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Short communication. Repellency and toxicity of oils from different origins on *Myzus persicae* Sulzer (Homoptera: Aphididae) in pepper

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Abstract

Three trials have been carried out to assess the repellency and toxicity of oils of different origins on *Myzus persicae* in pepper plants: a summer mineral oil, refined oils of soya and rapeseed and a crude fish oil. The results obtained suggest that tested oils, in particular fish oil, are feeding deterrents for apterous aphids. These oils did not cause repellency to alate aphids, since the alates freely chose to settle on either oil treated or untreated plants. Only plants treated with the standard insecticide (Cypermethrin) were not colonized by the alates and their nymphs. On the contrary, direct spray of pepper leaves infested with apterous aphids showed that oils were toxic for the aphids, since 12 h after spraying the mortality rates were equal or above 70% in all cases. By this time, among all the oils, the mineral oil showed the best control efficacy (12 h; 89% mortality rate, statistically comparable with the standard). However, at 36 h mineral oil mortality rate (90%) was similar to that of fish and rapeseed oils.

Key words: mortality, pest control, soya oil, rapeseed oil, summer mineral oil, fish oil, Cypermethrine.

Resumen

Repelencia y toxicidad de aceites de distinto origen sobre *Myzus persicae* Sulzer (Homoptera: Aphididae) en cultivo de pimiento

Se han llevado a cabo tres ensayos con el fin de evaluar la toxicidad y efecto de repelencia de aceites de distinto origen (mineral de verano, colza y soja refinados, y un aceite bruto de pescado) sobre el pulgón *Myzus persicae* en cultivo de pimiento. Los resultados obtenidos parecen sugerir que los aceites ensayados, y en especial el de pescado, actúan en cierta medida como deterrentes alimenticios sobre los ápteros de *M. persicae*. Estos aceites aparentemente no repelen a los pulgones alados, puesto que éstos se establecen indistintamente sobre plantas tratadas con aceites y plantas testigo, siendo las sometidas al tratamiento con el estándar (Cipermetrina), las únicas que no fueron colonizadas por alados y por ninfas nacidas de ellos. Por el contrario, sí quedó demostrada la toxicidad de todos los aceites en pulverización directa sobre hojas de pimiento infestadas por pulgones. Doce horas después de la pulverización, todos los tratamientos con aceites dieron lugar a porcentajes de mortalidad iguales o superiores al 70%. Entre todos los aceites, el mineral fue el que alcanzó una mayor eficacia de control a corto plazo (a 12 h: 89% de mortalidad, comparable estadísticamente al estándar) aunque a las 36 h la eficacia de este tratamiento (90%) fue similar a la alcanzada por los aceites de pescado y colza.

Palabras clave: mortalidad, control de plagas, aceite de soja, aceite de colza, aceite mineral de verano, aceite de pescado, Cipermetrina.

The insecticide properties of mineral oils derived from petrol have been known for some time (Chapman, 1967) and have been used traditionally to control wintering forms of woody pest species, especially homoptera and acarids. In spite of these posing no danger to the environment and the auxiliary fauna, the potential of these oils has not been fully exploited, especially for use in the summer months during the vegetative period. New trends in crop protection are encouraging the search for harmless pesticides or pesticides with very low toxicity that have renewed the interest for the use of oils in agriculture. Recent research has studied toxicity, repellence and the potential use of summer mineral oils to inhibit oviposition in different pest species in several cases of homoptera insects (Larew and Loc-

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ke, 1990; Liu and Stansly, 1995a, 1995b; López *et al.*,1998; Rae *et al.*,1997; Sieburth *et al.*,1998). However, to date, few authors have studied the effects of other vegetable and fish oils on these same insect species (Hernández *et al.*, 2002; Puri *et al.*,1994). The present work shows the results of three experiments carried out to evaluate the effect of repellence and toxicity of four oils of different mineral, vegetable and fish origins against the aphid *Myzus persicae* Sulzer. It should be noted that the methodology used in repellence experiments responds to two different models of «choice-test» that have the common objective of establishing the preference of released insects confined to a plate or cage for different treated and untreated plants or plant parts (Larew and Locke, 1990; Riedl *et al.*,1995; Liu and Stansly, 1995b; Isman, 2000; Hummelbrunner and Isman, 2001).

The oils studied were: a summer mineral oil; two refined vegetable oils, rapeseed and soya oil; and a raw fish oil. The mineral oil, a highly refined paraffin oil, is commercialized by the company Agrichem S.A. under the brand name of SUNSPRAY® Ultrafine (summer mineral oil 85% w/v), and is formulated as a emulsionable concentrate. The manufacturer's recommended dose for horticultural crops, including pepper, is 0.75%-1.5%. A dose of 1% was applied, as for the other oils, based on efficacy and phytotoxicity data obtained in other experiments. For vegetable and fish oils, when preparing the spray mixture Tween 20® was added as an emulsifying agent in a ratio of 10% Tween 20®, for the total volume of oil added.

Cypermethrine 10% w/v (SELENE®), a pyrethroid formulated as an emulsionable concentrate and marketed by the laboratory Key S.A. was used as the standard insecticide. Its use is recommended for the control of different pests including aphids. The dose used was the maximum dose recommended by the manufacturer, 100 cm^3 hl^{-1}.

The aphids used in the experiment came from a clone of the species *M. persicae* Sulzer, kept on pepper plants of the variety California Wonder, in a temperature chamber with a photoperiod of 16:8 h (light:dark) and temperatures of 22:16ºC (day:night).

Pepper plants cv. California Wonder were grown in individual pots or in feed trays, using turf mixed with vermiculite in equal quantities. Plants were kept in a temperature chamber under constant environmental conditions with a photoperiod of 16:8 h (light:dark) and temperatures of 24:16ºC (day:night). In the case that whole plants were required for the experiment these were used in the four true leaf stage, and when pepper leaves were used these were taken in the 8-10 true leaf stage, always using the central leaves of the plant.

First, a repellence experiment was carried out to assess the selection response of aphid *M. persicae* individuals when these are exposed in equal conditions to plant leaves treated with four oils. To do this, emulsions of the oils and the standard were prepared in water and 10 leaves of the pepper plant were submerged in each of these. After leaving the leaves to dry, using a 1 cm diameter perforating punch, 2 discs of leaves were obtained for each treated leaf. A total of 10 petri dishes (10 cm in diameter) were prepared by placing on them two layers of damp filter paper, then two leaf discs for each treatment, randomly forming a circle around the edge of the dish. Also, using a paintbrush, 200 aphids of the same age (7-9 days) were collected and were submitted to 1 h of fasting. After this, 20 aphids were released in the center of each dish. The dishes were then covered and sealed to prevent the aphids' escape and were placed on a flat surface with uniform lighting, at room temperature (22-24ºC). The assessments consisted in counting at different times (1, 3, 6, 12, 24 and 48 h) the number of aphids on each leaf disc, and recording whether these were alive or dead. The nymphs born on the leaf discs were not included in the counts and were removed to preserve the turgency of the leaf discs for as long as possible. Data were examined using analysis of variance or ANOVA (for a significance of 5%), followed by a comparison of means by the Scheffé multiple range test (Day and Quinn, 1989), before changing the variable «$\sqrt{(x+1)}$», to obtain a better fit of the normality hypothesis and homoscedasticity.

Table 1 shows the results of this experiment. The aphids showed a clear preference for the control leaf discs and a growing rejection of the discs treated with any of the oils. In accordance with this, until 24 h after release of the aphids, the number of those on the discs treated with fish, rapeseed and soya oils was significantly lower than those on controls. The same occurred for the mineral oil but only at 1, 3 and 12 h. This oil in general showed a worse long term behavior. In contrast, the fish oil was the most repellent to aphids and was the only treatment which showed significant differences compared to control discs in the last experiment (at 48 h) in spite of the reduced number of aphids on these discs, which were the most dehydrated owing to their having served as a food source to a larger number of aphids during the experiment.

Table 1. Number of live aphids (mean ± standard error) at different times since their release on petri dishes, on the leaf discs treated with the oils studied (n = 20)

Treatment	1 h	3 h	6 h	12 h	24 h	48 h
Rapeseed oil	1.0 ± 0.09 a	0.4 ± 0.05 a	0.3 ± 0.05 a	0.3 ± 0.04 a	0.3 ± 0.05 a	0.2 ± 0.04 ab
Mineral oil	0.7 ± 0.06 a	0.3 ± 0.05 a	0.7 ± 0.07 ab	0.5 ± 0.06 a	1.1 ± 0.09 ab	0.5 ± 0.08 ab
Fish oil	0.7 ± 0.08 a	0.4 ± 0.05 a	0.5 ± 0.06 a	0.2 ± 0.03 a	0.6 ± 0.07 a	0.1 ± 0.02 a
Soya oil	0.8 ± 0.09 a	0.5 ± 0.05 a	0.5 ± 0.06 a	0.5 ± 0.06 a	0.3 ± 0.05 a	0.2 ± 0.04 ab
Cypermethrine	0.4 ± 0.06 a	0.4 ± 0.06 a	0.2 ± 0.04 a	0.3 ± 0.05 a	0.1 ± 0.03 a	0.1 ± 0.03 ab
Control	3.0 ± 0.14 b	2.5 ± 0.15 b	1.9 ± 0.13 b	1.9 ± 0.12 b	2.8 ± 0.16 b	0.8 ± 0.07 b

Different letters in the same column indicate significant differences ($P \leq 0.05$) between treatments according to ANOVA and Scheffé test.

On the other hand, it is noteworthy that in all cases the standard insecticide revealed a statistically comparable behavior to that of oils in relation to the number of aphids on the leaf discs. In contrast, a large number of dead aphids was observed around these discs. Pyrethroids are compounds with insect repellent properties but there was a surprising and continuous mass displacement of aphids towards the leaf discs treated with Cypermethrine. Therefore, in the last assessment around 70% of all the aphids released, most of which were dead, were situated around or near these. Dead aphids in each assessment were found in a percentage of 50 to 100% on or around the leaf discs treated with insecticide. Dead aphids were not found on or around the leaf discs treated with oils until the last assessment with the exception of mineral oil, for which a low degree of mortality was recorded, much lower than that recorded with Cypermethrine.

In a second experiment, oil repellence on winged aphids was studied. To do this, 120 winged specimens (previously submitted to 1 h of fasting), were released into the cage that contained pepper plants sprayed previously with the oils studied, as well as control plants non-treated, and plants treated with the standard insecticide. The distribution of plants inside the cage followed a random block design with four replicas. Four plants were used for each treatment, arranged in two rows for each block. Plants were treated with 1 liter manual sprays, wetting them well over their whole surface to run off. After drying the plants these were placed in a cage that was made of a wooden frame 53 cm (high) × 46 cm (wide) × 66 cm (long). The cage, where three of its four sides were made of glass, was also closed with a transparent glass top. The remaining side was covered by a net curtain attached to the wooden frame by a strip of adhesive Velcro. In the inside upper part of the cage a structure was hung in which the boxes containing the captured and fasting aphids were placed. After locating the plants in position, the tops were taken off the boxes to allow the aphids to escape. The experiment was carried out at room temperature ranging from 18 to 30ºC. Experimental assessments consisted in counting at 1, 2, 3 and 7 days, the number of winged aphids on each plant and the number of nymphs born. The statistical analysis of the data was the same as in the previous case.

As can be observed in Table 2, significant differences were not obtained between the different oil treatments, or between these and controls for the two variables studied. The treatment with the best behavior

Table 2. Winged insects on the pepper plants and nymphs born and alive (mean ± standard error), 1, 2, 3 and 7 days after treatment (dat) and release of winged insects inside the cage (n = 16)

Treatment	Winged insects				Nymphs			
	1 dat	2 dat	3 dat	7 dat	1 dat	2 dat	3 dat	7 ddt
Rapeseed oil	1.6 ± 0.15 ab	1.8 ± 0.07 ab	1.5 ± 0.20 ab	0.6 ± 0.07 a	1.1 ± 0.22 a	6.1 ± 0.38 ab	7.6 ± 0.45 ab	5.4 ± 0.42 a
Mineral oil	1.1 ± 0.08 ab	1.1 ± 0.08 ab	0.8 ± 0.07 ab	0.6 ± 0.13 a	0.5 ± 0.10 a	3.5 ± 0.23 ab	4.3 ± 0.22 ab	2.8 ± 0.27 a
Fish oil	1.1 ± 0.15 ab	1.0 ± 0.14 ab	1.0 ± 0.14 ab	0.4 ± 0.09 a	1.3 ± 0.19 a	4.0 ± 0.38 ab	5.1 ± 0.46 ab	3.0 ± 0.22 a
Soya oil	2.1 ± 0.14 a	2.0 ± 0.14 a	2.4 ± 0.14 a	0.3 ± 0.07 a	1.3 ± 0.24 a	6.5 ± 0.36 a	8.8 ± 0.44 a	3.0 ± 0.26 a
Cypermethrine	0.0 ± 0.00 b	0.0 ± 0.00 b	0.1 ±0.05 b	0.1 ± 0.05 a	0.0 ± 0.00 a	0.0 ± 0.00 b	0.0 ± 0.00 b	0.0 ± 0.00 a
Control	1.1 ± 0.15 ab	1.5 ± 0.16 ab	1.4 ± 0.14 ab	0.4 ± 0.07 a	1.4 ± 0.19 a	5.4 ± 0.36 ab	7.0 ± 0.42 ab	5.4 ± 0.39 a

Different letters in the same column indicate significant differences ($P \leq 0.05$) between treatments according to ANOVA and Scheffé test.

was the standard insecticide, Cypermethrine. During the two days after release of the aphids, none of these were found on plants treated with Cypermethrine and live nymphs were not observed on these plants at any time. Nevertheless, differences were not obtained between treatments with oils and Cypermethrine except for soya, the oil with the poorest behavior during the whole experiment, coinciding in general with the highest numbers of winged insects and nymphs. Fish oils and mineral oils were the ones that gave the best and very similar results, except that reproduction of winged insects was slightly lower on plants treated with mineral oil. Rapeseed showed an intermediate behavior that was very similar to that of soya in relation to the number of live nymphs counted on the plants.

In a third experiment, the insecticide power of the oils applied in a direct spray on *M. persicae* was studied. To do this, 60 uniform-sized leaves of California Wonder pepper plants were collected. After wrapping their petioles in cotton wool wet with distilled water they were individually placed in the bases of Petri dishes on two layers of slightly dampened filter paper. Next, the leaves were infested with 10 adult aphids of 7-9 days old. After, leaves with aphids were sprayed uniformly on the upper and lower sides. Immediately afterwards, the dishes were covered and sealed with transparent film and left in a temperature chamber with a photoperiod of 16:8 h (L:D) at temperatures of 22:16°C (D:N). In total, 10 aphid infested leaves were sprayed per treatment, including the standard insecticide and control, that were sprayed with water. Counts were carried out at 12 and 36 h, and the number of dead aphids on each leaf was estimated. The statistical analysis of the data consisted in a one-way analysis of variance or ANOVA (with a significance of 5%), followed by a comparison of means by the Bonferroni test (Zar, 1999), before transforming the data by changing the variable «arcsen$\sqrt{x}$» to obtain a better fit of the normality hypothesis and homoscedasticity. These tests were applied in all cases to mortality percentages corrected by Abbot's formula.

Table 3 shows the corrected mortality data obtained in the two experimental evaluations. It is clear that 12 h after spraying the leaves with aphids, all treatments produced a high mortality (equal to or higher than 70%), with significant differences compared to the control in all cases. The most effective treatment was the insecticide Cypermethrine that caused 100% mortality of the aphids on the treated leaves. In the first experiment, the only oil comparable to Cypermethrine was mineral oil. This showed the best short-term behavior with a mortality of around 90%. Nevertheless, this percentage or a similar one, was also reached by fish and rapeseed oils at 36 h. Soya oil is once again the least effective treatment and the only one not comparable to Cypermethrine in the second experiment.

Table 3. Percentage of aphid mortality corrected by Abbot's formula at 12 and 36 h after spraying the infested leaves

Treatment	Mean ± SE	
	12 h	36 h
Rapeseed oil	78.5 ± 0.08 bc	89.4 ± 0.08 bc
Mineral oil	88.6 ± 0.08 cd	90.2 ± 0.09 c
Fish oil	81.4 ± 0.08 bc	88.0 ± 0.09 bc
Soya oil	70.0 ± 0.04 b	74.6 ± 0.03 b
Cypermethrine	96.9 ± 0.05 d	100.0 ± 0.00 c
Control	3.5 ± 0.05 a	16.4 ± 0.07 a

Different letters in the same column indicate significant differences ($P \leq 0.05$) between treatments according to ANOVA and Bonferroni test. SE: standard error.

From the above it can be concluded that all the oils tested have shown a degree of deterrence against the feeding of *M. persicae* aphids. However, the treatment of pepper plants with these oils does not seem to prevent colonization by winged insects which feed or reproduce on treated leaves. Other works have shown repellency activity on winged homoptera that tended to avoid areas or plants treated with oils. Larew and Locke's (1990) observed how whiteflies of the species *Trialeurodes vaporiarorum* landed on and then immediately abandoned chrysanthemum plants treated with summer mineral oil, suggesting that this repellence was more tactile than olfactory. As well as this tactile repellence that impedes movement and, in the case of homoptera, penetration of the plant with the stylus, for some of the oils, such as the fish oil, there also seems to be olfactory repellence since the strong odor that this oil emits in treated vine plots caused the difference in captures of the thrips, grape berry moth and grape leaf-hoppers in treated vine plots in comparison with those treated with mineral oil and raw soya oil, as shown in a previous work (Hernández *et al*., 2002). Also, in vine, and on *Jacobyasca lybica*, López *et al*. (1998) observed important declines in insect populations in plots treated with summer mineral oil attributable to a direct control effect possibly in addition to a repellence effect. In previously mentioned works, experiments were carried out on large insect populations and clear differences were observed between treated and untreated plants or plots. Better results than those obtained in this study could be achieved using a greater density of alate *M. persicae* and by increasing the separation between plants to reduce interferences between treatments with the different oils.

Numerous studies about the direct toxic effect of oil sprays on different insect pests have, in general, like our study, given good results. As well as those cited previously, for homoptera and summer mineral oils it is worth noting the work of Rae *et al*. (1997), who observed high levels of mortality on the psylla *Diaphorina citri*. The control efficacy of mineral oil treatments on different whitefly species has also been demonstrated (Larew and Locke, 1990, Liu *et al*., 1995a; Sieburth *et al*., 1998) and of oils from other origins such as cotton seed oil (Puri *et al.*, 1994). Although the main mode of action of the oils on insects is by asphyxia, it should be taken into account that vegetable oils may also contain toxic compounds that can increase the efficacy of treatments and have the advantage compared to mineral oils of their natural origin. It is, therefore, interesting to continue studying these substances to find those with the best properties for use in crop protection.

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