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Full Length Article

Insecticidal Effect of *Usnea longissima* (Parmeliaceae) Extract against *Sitophilus granarius* (Coleoptera: Curculionidae)

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ABSTRACT

Two secondary metabolites (diffractaic acid & usnic acid) and extract of a lichen species, *Usnea longissima* Ach. were tested against adults of *Sitophilus granarius* (L.) on Petri dishes. After exposure, mortality of the adults was determined at 24th, 48th and 96th h. The results showed that secondary metabolites and extract of *U. longissima* have an insecticidal effect on adults of *Sitophilus granarius* (L.) in comparison with controls. The insecticidal effect was influenced by the concentrations of the extracts and the exposure time. Higher concentrations and longer exposure time resulted in maximum toxicity on *S. granarius*. Treatment with extract and lichen compounds of *U. longissima* pointed out equal mortality against adults of *S. granarius*. The mortality rates after 96 h of treatment with the maximum concentration (10 mg mL^{-1}) of *U. longissima* extract, diffractaic acid and usnic acid were determined as 98.98, 91.91 and 94.94% for *S. granarius,* respectively. However, there was no mortality in the controls. The mortality rates after 96 h of treatment with the 10 mg mL^{-1} in concentrations of the extract of *U. longissima* were established and the highest mortality rate was found against *S. granarius* with 98.98%. © 2012 Friends Science Publishers

Key Words: *Sitophilus granarius*; *Usnea longissima*; Lichen extracts; Insecticidal effects

INTRODUCTION

Wheat weevils (*Sitophilus granarius* L.), also known as grain weevils or granary weevils, occur all over the world and are a common pest in many places. They can cause significant damage to harvested grains that are being stored and may drastically decrease yields. Usage of chemical insecticides in the fight against stored-product pests is preferred more. Moreover, giving result in a large extent of the chemical insecticides is the reason of this situation. Not only the target organisms but also the environment is damaged by the chemical insecticides. Therefore, in recent years, many researchers have been looking for new biological insecticides (Gandhi *et al.,* 2011).

Lichens are very significant source within biological sources of insecticides. Lichens produce a great number of secondary metabolites that participate in ecological interactions and respond to environmental changes. They are formed through symbiosis between fungi and algae and/or cyanobacteria. They are used for many areas such as pollution monitoring, medicinal, perfumery and cosmetic and dyeing clothes.

In recent years, many scientists have begun to benefit from lichens in insecticide area. Lichens have these various characteristics through produced a great number of secondary compounds by them and they generate these compounds in extreme conditions. Lichen substances could play a multiple biological role, also in response to different ecological factors. These compounds are also known as lichen acids. Antiviral, antiprotozoal, antiproliferative, analgesic, anti-inflammatory and antipyretic activities of usnic acid known as secondary metabolite have been reviewed. Among the lichen compounds, usnic acid is the well known and studied metabolite. This metabolite previously reported as antibiotic, antimycotic, antiherbivorous, phytotoxic, photobiont-regulating, and as a UV-filter (Cocchietto *et al*., 2002; Ingolfsdottir *et al*., 2002; Rattan, 2010).

Previously researchers reported that lichens contain many substances but in general they had one or two major substances that found in high concentrations. Concentrations of lecanoric acid in some *Parmelia* species, such as *P. carphorrizans* and *P. tinctorum,* vary from 2.6 to 4.8% of dry weight (Culberson *et al.,* 1977). Huneck and Hoefle (1978), reported that a lichen specie, *Pertusaria alaianta* contains up to 20% of a mixture of

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chloroxanthones. Lichens are well known for their slow growing characteristics. Therefore, the synthesis of large amounts of energetically expensive metabolites could be more important. In fact, several of them have proved to be endowed with diversified biological activities. Many researchers have reported that lichen metabolites have insecticidal effects (Bombuwala, 2001; Kathirgamanathar *et al.,* 2006; Nimis & Skert, 2006; Balaji *et al.,* 2007; Cetin *et al.*, 2008; Sahip *et al.,* 2008; Silva *et al.,* 2009).

The granary weevil, *S. granarius* (L.) (Coleoptera: Curculionidae) were well-known pests causing economically important yield lost in stored products in Turkey and many other countries (Yildirim *et al.,* 2001). The aim of the present study was to evaluate the insecticidal effect against adults of *S. granarius* of extract and two secondary metabolites (diffractaic acid & usnic acid) of a lichen species, *Usnea longissima* Arc. (Parmeliaceae) in *in-vivo* conditions.

MATERIALS AND METHODS

Insects and rearing conditions: In this study, insects were collected from storage houses located in Erzurum region. The health grains of wheat were obtained from a local market in same region and this material quickly stored in a freezer at -20°C. *S. granarius* adults were reared in laboratory at the condition of 25 ± 1°C, 64 ± 5 relative humidity and L:D = 12 h:12 h in the Department of Plant Protection of Atatürk University. The adults obtained from this laboratory cultures stored in separate insect cages including wheat grains.

Plant material and isolation of lichen extract: *U. longissima* Ach. were collected in July 2009-2010 from Trabzon in Turkey. After collecting these materials were exposed to dry in room conditions. Herbaria of these lichens were made and then species of them were identified. Air-dried lichen samples were pulverized and extracted by Soxhlet extractor. Each lichen samples (30 g) were extracted by distilled n-haxane, diethyl ether, acetone, and methanol solvents, respectively. It was used 300 mL from each solvent for extraction. Extraction by n-haxane and diethyl ether solvents were maintained two days at 25°C and extraction by acetone and methanol solvents were maintained three days at 25°C. At the result of extraction, solutions were put together and then solvent in solution was evaporated by evaporator. In this way total lichen substances were obtained. Total lichen substances were dissolved in acetone-water solvent existed 80% distilled acetone. The solutions composed for extract of each lichen species were prepared at 1.25, 2.5, 5 and 10 mg mL^{-1} concentrations.

Isolation lichen secondary metabolites: An air-dried sample of the lichen, *U. longissima* (250 g) was extracted with 500 mL of diethyl ether using a Soxhlet apparatus at 40°C. The crude extract of lichen sample was filtered and stored at 4°C for 24 h to precipitate usnic acid (UA). The UA precipitates were collected and subjected to silica gel (70-230 mesh) column chromatography (CC) by eluting with a $CHCl_3$: *n*-hexane (8:2) solvent system. At the end of this process, 2.10 g of usnic acid (Fig. 1) was obtained with a yield 0.84% (w/w). After the usnic acid precipitates were removed, the solution was concentrated using an evaporator under reduced pressure. The extract (18.75 g) was subjected to CC using silica gel (70-230 mesh) eluting with $CHCl_3$: *n*-hexane (7:3, 7.5:2.5, 9:1 & 10:0) and $CHCl_3$:CH_3OH (9:1) solvent systems. Thus, 5.75 g of diffractaic acid (Fig. 1) was purified. The spectral data have been previously reported (Bayir *et al.,* 2006; Odabasoglu *et al.,* 2006).

Determination of the age of the adults: In the present study, 4-6 day-old adults of *S. granarius* were used as the test. To obtain adult same age, in the same time emergence adults were collected and used.

Bioassays: In order to test the toxicity of the extracts and lichen compound against to the pest adults, 33 adults of the insects with 33 grains of wheat were placed to Petri dishes (9 cm). The extract and lichen compounds solutions were applied by spraying liquid. Each from dose was used 0.8 mL liquid for each Petri dish. Initial tests were done to establish the appropriate dose and exposure time ranges. The amounts of extract solutions applied were 1.25, 2.5, 5, and 10 mg mL^{-1} in each Petri dish. After exposure time of 24, 48 and 96 h, the mortality ratio was determined. The control group were grown on 80% acetone in a petri dishes. We used three replicates for each treatments and mortality were expressed as %.

Statistical analysis: The differences among the insecticidal activities of lichen extracts tested were determined according to analysis of variance (ANOVA) test by using the SPSS 15.0 software package. Tukey HSD Test was used for comparison of means. The results showed significant differences ($p < 0.01$).

RESULTS AND DISCUSSION

The toxicity effects of two secondary metabolites (diffractaic acid & usnic acid) and extract obtained from *U. longissima* Ach. on adults of *S. granarius* are summarized in Table I and II. The results show that secondary metabolites and extract of *U. longissima* have an insecticidal effect on adults of *S. granarius* (L.) in comparison with controls (Figs. 2, 3 & 4). Higher concentration and longer exposure time resulted in maximum toxicity on adults of *S. granarius*. The mortality rates after 24, 48 and 96 h treatment with different concentration of lichen extracts and secondary metabolites have been given in Fig. 4.

The mortality is only due to increasing concentration caused by the extract, diffractaic acid and usnic acid isolated from *U. longissima* have been given Fig. 2 and once exposure time was considered occurring mortality were given Fig. 3. The analysis of variance demonstrates that the effects on the mortality rate of adults of *S. granarius* are highly significant on the basis of concentrations and

Table I: Mortality effects of a lichen species extract and the two secondary metabolites on *Sitophilus granarius* adults

Treatments	Dose (mg mL^{-1})	Mean mortality[a] 24[b]	48[b]	96[b]
Extract	1.25	0.00±0.00a	2.67±0.33b	25.33±0.33b
	2.5	0.00±0.00a	3.67±0.88bcd	26.00±2.08b
	5.0	1.00±0.00ab	6.33±0.33fg	29.67±0.67def
	10.0	1.67±0.7abc	8.00±1.53g	32.67±0.33g
Diffractaic acid	1.25	0.00±0.00a	2.67±0.88b	26.33±0.88bc
	2.5	0.67±0.67a	4.33±0.33bcde	27.33±0.33bcd
	5.0	1.67±0.88abc	5.00±0.00cdef	28.00±1.15bcde
	10.0	2.67±0.68c	6.00±0.00ef	30.33±0.88efg
Usnic acid	1.25	0.33±0.33a	3.33±0.33bc	26.00±0.58b
	2.5	0.67±0.33a	3.67±0.33bcd	27.33±0.33bcd
	5.0	1.33±0.33abc	5.33±0.33def	29.00±0.58cdef
	10.0	2.33±0.88bc	6.33±0.33fg	31.33±0.88fg
Control	-	0.00±0.00a	0.00±0.00a	0.00±0.00a

[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≤0.05

Table II: The results of ANOVA belonging to concentration and exposure time of *Sitophilus granarius* adults

(a) Extract, Secondary Metabolites	(b) Extract, Secondary Metabolites	Mean Difference (a-b)	Std. Error	Sig. (p)
Extract	Usnic acid	0.00	0.284	1.000
	Diffractaic acid	0.17	0.284	0.936
	Control	11.42(*)	0.725	0.000
Usnic Acid	Extract	0.00	0.284	1.000
	Diffractaic acid	0.17	0.284	0.936
	Control	11.42(*)	0.725	0.000
Diffractaic Acid	Extract	-0.17	0.284	0.936
	Usnic Acid	-0.17	0.284	0.936
	Control	11.25(*)	0.725	0.000
Control	Extract	-11.42(*)	0.725	0.000
	Usnic Acid	-11.42(*)	0.725	0.000
	Diffractaic Acid	-11.25(*)	0.725	0.000

Dependent variable: The dead individual
*The mean difference is significant at the 0.01 level (p<0.01)

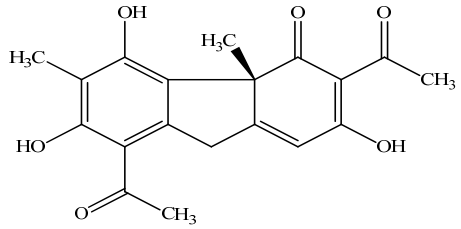
exposure time tested in these extracts (Table I & II). Higher concentrations and longer exposure times resulted in maximum toxicity on *S. granarius.* Treatment with extract and major compounds of *U. longissima* pointed out equal mortality against adults of *S. granarius* (Table I & II).

The mortality rates after 96 h of treatment with the maximum concentration (10 mg mL^{-1}) of *U. longissima* extract, diffractaic acid and usnic acid was determined as 98.98, 91.91, and 94.94% for *S. granarius,* respectively. However, there was no mortality in the controls of each species (Fig. 4).

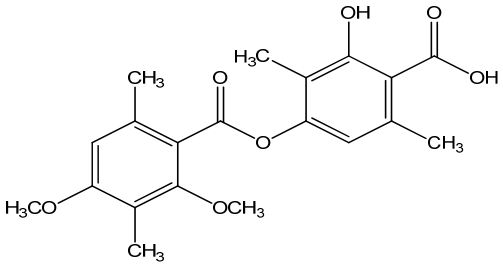
The mortality rates after 96 h of treatment with the 10 mg mL^{-1} in concentrations of extract of *U. longissima* was established to the highest mortality rate in *S. granarius* with 98.98% in comparison with two other lichen compounds.

The results revealed that the used two metabolites and extract of *U. longissima* have different insecticidal activity

Fig. 1: The chemical structures of usnic and diffractaic acids

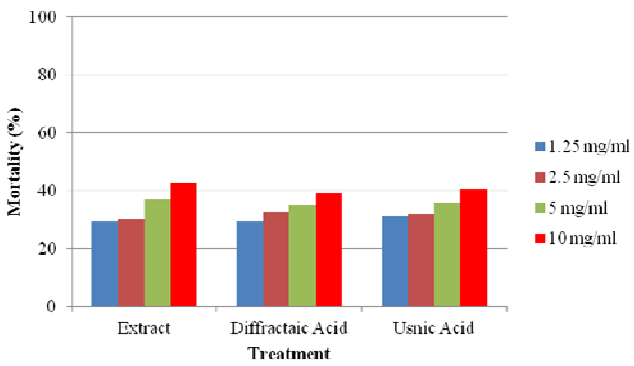


(+)-Usnic acid



Diffractaic acid

Fig. 2: Mortality of *Sitophilus granarius* (L.) exposed to the extract and two secondary metabolites of *Usnea longissima* at different doses



on *S. granarius*. The insecticidal activity increased with increasing concentration and exposure times. The extract and secondary components caused significant mortality (Figs. 2 & 3). The results suggest that lichen compounds could be useful in the search of new insecticides. Previous some studies demonstrated that in general the toxicity of extracts isolated from lichen samples against pests related to their secondary components (Balaji *et al.,* 2007; Cetin *et al.*, 2008; Sahip *et al.,* 2008; Silva *et al.,* 2009).

In this study, insecticidal effects of the total extract and two secondary metabolites isolated from *U. longissima* on adults of *S. granarius* were compared. The results of three samples are remarkable. Giving a close insecticide rate of the total extract and the two secondary metabolites, usnic acid or diffractaic acid responded as effective substances causing the death of *S. granarius*. Lichen substances could play a multiple biological role. Especially, usnic acid as a lichen metabolite controls its toxic features (Cocchietto *et*

Fig. 3: Mortality of *Sitophilus granarius* (L.) according to treatment times of the extract and two secondary metabolites of *Usnea ongissima*

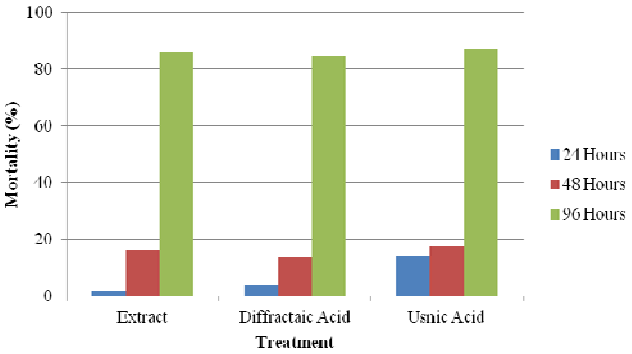
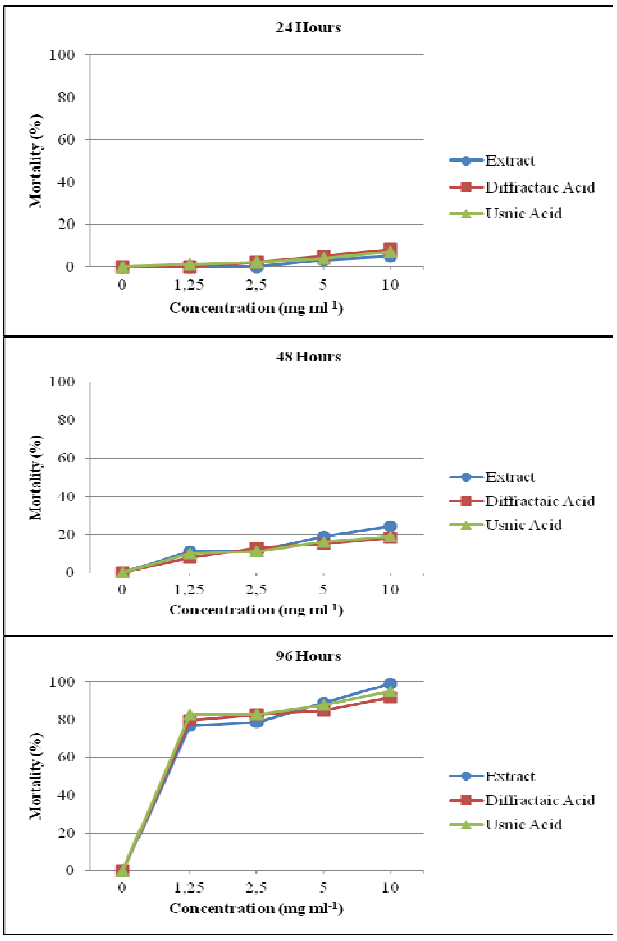


Fig. 4: Mortality of *Sitophilus granarius* (L.) in relation to exposure time and concentration of the extract and two secondary metabolites of *Usnea longissima*



al., 2002). Previously usnic acid was used against the larvae of *Culex pipiens* L. and exhibited 100% mortality (Cetin *et al.,* 2008).

Consequently, it is established that there is not a significant difference among lichen substances and the extract tested for insecticidal effects due to p>0.01 according to values computed at 99% confidence interval. It means that the lethal effect rates indicated on adults of *S. granarius* of this lichen species exhibited considerable difference from each other. However, it was seen that three samples studied have mortality in high degree, too.

Considering on all these results, it can be reported that *U. longissima* Ach. has insecticidal activity in a very large extent owing to its acidic ingredients. Therefore, *U. longissima* may be used as potential insecticidal agent against adults of *S. granarius*. These results suggest that lichen compounds may be useful in the search of new insecticides.

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