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Insecticidal Effect of the Extracts of *Cladonia foliacea* (Huds.) Willd. and *Flavoparmelia caperata* (L.) Hale AgainstAdults of the Grain Weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae)

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ABSTRACT

Four different concentrations of extracts, obtained from the two lichen species (*Cladonia foliacea* (Huds.) Willd. and *Flavoparmelia caperata* (L.) Hale), were tested against the grain weevil, *Sitophilus granaries* (L.) under laboratory conditions. Mortality rate of adults was determined at 24, 48, and 96 h. Obtained results showed that the extracts of *C. foliacea* and *F. caperata* had insecticidal effects on the adults of *S. granarius* in comparison with control. The insecticidal effect was influenced by the concentration of the extract and the exposure time. Higher concentrations and longer exposure time resulted to highest toxicity levels on *S. granarius*. Mortality rates after 96 h of treatment with highest concentration (20 $mg.ml^{-1}$) of the extracts of *F. caperata* and *C. foliacea* were determined as 91 and 83%, respectively. Bioassay revealed that 96 h LC_{50} values were 0.107 and 0.354 $mg.ml^{-1}$, respectively. The results suggest that lichen compounds could be useful as alternatives of pesticides.

Key words: Lichen, Biological control, Insecticidal effect, *Sitophilus granaries*.

INTRODUCTION

Wheat weevil, *Sitophilus granarius* (L.) also, known as grain weevil or granary weevil, has been recorded as common pest of stored products all over the world. It is a well-known pest that causes an economically yield loss in stored products in Turkey (Yildirim 2012).

Usage of chemical insecticides in the fight against stored-product pests is still preferred because of its ready availability and large scale use. However, they attack not only the target organisms but also cause environmental damages. Therefore, in recent years, researchers have been searched for safe alternatives such as biological insecticides. Lichens are very significant insecticidal sources of bioinsecticides (Emmerich *et al*., 1993). They are formed through symbiosis between fungi and algae and/or cyanobacteria. Lichens have been used to monitor pollution as well as sources of compounds that have used in medicines, perfumes, cosmetics and dyes (Cetin *et al*., 2008). Some of the compounds, known as lichen acids, have effects as antiviral, antiprotozoal, antiproliferative, analgesic, anti-inflammatory, and antipyretic activities of usnic acid (Cocchietto *et al*., 2002 and Ingolfsdottir, 2002).

In medicinal uses, lichens have been used on the basis of their morphological characteristics in the past. *Lobaria pulmonaria* (L.) Hoffm. had been used in lung diseases because this species is similar to lung. *Xanthoria parietina* (L.) Beltr. had been used to treat jaundice disease, *Peltigera apthosa* (L.) Willd. that has structures similar to warts on its thallus, has been used in the treatment of thrush (Uysal *et* al., 2009). It is known that more than 60 compounds of lichens possess antibiotic activity. Lichen acids such as; usnic and vulpinic had powerful antibiotic effect against some bacteria. Furthermore, *Letharia vulpine* (L.) Hue and *Vulpicida pinastri* (Scop.) J.-E. Mattsson lichen species were used to kill wolves and foxes that harm animal herds in some European and Scandinavian countries in the winter (Aslan *et al*., 1998).

Several studies indicated that lichen metabolites have insecticidal effects. In these studies, some lichens had antifeedant and lethal characteristic on insects (Bombuwala, 2001; Kathirgamanatkar *et al*., 2006; Balaji *et al*., 2007; Cetin *et al*., 2008 and Silva *et al*., 2009). On the other hand, insecticidal activity of many plant derivates against some pests has been well demonstrated (Yildirim *et al*., 2005 and Pavela, 2010).

The aim of the present study was to evaluate the insecticidal effect of the extracts; *Cladonia foliacea* (Huds.) Willd. and *Flavoparmelia caperata* (L.) Hale against adults of *S. granarius* in in-vivo conditions.

MATERIALS AND METHODS

Insects and rearing conditions

S. granarius adults were collected from Erzurum storage house in Turkey. Wheat grains were purchased from the local market and stored in a freezer at -20 °C. The wheat was washed by tap water, dried and heated at (30 °C) to prevent pre–infestation. *S. granaries* culture was maintained under the laboratory conditions of 25 ± 1 °C, 64 ± 5% R.H. and L:D 12 h:12 h at the Department of Plant Protection, Atatürk University, Turkey. Adults obtained from the laboratory culture were stored in separate insect cages (diameter 25 cm, height 30 cm) ,provided with wheat grains. A little grain of wheat, included larvae and pupae, were placed in separate Petri dishes. Adults that emerged at the same day (same age) were collected and used for experiments.

Isolation of lichen extracts

C. foliacea and *F. caperata* were collected in June 2009-10 from Erzurum, Turkey. All samples were identified and stored in the herbarium of Kazım Karabekir Education Faculty, Atatürk University-Erzurum. These materials were allowed/ left to dry under room conditions. Air-dried lichen samples were pulverized and extracted by Soxhlet apparatus. Each lichen sample (30 g) was extracted by distilled n-hexane, diethyl ether, acetone, and methanol solvents, respectively. 300 ml from each solvent was used for extraction. Extractions by n-hexane and diethyl ether solvents were maintained for two days at 25°C and extractions by acetone and methanol solvents were maintained for three days at the same temperature. As the result of extraction, solutions were mixed together and the solvents in the solutions were evaporated by rotary evaporator. By this way, total lichen substances were obtained. They were dissolved in acetone-water solvent, existed 80% distilled acetone. The extracts of each lichen species were prepared at 2.5, 5, 10, and 20 $mg.ml^{-1}$ concentrations. Extraction of *C. foliacea* and *F. caperata* yielded 9.65 and 8.42% (w/w) of lichen substances, respectively. The yields were based on dry materials of plant samples.

Bioassay

In order to test the toxicity of the extracts against the pest adults, 33 adults, provided with 33 grains of wheat, were placed in Petri dishes (9 cm). The extracts were applied with a spray gun. From each dose 0.8 ml liquid was used for each Petri dish. Initial tests were carried out to establish the appropriate dose and exposure time ranges. The amounts of extracts applied were 2.5, 5, 10, and 20 $mg.ml^{-1}$ in each Petri dish. After exposure, adults' mortality was determined at 24, 48, and 96 hours. A Petri dish applied with only 80% acetone solution, was used as a control group. Three replicates were used for each concentration as well as for the control. Exposure time combination and insecticidal effect of the extracts were expressed as % mean mortality of the adults.

Statistical analysis

Differences among the insecticidal activities of the tested lichen extracts were determined according to analysis of variance (ANOVA) test by using the SPSS 15.0 software package. Duncan test was used for comparison of means. The significance of the results was estimated at $p<0.01$. LC_{50} values in 96 h were calculated, following the method of Finney (1971). Probit analysis of concentration-mortality data was conducted to estimate the LC_{50} values and associated 95% confidence limits for each treatment (EPA Probit Analysis).

RESULTS AND DISCUSSION

Toxicity effects (mortality %) of the extracts obtained from *C. foliacea* and *F. caperata* on adults of *S. granaries* were summarized in table (1). At 24 h, the extracts of *F. caperata* didn't cause mortality, while the extract of 20 $mg.ml^{-1}$of *C. foliacea* caused about 4% mortality.

The mortality rates after treatments with different concentrations of lichen extracts were illustrated in fig. (1). Mortality rates after 24, 48, and 96 h exposure time were given in fig. (2). Longer exposure time resulted to high toxicity on *S. granaries* (Figs. 2 and 3). At the same time, these extracts showed a concentration dependent.

Analysis of variances showed that the effect on mortality rate of *S. granarius* adults, on the basis of concentration and exposure time tested, was highly significant ($p<0.01$). Treatments with the extracts of *F. caperata* led to highest mortality of *S. granarius* adults (Table 1).

Mortality rates after 96 h of treatment with the highest concentration (20 $mg.ml^{-1}$) of the extracts of *F. caperata* and *C. foliacea* attained 92 and 84% of *S. granarius* adults, respectively. However, there was no mortality in the control (Table 1). Significant differences among insecticidal effects of extracts at different concentrations of the two lichens were observed.

The present results showed that the extracts of *F. caperata* and *C. foliacea* had varying degrees of insecticidal effects against the adults of *S. granarius*. The insecticidal activity increased with increasing the concentration and exposure time, as the extracts caused significant mortality rates (Figs. 1, 2 and 3). Bioassay of the extracts showed that 96 h LC_{50} values were; 0.107 (0.000 - 0.888), with the slope of 0.419 (0.209) (0.008 - 0.830) for *C. foliacea* and 0.354 (0.009 - 1.032), with the slope of 0.840 (0.241) (0.366 - 1.314) for *F. caperata*. *C. foliacea* was the most potent insecticidal one (by 92%).

Table (1): Effects (Mortality %) of the extracts of the two lichen species; *Cladonia foliacea* (Huds.) Willd. and *Flavoparmelia caperata* (L.) Hale on *Sitophilus granaries* adults under laboratory conditions

Lichen species	Concentration ($mg.ml^{-1}$)	Mean mortality (%)[a]		
		24[b]	48[b]	96[b]
Control	-	0.00±0.00a	0.00±0.00a	0.00±0.00a
Cladoniafoliacea	2.5	0.00±0.00a	6.06±0.58ab	72.73±0.58b
	5	0.00±0.00a	10.10±0.67abc	74.75±0.33b
	10	0.00±0.00a	12.12±0.58bc	78.79±0.58bc
	20	4.04±0.33b	24.24±1.53d	83.84±1.45cd
Flavoparmeliacaperata	2.5	0.00±0.00a	6.06±0.00ab	73.74±0.33b
	5	0.00±0.00a	8.08±0.33abc	86.87±0.33de
	10	0.00±0.00a	10.10±0.33abc	88.89±0.33de
	20	0.00±0.00a	18.18±1.53cd	91.92±0.33e

[a] Mean±S.E. of three replicates, each set-up with 33 adults
[b] Exposure time (h)
Values followed by different letters in the same column differ significantly at $p<0.01$.

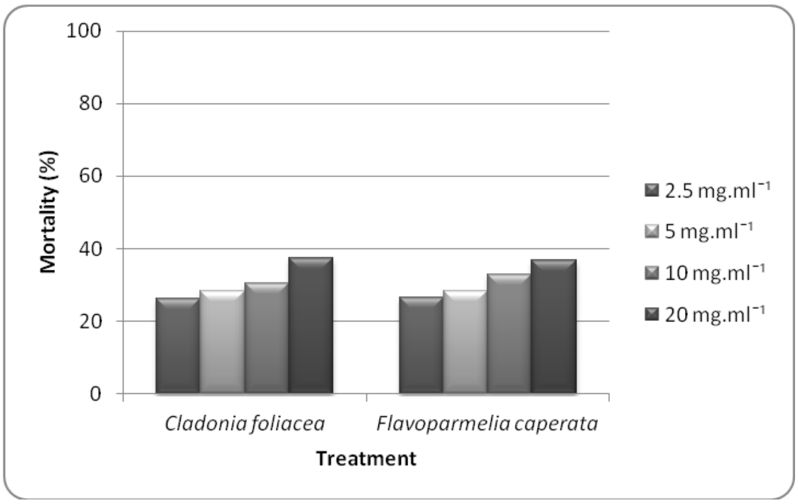

Fig. (1): Mortality rates of *Sitophilus granarius* exposed to different concentrations of the extracts of *Cladonia foliacea* (Huds.) Willd. and *Flavoparmelia caperata* (L.) Hale ichen species under laboratory conditions.

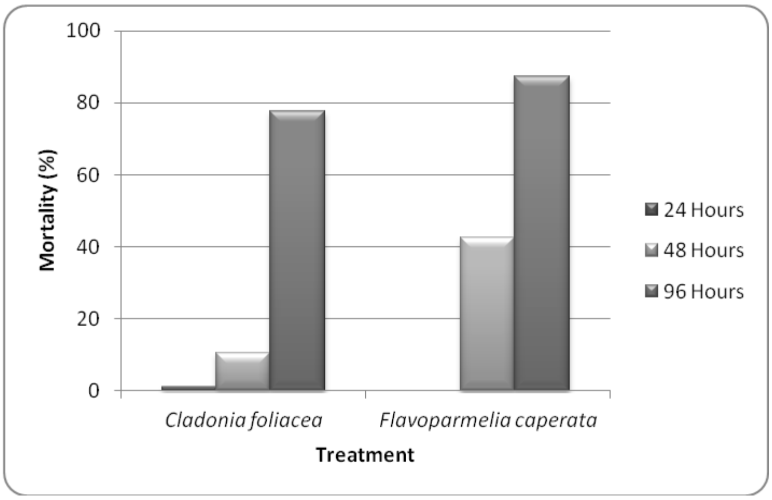

Fig. (2): Mortality rates of *Sitophilus granaries* at intervals of time after treatment with the extracts of *Cladonia foliacea* (Huds.) Willd. and *Flavoparmelia caperata* (L.) Hale lichen species under laboratory conditions.

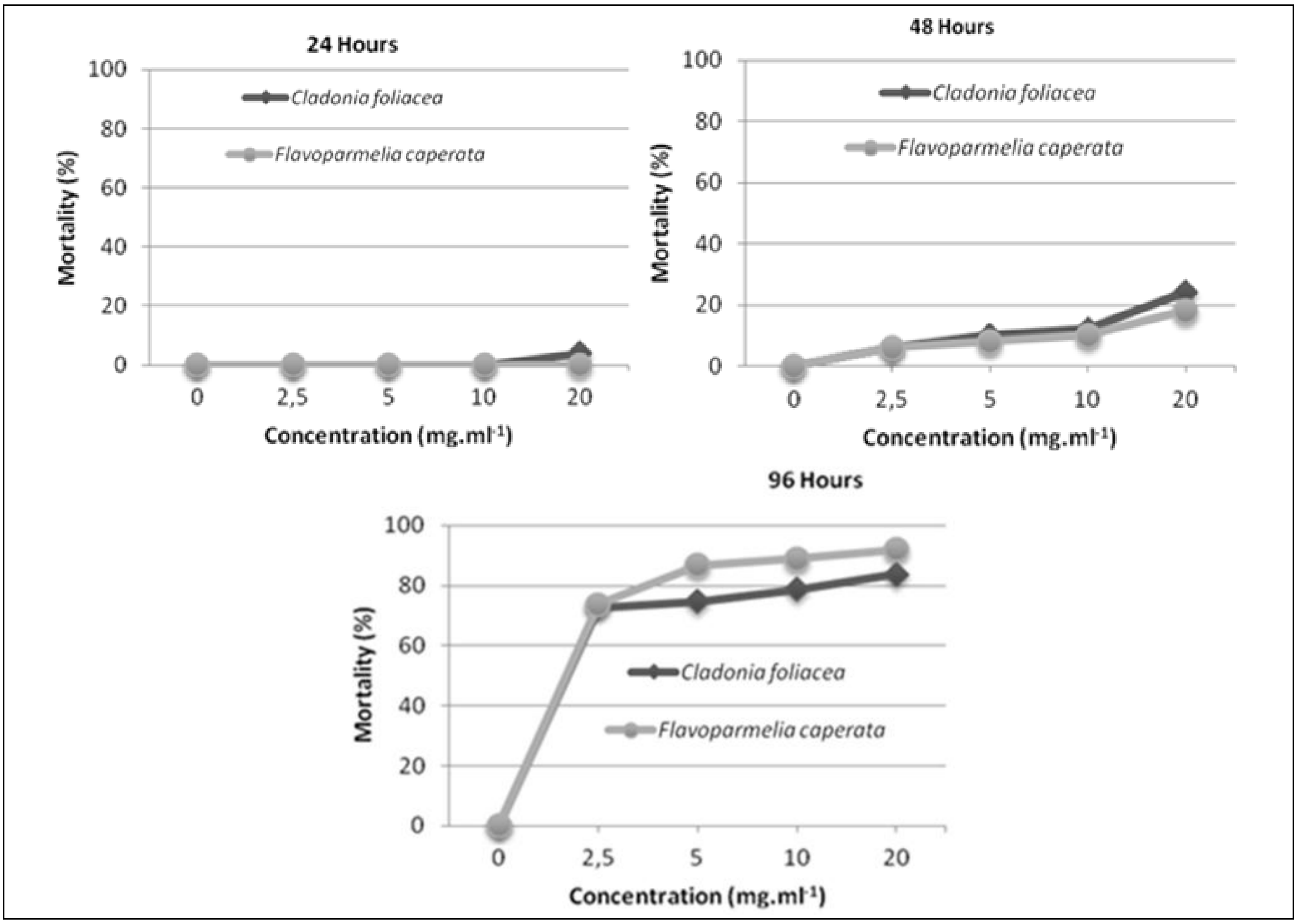

Fig. (3): Mortality rates of *Sitophilus granarius*in in relation to exposure time and concentration of the extracts of *Cladonia foliacea* (Huds.) Willd. and *Flavoparmelia caperata* (L.) Hale lichen species under laboratory conditions.

Natural products are now being considered as an alternative to the arsenal of synthetic compounds currently available. Previous studies demonstrated that in general, the toxicity of extracts isolated from lichen samples against pests was related to their secondary components (Cetin *et al*., 2008; Sahib *et al*., 2008 and Silva *et al*., 2009). These results suggested that the extracts isolated from different lichen species might have different toxicity levels, which can be attributed to their different chemical composition and different components (Karagoz and Aslan, 2005; Aslan *et al*., 2006; Gulluce *et al*., 2006; Sahib *et al*., 2008; Yildirim *et al*., 2012 and Emsen *et al*., 2012). In these studies, some lichens had antifeedant and lethal characteristics on the insects. Lichens usually contain only one or two major substances, often found in high concentrations. Concentrations of lecanoric acid in some *Parmelia* species, such as *P. carphorrizans* and *P. tinctorum*, varied from 2.6 to 4.8% of dry weight (Culberson *et al*., 1977), in *Cetrariais landica* contents of fumarprotocetraric acid could reach 11% (Ingolfsdottir, 2002), while *Pertusaria alaianta* contained up to 20% of a mixture of chloroxanthones (Huneck and Hoefle, 1978). For slow-growing organisms such as lichens, the synthesis of large amounts of energetically expensive metabolites must be of some adaptive values. In fact, some of them have proved to be endowed with diversified biological activities.

Obtained results suggest that the extracts isolated from different lichen species might have different toxicity effects, which can be attributed to their different chemical composition and different major and/or minor components. These data point out that lichen species may be used as potential insecticidal agents against adults of *S. granarius*.

REFERENCES

Aslan, A., A. Oztürkand and E. Kaya 1998. Economic importance of lichens and important lichen species determined in Oltu region. Proceedings of the Symposium of Oltu and its Vicinity from Past to Future, 1-3 July 1998, Oltu. Erzurum: 356-365.

Aslan, A., M. Güllüce, M. Sökmen, A. Adıgüzel,

F. Sahinand H. Özkan 2006. Antioxidant and antimicrobial properties of the lichens *Cladonia foliacea*, *Dermatocar ponminiatum*, *Everinia divaricata*, *Evernia prunastri*, and *Neofus cellapulla*. Pharm. Biol. 44:247-252.

Balaji, P., S. Malarvannan and G. N. Hariharan 2007. Efficacy of *Roccellamontagnei* extracts on *Helicoverpa armigera* (Lepidoptera: Noctuidae). J. Entomol. 4:248-252.

Bombuwala, B. D. K. 2001. Isolation and bio activity studies of lichen substances from Sri Lankan lichens. News Internet Edition, Ph D. Thesis Department of Chemistry.University of Peradeniya, Sri Lanka.

Cetin, H., O. Tufan, A. O. Turk., T. Tay., M. Candan., A. Yanikoglu and H. Sumbul 2008. Insecticidal activity of major lichen compounds, (−)- and (+)- usnic acid, against the larvae of house mosquito, *Culex pipiens* L. Parasitol. Res. 2:1277-1279.

Cocchietto, M., N. Skert., P.L. Nimisand G. Sava 2002. A review on usnic acid, an interesting natural compound. Naturwissenschaften 89:37-146.

Culberson, C. F., W. L. Culberson and A. Johnson 1977. Second supplement to "Chemical and botanical guide to lichen products". Am. Bryol. Lichenol. Soc. Missouri Botanical Garden, Saint Louis, p. 400.

Emmerich, R., I. Giez., O. L. Lange and P. Proksch 1993. Toxicity and antifeedant activity of lichen compounds against the polyphagous herbivorous insect *Spodoptera littoralis*. Phytochemical 33: 389-1394.

Emsen, B., Y. Bulak, E. Yildirim, A. Aslan and S. Ercisli 2012. Activities of two major lichen compounds, diffractaic acid and usnic acid against *Leptinotarsa decemlineata* Say, 1824 (Coleoptera: Chrysomelidae). Egypt. J. Biol. Pest Co. 22:5-10.

Finney, D. J. 1971. Probit analysis. 3rd edition. Cambridge Univ. Press, Cambridge.

Gulluce, M., A. Aslan, M. Sokmen, F. Sahin, A. Adigüzel, G. Agar and A. Sokmen 2006. Screening the antioxidant and antimicrobial properties of the lichens *Parmelias axatilis*, *Platisma tiaglauca*, *Ramalinap ollinaria*, *Ramalinap olymorpha* and *Umbilicariany landeriana*. Phytomedicine 13:515-521.

Huneck, S. and G. Hoefle 1978. Struktur und ^{13}C-NMR-specktroskopie von chlorhaltigen Flechtenxanthonen. Tetrahedron 34: 2491-2502.

Ingolfsdottir, K. 2002. Usnic acid. Phytochemistry 61:729-736.

Karagoz, A. and A. Aslan 2005.Antiviral and cytotoxic activity of some lichen extracts. Biologia 60: 281-286.

Kathirgamanathar, S., W. D. Ratnasooriya., P. Baekstrom., R. J. Andersen and V. Karunaratne 2006. Chemistry and bioactivity of physciaceae lichens *Pyxineconsocians* and *Geterodermia leucomelos*. Pharm. Biol. 44:217-220.

Pavela, R. 2010. Antifeedant activity of plant extracts on *Leptinotarsa decemlineata* Say. and *Spodoptera littoralis* (Bois.) Larvae. *Ind. Crop. Prod*. 29:1-7.

Sahib, K., N. S. Kularatne, S. Kumar and V. Karunaratne 2008. Effect of (+)-usnic acid on the shot-hole borer (*Xyleborus fornicates* Eichh.) of tea. J. Natl. Sci. Found. Sri. 36: 335-336.

Silva, M. D. C., R. A. Sa., T. H. Napoleao., F. S. Gomes., N. D. L. Santos., A. C. Albuquerque., H. S. Xavier., P. M. G. Paiva and M. T. S. Correia 2009. Purified *Cladonia verticillaris* Lichen Lectin: Insecticidal activity on *Nasutitermes corniger* (Isoptera: Termitidae). Int. Biodeter. Biodeg. 63:334-340.

Uysal H., D. Altun and A. Aslan 2009. The effects of *Lobaria pulmonaria* (L.) Hoffm. on the longevity on *Drosophila melanogaster*. Tr. Sci. Res. Found.2:271-276.

Yildirim E. 2012. Pests of Stored Productand their Control Methods. Third Edition. Ataturk University Agricultural Faculty Press 191, Erzurum, 123 p.

Yildirim, E., M. Kesdek and S. Kordali 2005. Effects of essential oils of three plant species on *Tribolium confusum* du Val and *Sitophilus granarius* (L.) (Coleoptera: Tenebrionidae and Curculionidae). Fresen. Environ. Bull.14:574-578.

Yildirim, E., A. Aslan, B. Emsen, A. Cakir and S. Ercisli 2012. Insecticidal effects of *Usnealongissima* (Parmeliaceae) Extract against *Sitophilus granarius* (Coleoptera: Curculionidae). Int. J. Agric. Biol.14: 303-306.