Insecticidal Activities of Extracts of Three Lichen Species on *Sitophilus granarius* (L.) (Coleoptera: Curculionidae)

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Abstract

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Four different concentrations of extracts obtained from three lichen species (*Lecanora muralis* (Schreb.) Rabenh., *Letharia vulpina* (L.) Hue, and *Peltigera rufescens* (Weiss) Humb) were tested against adults of *Sitophilus granarius* (L.) in Petri dishes. After treatments, mortalities of the adults were determined after 24, 48, and 96 h. Expectedly, higher concentration and longer exposure time resulted in higher *S. granarius* mortality. Mortalities 96 h after treatments with the highest concentration (20 mg/ml) of extracts of *L. vulpina, P. rufescens,* and *L. muralis* were determined as 100, 100, and 86.86%, respectively. However, there were no dead insects in the control group. Values of LC_{50} after 96 h for *L. muralis, L. vulpina,* and *P. rufescens* extracts were 0.666, 0.505, and 0.328 mg/ml, respectively.

Keywords: granary weevil; insecticidal effect; lichen extract

The wheat weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), also known as grain weevil or granary weevil, is a common economic pest all over the world. *S. granarius* decreases the post-harvested grain yield at storage significantly (Yildirim 2012). Chemical insecticides are the method of choice for controlling stored grain pests. A rapid and efficient control of the pests is generally provided with chemical insecticides. Nevertheless, the chemical insecticides cause many environmental hazards and are detrimental to human health (Liess & von der Ohe 2005; Frampton *et al*. 2006; Starks *et al*. 2012). In order to overcome the undesirable effects of insecticide use, many researchers are looking for new biological insecticides, which provide pest control with minimal environmental hazards (Emsen *et al.* 2012a,b; Yildirim *et al*. 2012a,b, 2013).

Lichens can be a good source of biological insecticides. They contain many secondary metabolites and are formed through symbiosis between fungi and algae and/or cyanobacteria (Galun 1988; Brodo *et al*. 2001; Nash 2008). Many metabolites obtained from approximately 60 lichen species are used in antimicrobial, anticancer, antiallergen, immunological, and expectoral drugs (Kirmizigul *et al*. 2007). In recent years, researchers have been focusing on the secondary compounds of lichens commonly known as lichen acids to be used as alternatives for chemical insecticides (Emsen *et al.* 2012a; Yildirim *et al*. 2012a). In addition, antiviral, antiprotozoal, antiproliferative, analgesic, anti-inflammatory, and antipyretic activities of usnic acid, known as a secondary metabolite, have been researched (Cocchietto *et al*. 2002; Ingolfsdottir 2002; Rattan 2010). However, to the best of our knowledge, studies have not yet been conducted to evaluate the insecticidal activities of lichens against *S. granarius*. Therefore, the present study was designed to evaluate the insecticidal effects of four different concentrations of extracts of *Lecanora muralis* (Schreb.) Rabenh., *Letharia vulpina* (L.) Hue, and *Peltigera rufescens* (Weiss) Humb. against adults of *S. granarius* under laboratory conditions.

MATERIAL AND METHODS

Insects. The culture of *S. granarius* used in the study was started using insects collected from the Erzurum storage house. Wheat grains were purchased from a local market and stored at −20°C. The wheat was washed using tap water, and dried by heating before use in the experiments in order to prevent infestation by insects. *S. granarius* adults were reared in the laboratory at 25 ± 1°C, 64 ± 5% relative humidity, and 12 h photoperiod in the Department of Plant Protection, Atatürk University, Turkey. The adults obtained from laboratory cultures were kept in separate insect cages that contained wheat diet.

Plant material and isolation of lichen extracts. *L. muralis*, *L. vulpina,* and *P. rufescens* were collected during the period June 2012–2013 from Erzurum, Turkey. All samples were identified and stored in the Kazim Karabekir Education Faculty at Atatürk University, Erzurum. After collection, lichen samples were dried under room conditions. Air-dried lichen samples were pulverised and extracted by Soxhlet extractor. Metabolites from each 30 g lichen sample were extracted by using 300 ml of distilled *n*-hexane, diethyl ether, acetone, and methanol solvents. Extractions by *n*-hexane and diethyl ether solvents were conducted over two days at 25°C. Extractions by acetone and methanol solvents lasted three days and were conducted at 25°C. Thereafter, all extracts were mixed together and the solvents of each extract were evaporated using a rotary evaporator in order to obtain the total crude extract. Through this process, total lichen extracts were obtained. Total lichen extracts were then dissolved in 80% acetone. The concentrations of the solutions for each lichen species were 2.5, 5, 10, and 20 mg/ml. Extraction of *L. muralis, L. vulpina,* and *P. rufescens* yielded 8.33, 7.50, and 6.62% (w/w) of lichen substances, respectively. The yields were based on dry materials of lichen samples.

Determination of the age of the adults. Four- to six-day old *S. granarius* adults were used. Larvae and pupa with wheat grain were placed separately in Petri dishes. After adult emergence from pupa, they were collected and used for the same tests.

Bioassays. To determine the toxicity of the extracts against *S. granarius* adults, 33 adults were placed in 9 cm Petri dishes that contained 33 kernels of wheat. The concentrations of the extracts used were 2.5, 5, 10, and 20 mg/ml with each Petri dish assigned a single concentration. For each Petri dish 0.8 ml of extract solution was used. The 0.8 ml of extract solution was applied using an airbrush spray gun. Mortalities of adults were determined 24, 48, and 96 h after exposure. A Petri dish sprayed with only 80% acetone solution was used as the control. There were three replicates for each concentration and exposure time combination and insecticidal activity of extracts was expressed as percentage mortality of the adults.

Statistical analyses. The differences among the insecticidal activities of lichen extracts tested were determined by the analysis of variance (ANOVA) test using SPSS software. Duncan and *LSD* tests were used for comparison of means.

The LC_{50} (median lethal concentration) values were calculated following the method of Finney (Finney 1971). Probit analysis of concentration-mortality data was conducted to estimate the LC_{50} values and associated 95% confidence limits for each treatment using the EPA Probit Analysis Program.

RESULTS

Extracts were toxic to adults of *S. granarius* at different concentrations and exposure times. Maximum mortalities were recorded after 96 h of exposure at all concentrations (Table 1, Figures 2 and 3). There were high positive correlations between concentration and insecticidal effect; exposure time and insecticidal effect (Table 2). For concentration-insecticidal effect correlation, *L. muralis* had the lowest correlation coefficient (0.446), whereas *P. rufescens* had the highest coefficient (0.513). For elapsed time-insecticidal effect correlation, *L. muralis* had very high positive correlation (0.832), whereas *P. rufescens* had the lowest correlation of 0.739 (Table 2).

Extracts from the three species of lichens were toxic to *S. granarius* adults (Tables 1 and 2). Treatments with extracts of *L. vulpina* and *P. rufescens* resulted in higher mortality of *S. granarius* adults compared to *L. muralis* which caused low mortality (Table 1).

After 24 h exposure, the *L. muralis* 20 mg/ml extract treatment caused significantly higher mortality than the control, the 5, 10, and 20 mg/ml extracts of *L. vulpina* and *P. rufescens* also caused significantly higher mortality (Table 1). Extracts of *P. rufescens* caused no significant increase in mortality after 48 h of exposure. Mortalities caused by 5, 10, and 20 mg/ml extracts of *L. vulpina* and 10 and 20 mg/ml extracts of *L. muralis* were significantly higher than
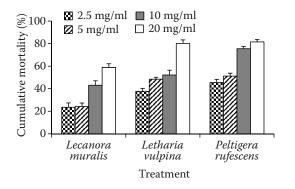
Figure 1. Percentage mortality of *Sitophilus granarius* exposed to extracts of three lichen species at different concentrations. Each value is expressed as mean ± standard deviation (*n* = 3)
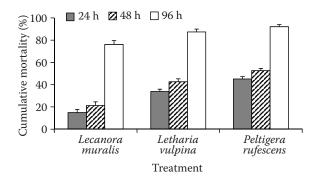

Figure 2. Percentage mortality of *Sitophilus granarius* according to the treatment times of extracts of three lichen species. Each value is expressed as mean ± standard deviation (*n* = 3)

those in the control. After 96 h of exposure, all lichen extracts resulted in significantly higher mortalities than the control (Table 1).

Based on data for all concentrations, toxicity of extracts of the three lichen species on *S. granarius* in decreasing order was *P. rufescens* > *L. vulpina* > *L. muralis*. At the highest concentration (20 mg/ml), insecticidal activities (cumulative mortalities) of *P. rufescens*, *L. vulpina,* and *L. muralis* extracts on *S. granarius* were 81.47, 80.13, and 58.91%, respectively (Figure 1).

The highest cumulative mortalities after 24, 48, and 96 h of exposure were caused by *P. rufescens* extract. After 96 h of exposure, insecticidal activities

(cumulative mortalities) of *P. rufescens*, *L. vulpina*, and *L. muralis* extracts on *S. granarius* were 92.16, 87.36, and 76.25%, respectively (Figure 2).

The rapid ability of insecticides in killing pests is important in postharvest loss mitigation. After 24 h exposure, although the extracts with concentration of 2.5 and 5 mg/ml obtained from *L. muralis* failed to exert any mortality effects, the extracts with all concentrations of *L. vulpina* and *P. rufescens* began to show insecticidal activity against *S. granarius* (Table 1 and Figure 3). Cumulative mortalities after 24, 48, and 96 h of exposure and cumulative mortalities of treatments with different concentration of lichen extracts on *S. granarius* were given in Figures 1

Table 1. Mortality effects of three lichen species extracts on *Sitophilus granarius* adults

Treatment	Concentration (mg/ml)	Mean mortality[a]		
		24[b]	48[b]	96[b]
Lecanora muralis	2.5	0.00 ± 0.00[D]	2.02 ± 0.57[E]	68.68 ± 1.52[D]
	5	0.00 ± 0.57[D]	3.03 ± 0.00[E]	69.70 ± 4.58[D]
	10	21.21 ± 3.00[BCD]	28.28 ± 2.08[D]	79.79 ± 1.1[CD]
	20	38.38 ± 3.05[B]	51.51 ± 3.60[BC]	86.86 ± 0.57[BC]
Letharia vulpina	2.5	10.10 ± 1.52[CD]	21.21 ± 6.08[DE]	81.81 ± 1.00[BC]
	5	27.27 ± 5.29[BC]	36.36 ± 3.60[CD]	81.81 ± 1.00[BC]
	10	31.31 ± 2.51[BC]	39.39 ±3.60[CD]	85.85 ± 0.57[BC]
	20	66.66 ± 7.00[A]	73.73 ± 1.52[A]	100.00 ± 0.00[A]
Peltigera rufescens	2.5	17.17 ± 3.21[BCD]	32.32 ± 3.21[CD]	86.86 ± 1.15[BC]
	5	29.29 ± 2.08[BC]	35.35 ± 2.08[CD]	88.88 ± 0.57[ABC]
	10	65.65 ± 1.52[A]	67.67 ± 2.30[AB]	92.92 ± 0.57[AB]
	20	68.68 ± 3.05[A]	75.75 ± 2.64[A]	100.00 ± 0.00[A]
Control	–	0.00 ± 0.00[D]	0.00 ± 0.00[E]	0.00 ± 0.00[E]

[a]mean ± standart deviation of three replicates, each set-up with 33 adults; [b]exposure time (h); values followed by different capital letters in the same column differ significantly at *P* < 0.01

Table 2. Correlation between different variables and three lichen species extracts

Treatment	Mortality–Concentration[a]	Mortality–Exposure time[a]
Lecanora muralis	0.446[b]	0.832[b]
Letharia vulpina	0.510[b]	0.764[b]
Peltigera rufescens	0.513[b]	0.739[b]

[a]Pearson correlation coefficient; [b]correlation is significant at the 0.01 level

and 2, respectively. Insecticidal activities after 96 h of treatment were recorded as 100, 100, and 86.86% at maximum concentration (20 mg/ml) of extracts obtained from *L. vulpina*, *P. rufescens,* and *L. muralis*, respectively. (Table 1 and Figure 3).
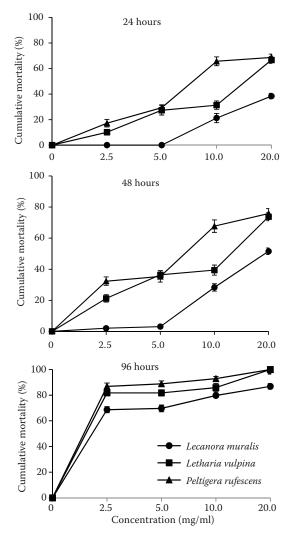

Figure 3. Percentage mortality of *Sitophilus granarius* in relation to the exposure time and concentration of extracts of three lichen species. Each value is expressed as mean ± standard deviation (*n* = 3)

Concentration-mortality data for each extract were subjected to probit regression analysis in order to determine LC_{50} values for 24, 48, and 96 h after exposure (Table 3). Among the three lichen samples, the most potent insecticidal lichen was *P. rufescens* with LC_{50} value of 0.328 mg/ml for 96 h after exposure. Whereas, the least potent lichen was *L. muralis* with LC_{50} value of 0.666 mg/ml for 96 h after exposure. Based on LC_{50} values of lichen extracts at 24, 48, and 96 h after exposure, the strength of the three species of lichens in decreasing order is *P. rufescens* > *L. vulpina* > *L. muralis* (Table 3).

DISCUSSION

Many chemical insecticides are broad-spectrum killing both target and non-target organisms, which could comprise invertebrates and vertebrates. Less than judicious application of pesticides results in conversion products of the pesticide or the pesticide itself remaining in food, soil, water, and air. Even though some pesticides are not viewed as toxic, many of them could cause deleterious effects on the nervous system, and could have carcinogenic influences and even mutagenic (Liess & 2005; Frampton *et al.* 2006; Starks *et al.* 2012). Based on the afore-mentioned hazardous effects of pesticides, natural insecticides that are highly efficacious and are of reduced risk, such as lichen extracts, should be sought for the control of pests. Many of the detrimental effects of traditional pesticides on human health and the environment would be reduced by a more widespread use of reduced risk pesticides such as natural insecticides.

Biological insecticides derived from plants and microorganisms are used against many pests including insects (Lacey *et al.* 2009; Kim *et al.* 2010; Raudonis *et al.* 2010; Sharifian *et al.* 2013). Extracts and secondary metabolites of lichens have been demonstrated as effective biological insecticides against pests such as *S. granarius* (Emsen *et al.* 2012a; Yildirim *et al.* 2012b), *Sitophilus zeamais* (Yildirim *et al.* 2012a), and *Leptinotarsa decemlineata* (Emsen *et al.* 2012b). However, before the current study, there were no reports on the insecticidal effects of *L. muralis, L. vulpina,* and *P. rufescens* extracts against *S. granarius*.

Our data suggest that the extracts isolated from the three different lichen species investigated might have different toxicity, and this could be attributed to

Table 3. The LC$_{50}$ values (mg/ml) of three lichen species extracts on adults of *Sitophilus granarius*

Treatment	Exposure time (h)	LC$_{50}$ (limits)	Slope ± standard error (limits)
	24	23.022 (19.329–30.083)	2.987 ± 0.420 (2.163–3.811)
*Lecanora murali*s	48	18.671 (15.864–23.282)	2.626 ± 0.307 (2.025–2.227)
	96	0.666 (0.028–1.641)	0.717 ± 0.212 (0.302–1.133)
	24	13.454 (11.079–17.413)	1.744 ± 0.218 (1.317–2.172)
Letharia vulpina	48	9.776 (7.950–12.581)	1.450 ± 0.203 (1.053–1.848)
	96	0.505 (0.045–1.175)	1.063 ± 0.270 (0.534–1.591)
	24	8.560 (7.197–10.343)	1.749 ± 0.208 (1.343–2.156)
Peltigera rufescens	48	6.231 (4.977–7.672)	1.439 ± 0.200 (1.047–1.832)
	96	0.328 (0.011–0.910)	1.139 ± 0.321 (0.510–1.768)

differences in their chemical composition differing major or minor components. Previous studies have demonstrated that the toxicity of extracts isolated from lichen samples against pests was related to their secondary components (Emsen *et al.* 2012a; Yildirim *et al.* 2012a).

Based on our data, the extracts of *L. vulpina* and *P. rufescens* were more toxic against adults of *S. granarius* than were the extracts of *L. muralis*. *L. vulpina* and *P. rufescens* have compounds that show insecticidal activity and have the potential for being used as biological insecticidal agents against *S. granarius*.

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