

Comparison the Effect Insecticide of the Oils of Six Plant Extracts on the Aphids in Alfalfa Green

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Abstract

The green alfalfa is the culture more used as livestock feed in the Ziz Tafilalt region in the south-east of Morocco. But the large aphid pest caused major damage to this culture, which pushes the peasants to use of synthetic insecticides in great quantity during the period of spring and the summer. The major problem in the production of alfalfa is the infestation by aphids. They transmit viruses of the tile to the plants. They are more likely to be a danger in the cool weather. The study presents the effects of products Kanuka, Ravintsara, Tea tree on the one hand and of products Thyme, Oregano, Neem on the other hand on the aphids of green alfalfa. The tests were conducted of the months from May to September on the aphids of the alfalfa fields green. It determines the percentage of deaths of aphids in function of the dose 1% and 5% of these products and the time 3, 7 and 11 hours after treatment. A comparison between the results obtained showed that the Oregano products followed by the Neem and thyme are more active.

Keywords: alfalfa, insecticide, aphid, kanuka, ravintsara, tea tree, oregano, thyme, neem

1. Introduction

In the Mediterranean regions such as Morocco, mainly in the luzernieres, it is feared the abundance of the Cagot, amateur snail of dry environment (to affinity Xerophilic). It creates a very large discomfort during of the mechanical harvesting of pods. Among the insects, some are real pests, responsible for losses in the quality and quantity of forage or productivity. The aphid of alfalfa is often present. It overwinters in collar and colonized then stems, leaves, inflorescences and pods.

In the culture of green alfalfa, parasites such as the aphids cause especially of damage to the leaves, causing discoloration. They attack the young shoots and the buds. A severe attack led to the fall of the leaves. In Morocco, a few species of aphids have been found in large quantities on the alfalfa fields green.

Based on studies in Quebec, the parasitic diseases the most important leading the development of foliar spots among the alfalfa. The symptoms appear first on the basal leaves. The spots are circular (diameter 2 mm), brown to dark brown, with an irregular margin (finely serrated). On the upper face of the spots, the fungus produces a fruiting which is in the form of a tiny point raised brown, located in the center of the stain. The affected leaves yellowing and fall (Michel Lacroix, 2002).

Of the larva to adult, all stages feed on the underside of the leaves. They can affect the plant in different ways: reduction of photosynthesis, injection of phytotoxic substances while they are fed, accumulation of faeces and paintings on the plants that can affect the appearance of plants. All of these effects have consequences for the decrease of the usable quantity of plants and therefore causes great economic losses (Johnson and Lyons, 1991).

Damage from aphids can be direct or indirect. Direct damage to plants occurs from the feeding activity of aphid nymphs and adults. Aphids pierce the plant tissue and extract sap, which results in a variety of symptoms, including decreased growth rates and reduced vigor; mottling, yellowing, browning, or curling of leaves, and wilting, low yields, and plant death. Indirect damage is also caused by the ability of some aphid species to serve as virus vectors. However, the viruses transmitted by aphids can cause severe losses. Under favorable conditions, these viruses can cause a high rate of crop failure and severe economic losses (Barbercheck, 2011).

Even if pesticides have contributed to increase crop yields notably fighting against pests, the side effects of the use of pesticides are many: effects on the health of the people, the wildlife and flora; contamination of the water, soil and air. The World Health Organization advance the impressive figure of 3 million poisonings each year in the world due to pesticides with 220 000 dead in total (Bouguerra, 1986).

Horticultural oils can also be applied against aphids and aphid-transmitted viruses. These oils are useful in interfering with the transmission of the viruses. They should be applied early in the growing season (two weeks after planting) as aphids are known to colonize plants shortly after germination. Tank mixes of horticultural oils and insecticides have also been used to enhance the control of non-persistently transmitted viruses (Katis, 2007). In organic systems, horticultural oils and soaps are effective when applied regularly with a drop nozzle (to get the undersides of leaves) and high pressure, but these must be applied regularly and may be cost prohibitive. Neem and pyrethrin are other options, but care must be taken when using these broad-spectrum insecticides to prevent harm to beneficial insects (Liburd and Nyoike, 2008).

Several plants provide natural insecticides, but their extent and their specific action often have led us to focus our research on the Kanuka, Ravintsara, Tea tree, Oregano, Thyme and Neem. These plants are also used for many uses. The growing interest in the use of pesticides based on extracts from these plants in the world is motivated by their effects comparable to those of chemical pesticides (Mouffok et al., 2007/2008).

This work has for objective to make a comparison the effect of insecticide products Kanuka, Ravintsara, Tea tree, Oregano, Thyme and Neem on the aphids of alfalfa green in order to reduce the damage caused by these parasites in protecting the environment and in assessing the effect of insecticide natural products used in this study.

2. Materials and methods Ionic

2.1 Alfalfa

The common name is Alfalfa (Lucerne) and latin name is *Medicago sativa*. The family name is Fabaceae/Leguminosae (Pea family). Its scientific name: *Medicago* is neighbor of the Latin designation: *medica* (Virgil and Pliny). This name is derived from the Greek: *Medike* which designated the origin of this plant, introduced of the Medes after the expedition of Darius, cited by Theophrastos in his book: *Research on the plants* (Remi Coutin, 2001).

Alfalfa has a root system swivel, very developed and deep (up to two meters): it allows to fragment the soil and improve its structure. These qualities induce farmers to place this culture in head of rotation. Alfalfa has many environmental benefits as the subtraction of inorganic nitrogen in the process of leaching, the treatment of effluents rich in nitrogen and the positive impact on biodiversity. It is also a strategic stake in economic independence and protein for the feeding (Thiebeau et al., 2003).

2.2 Substances used as natural insecticides

Reagents used in this work have been provided by Herb'Atlas, supplier of natural products, organic and conventional essential oils.

2.2.1 Kanuka essential oil (EO)

Kanuka essential oil is anti-bacterial, anti-fungal and anti-inflammatory. Kanuka (*Kunzea ericoides*) belongs to the Myrtaceae family and is originally from New Zealand. The major components of the oil are: α -pinene + α -thujene (62.26 %), globulol + viridiflorol (5.40 %), 1,8-cineole (4.25 %) and para-cymene (3.89 %).

2.2.2 Ravintsara essential oil (EO)

Ravintsara (*Cinnamomum camphora*), grown in Madagascar, is a large evergreen tree from the Lauraceae family. It has a broad spectrum of properties, being anti-viral, anti-bacterial, anti-fungal, immunostimulant, etc. The oil is obtained from the leaves by steam distillation. Its major components are 1,8-cineole (48.2 %), sabinene (7.05 %), α -terpineol (6.25 %), α -pinene (3.4 %), terpinen-4-ol (3.15 %) and β -pinene (2.4 %).

2.2.3 Tea tree essential oil (EO)

Tea tree oil, also called melaleuca is taken from the leaves of the *Melaleuca alternifolia*, which is native to the northeast coast of New South Wales, Australia. The major components of the oil are terpinen-4-ol + β -caryophyllene (42.72 %), γ -terpinene (18.44 %) and α -terpinene (8.61 %). The Tea tree belongs to the botanical family of the Myrtaceae.

2.2.4 Oregano essential oil (EO)

The Oregano used, *Origanum compactum*, is widely available in the North of Morocco. The method used for obtaining the essential oil of Oregano is hydro-distillation by steam distillation. Its major constituents are carvacrol (32.14 %), thymol (21.42 %) and γ -terpinene (18.80 %).

2.2.5 Thyme essential oil (EO)

The species used in this work is *Thymus satureoides* (Moroccan red Thyme), an endemic plant found in forest clearings, scrub and matorrals of low and medium mountains. The thyme essential oil is obtained by hydro-distillation by steam distillation. The major components of the oil are α -terpineol + borneol (39.23 %), camphene (9.25 %), carvacrol (7.93 %) and terpinen-4-ol + β -caryophyllene (7.06 %).

2.2.6 Neem vegetable oil (VO)

The botanical name of Neem, also known as Indian Lilac, is *Azadirachta indica*. Neem is an evergreen tree native to India, Burma, Java and the Lesser Sunda Islands (Mouffok et al., 2007/2008). Neem oil is obtained by cold pressing and sand filtration. The active molecule is azadirachtin (0.29 %).

2.3 Description and characterization of the aphids

2.3.1 Name and identification of aphid

The latin name are Adelgides, Aphidides, Eriosomatides, Phylloxerides and the Common name is Aphids. Aphids belong to the insects, more precisely to the Homoptera order and Aphididae family. They are polyphagous, sucking biting insects. Aphids are usually soft body, pear-shaped. A single morphological character distinguishes them from other insects is the presence of cornicles.

They were identified with a magnifying glass of 8x and they present the following characteristics: 0.25 mm – 2.5 mm long, dark and light green head, dark and light green chest, yellow-green and light green abdomen.

2.3.2 Aphids in the alfalfa

The aphids in the alfalfa are the alfalfa aphid (*Macrosiphum creelii*), blue alfalfa aphid (*Acyrtosiphon kondoi*), green peach aphid (*Myzus persicae*), pea aphid (*Acyrtosiphon pisum*) and spotted alfalfa aphid (*Therioaphis maculata*) (Knowles, 1998). The information in Table 1 and Figure 1 (page 7 and 8) can be used to assist in the identification of aphids commonly found on alfalfa (Kinney and Peairs, 2011).

2.4 Experimental conditions and method

2.4.1 Conditions

The tests have been done from early May to late September in alfalfa green fields. The geographical area chosen is near the town of Erfoud; Ziz Oasis named Tafilalt southeast Morocco. The area of fields ranged from 0.1 to 0.5 hectare. In order to carry out these experiments random plots of 1 m² were taken, mutually separated by 10 m for sufficient insulation.

2.4.2 Experiments and procedures

The experiments consist of evaluating the mortality of aphids in the presence of dilute solutions of oils using a methodology inspired by the protocol of the World Health Organization (WHO, 1985). In that way, aphids parasitizing fields of 1 m² surface were taken immediately after treatment in 25×40 cm² clear plastic bags for later counting in the laboratory.

Previous experiments allowed selecting a range of concentrations for the tests. According to this, stock solutions of each oil sample were prepared in pure water, and from these solutions the final test dilutions were made at different concentration percentages (v/v) (1 % and 5 % oil in pure water).

Each plot was sprayed with 100 ml of a solution (oil + water + 1 ml of liquid soap per liter of solution as an emulsifier) by use of a manual sprayer. In order to verify the reproducibility of the results each test was repeated four times. A control sample of 100 ml of pure water and emulsifier enables to measure the natural mortality at the same experimental conditions. The count of dead aphids on the last 20 cm of plants taken in a 1 m² surface area has been accomplished by means of a magnifying glass 8x, and this 3, 7 and 11 hours after treatment. The same procedure was conducted for the other plots and concentrations (1 % and 5 %).

3. Results and Discussion

3.1 Results

The application of the studied oils on the aphids at different concentrations causes mortality during the first hours after treatment as shown in table 2 (page 8). Each mortality percentage ($m \pm SEM$ where m is the mortality and SEM is the Standard Error of Measurement) presented in table 2 is the average of sixteen tests which have the unavoidable uncertainty of the measurement.

Table 2 shows that after hours of experience the control did not exceed 14.43 % mortality in all tests. We see that by the dose 1 % or 5 % mortality is low in 3 h for the Neem oil and it is strong for the Oregano. These mortality rates are almost stabilized at the end of each test, which proves that the effect of the products is fast compared with that of other extracts such as *Melia volkensii* or the effect is observed on two weeks (Diop and Wilps, 1997).

After three hours of treatment, for each product the mortality rate increases as a function of the concentration but does not exceed 44.38 % for the oil of Oregano to low concentration, Ravintsara (43.33 %), Thyme (40.64 %), Tea tree (39.16 %), Kanuka (37.5 %) and Neem (36.94 %). But to the dose 5 % the mortality rate is 54.43 % for the Oregano and Thyme, Neem, Ravintsara, Tea tree and in the last of Kanuka oil with 35 % of mortality. In addition, the essential oil of Oregano seems more active.

After eleven hours of treatment the variation of the mortality rate of aphids in function of the concentration evolved weakly by comparing with the previous case (three hours). The mortality for the dose 5 % is the 95.27 % of the Oregano and then Neem, Thyme, Tea tree, Ravintsara and Kanuka with 70 % of mortality. But for the dose 1 % the mortality is 94.7 % of Oregano then Neem, Thyme, Ravintsara, Kanuka and Tea tree. The Oregano remains more active followed by the Neem which becomes active after long duration.

From these results, the essential oil of Oregano and Thyme seem to be the most active in first hour. Also the Oregano is still active but the Thyme is not or the Neem becomes active in the long term. It is observed that the mortality varies little even at a high dose and long duration. It is likely to remain far from the great values of fatalities for the Kanuka oils, Ravintsara and Tea tree.

To evaluate more precisely the insecticide activity of these products against aphids, it was calculated the TL_{50} and the TL_{90} , defined as the time causing lethal respectively 50 % and 90 % of mortality in the population of aphids treated. These values were determined from an experimental curve giving the variations of the percentage of mortality to 1% and 5% depending on the increasing time in the table 3 (page 9). In addition we calculated the LC_{50} and LC_{90} as the lethal concentrations causing 50 % and 90 % of mortality in the population of aphids treated in table 3.

3.1.1 Lethal concentration causing 50 % and 90 % of mortality (LC_{50} and LC_{90})

After eleven hours of the treatment we reached a 50 % mortality of aphids to low dose 0.2 % of Kanuka and then

to 0.5 % of Ravintsara but it was 90 % of mortality that at a dose close to 7 %. It was the mortality of 50 % of the aphid after eleven hours of treatment from the concentrations close to 1 % of Oregano and Neem products. We reached a 90 % mortality of aphids after eleven hours of the treatment from the low dose from 4 % of the Oregano and then the Neem to 4.1 %. Then the product Kanuka seems more active followed by Ravintsara to low dose.

3.1.2 Lethal time causing 50 % and 90% of mortality (TL₅₀ and TL₉₀)

The mortality of aphids reached 50 % for the dose 1 % of Oregano from 4 hours and then the Neem products from 4.5 hours. Also for the dose 5 % of Oregano from 3.5 hours then the Neem from 4.25 hours. For the dose 1 % we have that the Oregano gives a mortality rate of over 90 % from 8.75 hours then the Neem from 9 hours. At a dose of 5 % was this mortality for the Oregano product from 8.5 hours and then the product Neem from 8.75 hours.

It can be inferred that the product of Oregano is the most deadly of aphids in alfalfa fields followed by the Neem. More than 13.75 hours after treatment to achieve mortality rate of 90 % for all products. This shows that all products even at low concentrations are already active after a dozen of hours.

After treatment, the mortality is quick, with the high dose and reached a value of close to 90 % in alfalfa green but remains less rapid for the low dose by contribution to the witness. The comments hour after hour show a very low activity of aphids treated with the dose 5 % which implies that the concentrated action of the oil on the ground can increase the rate of mortality. However, the death of aphids is preceded by a paralysis of the first hours. To the control the vegetation has not been renewed among aphids that had continued to consume their food.

3.2 Discussion

It can be assumed that the mortality is mainly due to the various active compounds containing in these products, the dose used and the processing time of aphids. The increase in dose makes the oil very active against aphids, this can lead to dilution and a modification of the metabolism. This is demonstrated by the assumption of Isman (Isman, 1999) or the essential oils act directly on the cuticle of insects and mites to soft body because they appear to be more effective on the arthropods to soft body as the case of the Facin who exercised a suppression satisfactory on thrips, aphids, the a whitefly and some mites.

Butler and Henneberry (Butler and Hennberry, 1990) have tested a solution of 5 to 10% of the oil from the seeds of cotton on the aphids of the cabbage, the couple, the thrips and the to legionnaire in the beet. The oil from the seeds of cotton has reduced up to 91 % the number of larval legionaries on the bette to carde. The couple have been significantly reduced for 7 days on the celery with a solution to 5 %. The populations of aphids were 86 % less on the seedlings of cabbages rosettes on the treaties that witnesses 16 days after the treatment.

In relation to the witness, it is the treatment of aphids by the Oregano, the Neem and the Thyme in alfalfa which are located the most affected. These two cases in which the concentrations were sufficient to cause the death which has engendered of disturbances in the behavior of the insect. The oils of Oregano, Basil, Marjoram, Thyme, Sage, Laurier, Rosemary, Lavender, of Anise, Mint, Celery, Cumin, Citrus Fruit, Coriander and Fennel have been tested and several have caused up to 100 % of mortality in the small borer of cereals, *Rhysopertha dominica* (F.), the saw-toothed grain beetle, *Oryzaephilus* are important for *Virola surinamensis* which is (L.), the red flour beetle, Beetle castaneum (Hbst.) and the rice weevil (Shaaya et al., 1991).

4. Conclusion

A low dose the Kanuka, Ravintsara and then the Tea tree seem active but are not at a high dose. It can have a complexation between the different compounds which gave a decrease of the insecticide activity. This will allow to observe the different changes on the qualitative and quantitative terms of the essential oils in order to estimate to what conditions or at what period and to what culture such or such essential oil could give a satisfactory performance or have an interesting activity.

The results of our study have allowed to say that the dose 1 % of the products applied on aphids does not have much of an impact, this may be due to two reasons: the dose is insufficient and/or these pests are more resistant. For the dose 5 %, all samples showed an interesting activity on aphids. With time, the extracts of the Oregano, Neem and Thyme in alfalfa green being the samples the most effective and reaches a mortality rate more than 90 % for the high dose.

In the Mediterranean basin, we are met by a very large number of aromatic plants. Its climate rich in brightness and heat, accompanied with the seasons marked, requires on the part of the plants of efforts adaptives favorable to a molecular evolutionary richness conferring on them to multiple properties, among other the insecticide effect (Penoel, 1994).

In effect, the natural extracts of plants are a true wealth and can be the cause of a large number of substances insecticides exploitable in the control of pests (Isman, 2001). In this context, the use of natural molecules of interest (ecological and economic) to the insecticidal properties of lesser toxicity in man, is proving to be an alternative approach to the use of insecticides of synthesis.

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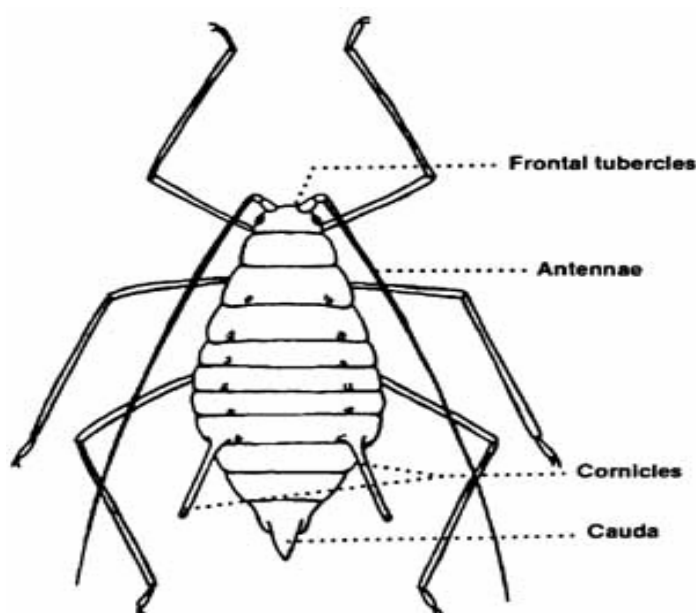


Table 1. Characteristics of common aphids on alfalfa

Species	Characteristics
Alfalfa aphid (<i>Macrosiphum creelii</i>)	pale green, darker along center line, cornicle dusky at tip, June-August, rare but found throughout state.
Blue alfalfa aphid (<i>Acyrtosiphon kondoi</i>)	blue, blackish-cornicles, towards tips, rare.
Clover aphid (<i>Nearctaphis bakeri</i>)	dull yellowish-green to pinkish, dark-green or rusty brown specks, pale yellow cornicles with orange blotch at base, found on clovers, March-December, common throughout state.
Cowpea aphid (<i>Aphis craccivora</i>)	polished black, black cornicles, found on various Crops, March-November, common throughout state.
Green peach aphid (<i>Myzus persicae</i>)	pale yellow to green, cornicles dusky at tips, common throughout state on many Crops rare on alfalfa, May-October.
Pea aphid (<i>Acyrtosiphon pisum</i>)	pale green, cornicles blackish towards tip. On alfalfa, clovers, peas, March-November, common throughout state.
Potato aphid (<i>Macrosiphum euphorbiae</i>)	very similar to alfalfa aphid. May-November, common throughout state.
Spotted alfalfa aphid (<i>Therioaphis maculata</i>)	pale yellow, spotted. Cornicles very short, on alfalfa, throughout state.

Table 2. Aphid mortality percentage (%) in terms of time after exposure and oil solution concentration

Time (h)	Concentration 1% (v/v)			Concentration 5% (v/v)		
	3	7	11	3	7	11
Oil						
EO Kanuka	37.5±3.61	65.62±2.7	68.75±1.8	35±1.44	67.5±2.15	70±2.87
EO Ravintsara	43.33±3.54	60.42±3.96	69.17±3.14	42.7±3.72	64.58±4.16	70.83±4.16
EO Tea tree	39.16±3.41	61.87±3.1	67.08±2.66	38.88±4.81	68.05±3.61	72.22±4.81
EO Thyme	40.64±0.93	86.29±0.92	90.79±0.98	45.33±0.78	89.23±1.68	92.33±1.84
EO Oregano	44.38±0.62	93.7±1.2	94.7±1.8	54.43±0.8	92.17±1.23	95.27±1.39
CO Neem	36.94±0.81	88.26±0.92	92.76±0.98	44.98±0.63	90.83±1.16	93.93±1.32
Control	6.25±1.28	11.6±0.99	14.43±1.05	4.8±0.41	6.89±0.85	14.06±1.01

Table 3. TL₅₀ and TL₉₀ for 1% and 5% product concentration LC₅₀ and LC₉₀ for 11 hours after treatment

	TL ₅₀		TL ₉₀		LC ₅₀	LC ₉₀
	1%	5%	1%	5%	After 11 hours	After 11 hours
EO Kanuka	5.75 h	5.5 h	13.25 h	12.75 h	0.2 %	7.6 %
EORavintsara	6.25 h	5.75 h	13.5 h	13 h	0.5 %	7.2 %
EO Tea tree	6.25 h	5.5 h	13.75 h	12.5 h	0.8 %	6.6 %
EO Thyme	4.75h	4.25 h	9.5h	9h	1.25 %	4.25 %
EO Oregano	4 h	3.5 h	8.75h	8.5 h	1 %	4 %
CO Neem	4.5 h	4.25 h	9 h	8.75 h	1.1 %	4.1 %