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Insecticidal Effect of Two Lichen Species Extracts *Ramalina farinacea* (L.) Ach. and *Parmelia acetabulum* (Neck.) Duby on adults of *Ceratitis capitata* Wiedemann, 1824 (Diptera: Tephritidae)

K Bachi*, F Sahmoune-Sidi Mansour, D Sadoudi-Ali Ahmed

PSEMRVC Laboratory, Mouloud Mammeri University of Tizi Ouzou, Algeria

ABSTRACT

The bioefficacy of the lichen substances of R. farinacea (L.) Ach and P. acetabulum (Neck.) Duby is evaluated against adults of the Mediterranean fruit fly Ceratitis capitata Wied., considered one of the most important pests of fruit crops. By performing a fumigation test, we found that these two extracts of lichens reveal a very highly significant insecticidal effect on the adults of the fruit fly. We obtained 100% adult mortality after 48 hours of exposure to the dose of 20 µl/L air of R. farinacea extract and at this same dose of P. acetabulum extract we obtained 100% adult mortality after 96 hours (20 µl/L of air are taken from lichen acetone extract with a concentration of 0.03 mg plant pouder per 1 µl of acetone).

Keywords: *Ramalina farinacea*, *Parmelia acetabulum*, *Ceratitis capitata*, Lichenic extract, Insecticide

INTRODUCTION

The Mediterranean fruit fly *Ceratitis capitata* Wiedemann, 1824 (Diptera: Tephritidae) is one of the most important fruit pests. It is the main obstacle to the production and exports of fruits, because of its wide distribution in the Mediterranean hence named "Mediterranean fruit fly" [1,2]. The damages they caused are twofold: on the one hand, they are those attempting nesting females; those bites give a bad appearance to the fruit. On the other hand, we retain the damage caused by larvae that feed on fruits and galleries are opening the way for the fungi, especially *Penicillium degitatum* [3]. To limit the damage caused by this pest, several methods of fight are used. Pesticides are often used in order to limit the damages despite the inconveniences related to their costs, their toxicity, their residues and to the resistance phenomenon that has appeared in several species of stored product pest insects [4]. The autocidal control, also called the sterile insect technique (TIS), allowed the eradication of this pest in some parts of the world such as California and southern Mexico as well as cultural control such as tillage and choice of resistant varieties also gave good results [5]. Many researchers had interest to alternative control by using biological insecticides. However, only a few components and formulations of biological origin are available and marketed worldwide [6]. In this study, the insecticidal effect of *R. farinacea* (L) Ach., 1810 and *P. acetabulum* (Neck.) Duby 1830 extracts have been tested on the longevity of adults of the Mediterranean fruit fly by adopting a fumigation test. Currently, lichen extracts are the subject of various studies, particularly in the biological control of insect pests [7].

Lichens are formed through symbiosis between fungi and algae and/or cyanobacteria [7]. They usually grow on rocks, bark and on the ground. They are widely used in various fields such as floral decoration, pollution bioindication, medicine and perfumery [8,9]. Lichens are known to produce a great number of secondary metabolites, most of these compounds have bioactive properties [10,11]. Usnic acid, one of the most common lichen compounds, is widely found in many genera, such as *Usnea*, *Cladonia*, *Ramalina* and *Parmelia* [11]. The biological activities of usnic acid such as antiviral, anti-protozoal, antiproliferate, analgesic, anti-inflammatory and antipyretic activity were examined by Cocchietto et al. [12] and Ingolfsdottir [13].

MATERIALS AND METHODS

Lichens

The lichen *R. farinaceae* was collected in the forest of Darna (Kabylia), on June 2012. It is a forest-type site that extends over an area of 450 ha and an altitude of 985 m. It is located southeast of the wilaya of Tizi-Ouzou having coordinates 36°28'-36.30"N latitude and 4°15'-04.17"E longitude. The lichen *P. acetabulum* was collected on the northern slope of the mountain forests of Tala-Guilef (Kabylia) in September 2014. This forest site is located at an altitude of 1337 m with angular coordinates as 36°28'48.44"N latitude and 4°00'15.07"E latitude. These two forest areas belong to the Djurdjura National Park.

Adults of C. capitata

Adults of *C. capitata* were obtained from the infested figs *Ficus carica* L. of the variety Zith elkhadem under controlled laboratory conditions at 27 ± 1°C, 70 ± 5% humidity and at light/darkness ratio of 11/12. These figs are placed in sieves, which are arranged in bowls containing about 2 cm of sand that is used to retrieve the pupae because the third-stage larvae leave the fruit of a sudden relaxation to sink in shallow soil where pupation takes places, giving the pupae. The strainers are covered with a muslin mesh diameter which is less than that of drosophila, and attracted by the fruit during fermentation, the pupae are collected daily by sieving the sand. The emerged adults were counted daily and those who are less than 24 h old are used in fumigation tests to highlight the bioinsecticide effect of *R. farinacea* and *P. acetabulum* extracts.

Acetone extracts of lichens

To obtain the lichens extracts of the two species, we adopted the protocol of Van Halluwyn & Lerond [14]. The lichen, dried at room temperature, is crushed in a mortar vessel.

To carry out the extraction by maceration, we introduced 300 mg of lichen powder into a test tube to which were added 10 ml of acetone. The mixture is left so macerated for at least 3 h with occasional stirring. The obtained liquid extract was concentrated and green in color because of the complete extraction of the lichenic compounds. It was from this crude acetonic extract that we took the different doses used in the fumigation test.

Fumigation test

The test was performed under laboratory conditions at 27 ± 1°C temperature and 70 ± 5% relative humidity. It consist in exposing five pairs of *C. capitata* adults, aged less than 24 h, to vapors of each essential oil inside of a glass bowl (of 1 L volume). The experimental lichens extracts dose was deposited on a mass of cotton fixed with a wire at the internal face of the cover equipped with a hermetic joint. Control is made without exposing the flies to lichens extracts.

For each tested dose and the control, counting of the dead individuals was performed after 24, 48 and 96 h of exposure. Three repetitions were performed.

Statistical analysis

Results obtained were subjected to variance analysis (ANOVA) tests based on classification criterion. When this analysis provided significant differences, it was completed by the test of Newman-Keuls at 5% (logiciel Statbox, version 6.1).

RESULTS

Effect of R. farinacea extract on longevity of C. capitata

The *R. farinaceae* extract exert an insecticidal effect on adults of *C. capitata* according with the doses tested and the exposure time compared to the control that shows no adults mortality of the *C. capitata* as described in Figure 1.

At the lowest dose of 5 µl/L air, we obtained 100% mortality after 96 h of exposure. On the other hand, the dose of 20 µl/L air proves to be more effective, since we obtained a mortality percentage of 100% after only 48 hours of exposure of adults to the lichenic extract of *R. farinacea* compared with the control that shows no adult mortality of the *C. capitata*.

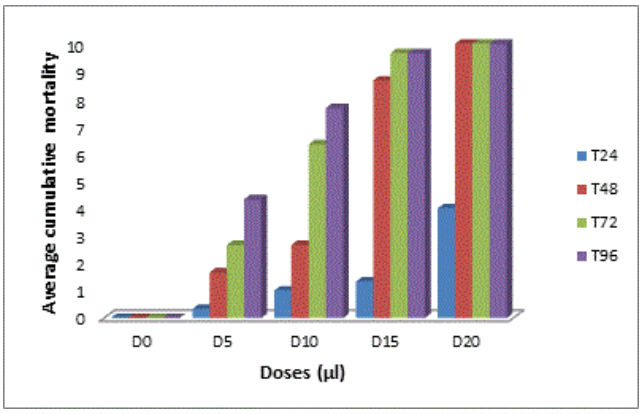

Figure 1: Mortality of *C. capitata* adults according to time exposure and doses of *R. farinacea* extract

Determination of the LC50

The regression line in Figure 2 is a positive linear form of y = 1.093x + 2.635 and R^2 is very close to 1 (R^2=0.9), which shows a strong positive link between the probits and the logarithm of the tested concentrations. It is the dose 8.69 µl/L air of the *R. farinacea* extract which causes the death of 50% of *C. capitata* adults.

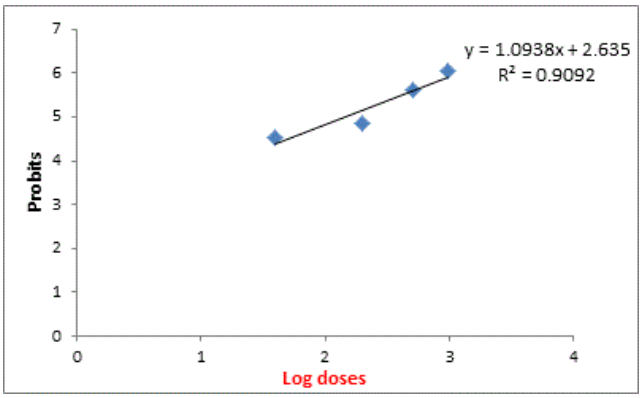

Figure 2: Linear regression of mortality in probits (fumigation test with *R. farinacea* extract)

Analysis of variance at the 5% threshold shows a very highly significant effect of the dose (P=0.000) and a very highly significant effect of the time (P=0.000) and their interaction (P=0.000) on adults longevity.

Effect of Parmelia acetabulum extract on longevity of C. capitata

The *P. acetabulim* extract revealed an effective insecticidal effect on adults of *C. capitata* than the average cumulative mortality increased with the doses tested and the exposure time compared to the control as shown in Figure 3.

The lowest dose of 20 µl/L air is effective because we obtained an average percentage mortality of 100% after 96 hours of exposure. However, the dose of 35 µl/L is the most effective because we recorded 100% mortality in only 24 hours compared with the control that shows no adult mortality.

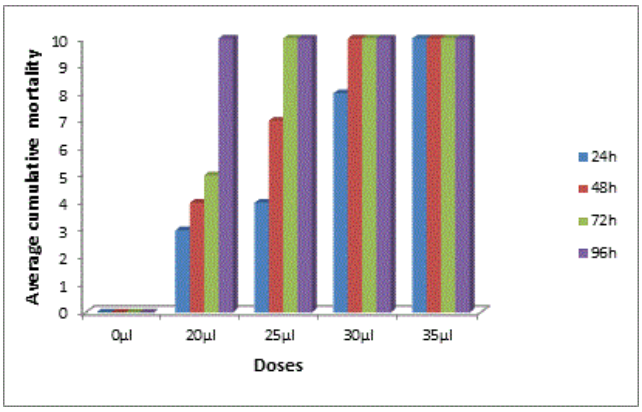

Figure 3: Mortality of *C. capitata* adults according to time exposure and doses of *P. acetabulum* extract

Determination of the LC50

The regression line in Figure 4 is positive. It expresses the probit of the corrected mortality percentage as a function of the logarithm of lichenic extract concentrations. It is the dose 18.82 µl/L air of *P. acetabulum* extract which causes the death of 50% of *C. capitata* adults.

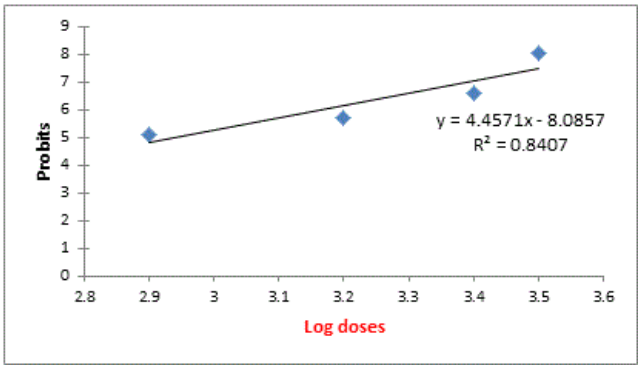

Figure 4: Linear regression of mortality in probits (fumigation test with *P. acetabulum* extract)

The analysis of variance at 5% threshold shows a very highly significant effect of the dose (P=1.63 e^{-05}) and a non-significant effect for the exposure time (P=0.7316) on adult longevity.

DISCUSSION

The results obtained, showed clearly that the lichen extracts exert a very highly bioinsecticidal effect in fumigation test against *C. capitata* adults. With the highest dose 20 µl/L air of *R. farinacea* extract, we recorded a mortality rate that reached 100% in 48 h compared to the control that showed no mortality. At this same dose (20 µl/L air) of *P. acetabulum* extract, we obtained a total mortality of *C. capitata* adults after 96 h of treatment. However the highest dose 35 µl/L air proved more effective because we obtained a total mortality of adults in 24 h compared to the control which showed no *C. capitata* adult mortality.

The different LC_{50} values of these lichen acetonic extracts are between 8.69 to 18.82 µl/L air. These results show that these extracts are active by fumigation test. Although the *R. farinacea* extract has been more effective with the lowest LC_{50} of 8.69 µl/L of air. The correlation coefficients obtained are between 0.84 and 0.90, which indicates a strong correlation between the *C. capitata* adult mortality and the doses of lichen extracts used.

This insecticidal activity would be due to the presence of secondary metabolites such as phenolic compounds, dibenzofurane, usnic acids, depsidones, depsones, lactones, quinines and pulvinic acid derivatives [15].

No bibliography reports studies or research on bioinsecticidal effect of use lichen extracts against the fruit fly *C. capitata*. But many references have revealed that usnic acid is the major compound of acetonic lichens extracts

[11,16]. Several authors have revealed the insecticidal effect of usnic acid on *Culex pipiens* mosquitoes, *Aedes aegypti*, and *Culiseta longiareolata* [7,12,17-21].

Antifeedant activity of lichen compounds (e.g. usnic acid, vulpinic acid) against the polyphagous herbivorous insects *Spodoptera littoralis* and *Spodoptera ornithogalli* was reported by Emmerich et al. [22] and Slansky [23], respectively.

Vinayaka et al. [24] found that *R. nervulosa*, *R. pacifica* (Ramalinaceae) and *Parmotrema tinctorum*, *Usnea galbinifera* (Parmeliaceae) reveal a larvicidal activity against second instar larvae of *A. aegypti*. These same authors report that usnic acid and sekikaic acid were detected in *R. nervulosa* but *R. pacifica* showed the presence of usnic acid and salazinic acid. In *P. tinctorum*, lecanoric acid and orsellinic acid were found to be present. Galbinic acid and norstictic acid were found in *U. galbinifera*.

Cetin et al. [7] reported that the insecticidal activity of lichens is linked to the major component called usnic acid which is isolated from two lichens *Cladonia foliacea* and *Ramalina farinacea*. Its larvicidal activity was then tested against larvae of *C. pipiens*. Larval mortality reached an average of 100% at the 3rd and 4th larval stage with both lichen species *R. farinacea* and *C. foliacea*, unlike the control that showed no mortality.

Cetin et al. [21] have obtained excellent results with lichen metabolites, among them usnic acid, atranorin, 3-hydroxyphysodic acid and gyrophoric acid against mosquito larvae of *Culiseta longiareolata* (Dipterous: Culicidae) that showed high larvicidal activity with values of 0.48, 0.52 and 0.97 µg/ml, respectively.

Several authors have demonstrated the insecticidal activity of lichenic compounds against mosquitoes and other insects [17,18,20,25]. Lichenic compounds such as cabraleadiol monoacetate, 4-o-methylcryptochlorophaeic, lichexanthone and 3,6-dimethyl-2-hydroxy-4-methoxybenzoic acid have been shown to be effective against *A. aegypti* larvae [19].

The larvicidal activity of the methanol extract, fractions and compound 2-hydroxyl-4-methoxy-6-propyl-methyl benzoate and usnic acid identified from the lichen *Ramalina usnea*, was tested against the third instar larvae of *A. aegypti* mosquito. The methanol extract and three fractions showed activity, killing 100% and 96.6% of the larvae at concentration of 150 g/ml at 24 h [16].

CONCLUSION

The *R. farinaceae* and *P. acetabulum* extracts revealed significant insecticidal effect in fumigation test, reducing the longevity of *C. capitata* adults with increasing concentrations and exposure time. But at the highest doses we record rates that sometimes reach 100% in less time. This diversity of action of the lichen extracts that we used could be due to the diversification of their chemical compounds or in other words the bioactive molecules of this lichen. These compounds can carry out a singular action of one of the components or a synergistic effect between several compounds on the Mediterranean fruit fly adults. It would be interesting to consider in perspective to isolate the active components of these extracts in order to determine their mechanism of action on the adults of *C. capitata* and to make trials in the orchards in order to substitute these natural insecticides to the chemical ones and protecting the ecosystems.

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