	$93.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$93.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$	$93.0^{a} \pm 0.0$	$100.0^{a} \pm 0.0$
Water + DMSO	$0.0^{c} \pm 0.0$	$7.0^{d} \pm 0.0$	$0.0^{d} \pm 0.0$	$7.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$7.0^{c} \pm 0.0$
(F-value, df)	(57.13, 12)	(48.92, 12)	(136.85, 12)	(44.76, 12)	(120.36, 12)	(72.51, 12)
Significance	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001

Data were arcsine-transformed before subject to ANOVA. Means within the same column that have the same letters are not significantly different (P ≥ 0.05) using Least Significant Differences LSD.

partially purified flavonoids obtained from *Calotropis procera* are toxic to adults and eggs of *Callosobruchus chinensis*, an insect that attacks grain legumes (Salunke *et al.*, 2005). They are found to alter molting in insects causing death (Stamp and Yang, 1996). They also recorded to have an ovicidal effect due to chemical toxicity (Singh *et al*., 1978).

After 72 h, both the acetone and ethanol extracts from *R. dispermus* showed a significantly higher insecticidal activity than that obtained by the hexane and chloroform extracts at the same concentration (10,000 ppm). This could be due to the hydrolysis of anthraquinones and flavonoid glycosides to their aglycone, or non-sugar components, by insect enzymes or by other factors and which lead to an increase in the concentration of aglycones and free phenolic OH-groups. The phenolic OH-groups enable anthraquinones to interact with proteins by forming hydrogen and ionic bonds (Wink and Schimmer, 1999). This explains the various interactions of emodin with enzymes, transporters, channels, and receptors. As these interactions are possible with every protein, we would expect multiple targets and multifunctional activities (Wink and Schimmer, 1999). Also, flavonoids have a catecholic B-ring that seems to be responsible for the toxicant activity to insects (Onyilagha *et al.*, 2004).

The insecticidal activity of *R. dispermus* extracts was enhanced by increasing the concentration of the extracts and the exposure time. This could be ascribed to the synergistic interaction of various anthraquinones and flavonoids when exposure time or/and their concentration is increased. Yang *et al.* (2007) reported a significant synergistic interaction between physcion and chrysophanol on plant powdery mildew when the ratio of physcion to chrysophanol ranged from 1:9 to 5:5. The synergistic degree increased with increases in the chrysophanol proportion in the combination.

In conclusion, extracts from *R. dispermus* could be used as botanical insecticides in the integrated pest management (IPM) programs. Since this plant is used as a source of natural compounds for various pharmacological activities, the extracts obtained are expected to be of low or no hazard to human beings or other animals.

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