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Toxicity Effects of Some Lichen Species Extracts against the Colorado Potato Beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae)

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ABSTRACT

Extracts of the three lichen species; *Lecanora muralis* (Schreb.) Rabenh., *Letharia vulpina* (L.) Hue and *Peltigera rufescens* (Weiss) Humb. were tested against the 4th larval instar and adults of the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). Results showed that the three extracts had insecticidal effects on larvae and adults of the pest. Treatment with the extracts of *L. vulpina* and *P. rufescens* led to highest percentages of mortality (reached 100%). The extract of *L. muralis* caused the least mortality rate. Mortality rates, 120 hrs post treatment with the highest extract concentration (20 mg/ml) of *L. vulpina*, *P. rufescens* and *L. muralis*, were estimated as 100, 100 and 76.66% for the 4th larval instar and 100, 100 and 63.33% for adults of *L. decemlineata*, respectively, compared to no mortality in the control.

Key words: Insecticidal effect, Lichen extracts, Colorado potato beetle, *Leptinotarsa decemlineata*.

INTRODUCTION

The Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) is a serious pest of potatoes. It may also cause significant damages to tomatoes and eggplants in Turkey and many other countries. Both adults and larvae feed on foliage and may eliminate completely the crop. Insecticides are currently the main method of the beetle control on commercial farms. However, many chemicals are often unsuccessful when used against the pest because of its ability to rapid develop of insecticidal resistance (Gillott, 2005 and Yildirim, 2010). The beetle has developed resistance to most of all major insecticide classes, although not every population is resistant to every chemical (Alyokhin *et al.*, 2008). Therefore, in recent years, many researchers have looked for new biological compounds (Aggarwal and Brar, 2006; Alvarez *et al.*, 2007; Figueroa-Brito *et al.*, 2011 and Kordali *et al.*, 2012).

Lichens are significant insecticidal sources within biological insecticides (Emmerich *et al.*, 1993; Emsen *et al.*, 2012a, b and Yildirim *et al.*, 2012a, b) as they are formed through symbiosis between fungi and algae and/or cyanobacteria. Lichens can be used also to monitor pollution as well as sources of formulations that have uses in medicines, perfumes, cosmetics and dyes (Cetin *et al.*, 2008). Some of the formulations are known as the lichen acids that have effects as antivirals, antiprotozoal, antiproliferative, analgesic, anti-inflammatory, and antipyretic activities of usnic acid (Cocchietto *et al.*, 2002 and Ingolfsdottir, 2002).

Some previous researches have recorded that lichens have antibiotic effect. It was known that there are more than 60 antibiotic substances in lichens. Many researches indicated that some lichen acids such as usnic and vulpinic have powerful antibiotic effects against some bacteria (Galun, 1988). Furthermore, it was defined that the lichen species; *Letharia vulpina* (L.) Hue and *Vulpicida pinastri* (Scop.) J.-E. Mattsson were used to kill wolves and foxes that harm animal herds in some countries of Europe and Scandinavian in winter (Aslan *et al.*, 1998). Many experiments indicated that lichen metabolites have insecticidal, antifeedant and lethal characteristic effects on insects (Emmerich *et al.*, 1993; Bombuwala, 2001; Kathirgamanathar *et al.*, 2006; Nimis and Skert, 2006; Balaji *et al.*, 2007; Cetin *et al.*, 2008 and Silva *et al.*, 2009).

Aim of the present study was to evaluate the insecticidal effect of extracts from three different lichens: *Lecanora muralis* (Schreb.) Rabenh., *Letharia vulpina* (L.) Hue and *Peltigera rufescens* (Weiss) Humb. against the 4th larval instar and adults of the Colorado potato beetle, *L. decemlineata* under laboratory conditions.

MATERIALS AND METHODS

Insect rearing

The beetles were collected from Erzurum in Turkey. *L. decemlineata* adults and larval instars were reared on potato plant leaves under laboratory conditions of 25±1°C, 64±5 % RH and L: D = 12:12 h, at the Department of Plant Protection, Atatürk University. The 4th instar larvae were determined according to their morphological characteristics (Yildirim, 2010). For adults' test,

3-5 day-old adults were used as test insect. All tests were conducted under controlled laboratory conditions.

Lichens

The lichen species; *L. muralis*, *L. vulpina* and *P. rufescens* were collected from Erzurum in Turkey in June 2009. All samples were identified and stored in the herbarium of Kazım Karabekir Education Faculty, Atatürk University-Erzurum. Collected materials were maintained dry at room conditions.

Extraction of lichens

Air-dried lichen samples were pulverized and extracted by Soxhlet extractor. Pulverized lichen's samples (30g) were extracted at 25°C by distilled n-haxane, diethyl ether, acetone, and methanol solvents, respectively. 300 ml from each solvent were used for extraction. The solvent of each extract was evaporated using rotary evaporator for obtaining the total crude extract. By this way, total lichen substances were obtained. Extraction of *L. muralis*, *L. vulpina* and *P. rufescens* yielded 8.33, 7.50 and 6.62% (w/w) of lichens substances, respectively.

Solutions

Total lichen crude extracts were dissolved in acetone-water solvent, existed in 80% distilled acetone. From each lichen sample, different concentrations were prepared at 2.5, 5, 10 and 20 mg/ml concentrations.

Bioassay tests

To test insecticidal effects of the tested lichens extracts, 10 individuals of 4th larval instar or 3-5 day old adults were introduced into Petri dishes (9 cm) and confined with a piece of potato plant previously sprayed with 0.8 ml of the extract solution at desired concentrations (2.5, 5, 10 and 20 mg/ml).

Initial tests were done to establish appropriate dose and exposure time ranges. After exposure periods, mortality percentage of the 4th larval instar and adults were determined after 24, 48, 96 and 120 hrs. Petri dish applied with only 80% acetone solution was used as control. Three replicates were used for each dose. Exposure time combination with insecticidal activity of the extracts was expressed by % mean mortality of the 4th instar larvae and adults.

Statistical analysis

Differences among the insecticidal activities of tested lichens extracts were determined according to analysis of variance (ANOVA) and were tested by using the SPSS 15.0 software package. Duncan Test was used for comparison of means. Significant differences were estimated at $p<0.05$. Lethal concentration, LC_{50} and LC_{95} values were calculated following the method of Finney (1971). Probit analysis of concentration-mortality data was conducted to estimate the LC_{50} and LC_{95} values and associated 95% confidence limits for each treatment (EPA Probit Analysis).

RESULTS AND DISCUSSION

Toxicity effects of the lichen extracts, obtained from *L. muralis*, *L. vulpine* and *P. rufescens* on the 4th larval instar and adults of *L. decemlineata*, are summarized in tables (1) and (2). Results showed that all the extracts of foregoing lichens had an insecticidal effect on the 4th larval instar and adults of *L. decemlineata*. Higher concentration and longer exposure time resulted to maximum toxicity on both tested stages of the pest. Mortality rates at 24, 48, 72, 96, and 120 hrs post treatment with different concentrations of lichens extracts were given in figures (1) and (2). Analysis of variances demonstrated that the effects on mortality rate of the 4th larval instar and adults of *L. decemlineata* were highly significant ($p<0.05$) on the basis of concentration and exposure time tested. Treatments with the extracts of *L. vulpina* and *P. rufescens* had higher mortality rates on the 4th larval instar and adults of *L. decemlineata*, while *L. muralis* had the least mortality rate (Tables 1 and 2).

Comparisons among lichen species used in the experiments were given by computing value of p in tables (1) and (2) at $p<0.05$. Consequently, there were significant differences among the three tested lichen species on larvae and adults of *L. decemlineata*. Obtained data were subjected to probit regression analysis. Some tabular and pictorial forms were generated post-statistical analysis. Lethal concentrations, LC_{50} and LC_{95}, were determined for each extract.

All concentrations (2.5, 5, 10 and 20 mg/ml) of *L. vulpina* lichen extract gave highest mortality rate (100%) for either larvae or adults of *L. decemlineata*. Mortality rates 120 hrs post treatment with the maximum concentration (20 mg/ ml) of the extracts of *L. muralis*, *L. vulpina* and *P. rufescens* were determined as 76.66, 100, and 100% for 4th larval instar and 63.33, 100, and 100% for adults of *L. decemlineata*, respectively compared to no mortality in the control (Figs. 1 and 2).

L. vulpina and *P. ruferscens*, according to their LC values, were the most effective ones (Tables 3 and 4). LC_{50} and LC_{95} values after 96 and 120 hrs of *L. vulpina* extracts for both the 4th larval instar and adults of *L. decemlineata* were almost zero because of their very high mortality rates. For this reason, *L. vulpina* lichen species had highest insecticidal activity (100 %) on the pest. *L. muralis* had also a strong insecticidal efficacy against 4th larval instar

Table (1): Mortality rates (%) of *Leptinotarsa decemlineata* adults caused by three lichen species extracts at different time intervals under laboratory conditions

		Mean mortality at different intervals (hours)				
Treatments	Concentration (mg/ml)	24[b]	48[b]	72[b]	96[b]	120[b]
Lecanora muralis	2.5	0.00±0.00a	0.00±0.00a	3.33±0.57ab	6.66±0.57a	13.33±0.57b
	5	0.00±0.00a	3.33±0.57a	13.33±0.57bc	16.66±0.57b	26.66±0.57c
	10	6.66±0.57ab	6.66±0.57a	20±0.00c	26.66±0.57c	33.33±0.57c
	20	13.33±0.57b	23.33±1.15b	36.66±0.57d	56.66±0.57d	63.33±0.57d
Letharia vulpina	2.5	53.33±0.57cd	60±0.00cd	83.33±0.57fg	100±0.00f	100±0.00f
	5	86.66±0.57f	100±0.00g	100±0.00h	100±0.00f	100±0.00f
	10	100±0.00g	100±0.00g	100±0.00h	100±0.00f	100±0.00f
	20	100±0.00g	100±0.00g	100±0.00h	100±0.00f	100±0.00f
Peltigera rufescens	2.5	46.66±0.57c	56.66±0.57c	63.33±0.57e	80±1.00e	86.66±0.57e
	5	50±0.00cd	66.66±0.57d	73.33±1.52ef	90±1.00f	96.66±0.57f
	10	56.66±0.57de	76.66±0.57e	93.33±0.57gh	100±0.00f	100±0.00f
	20	63.33±0.57e	86.66±0.57f	100±0.00h	100±0.00f	100±0.00f
Control	-	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a

[a] Mean ±SE of three replicates, each set-up with 10 adults; exposure time (hr) [b] Exposure time (h)
Values followed by different letters in the same column differ significantly at $p<0.05$

Table (2): Mortality rates (%) of 4th instar larvae of *Leptinotarsa decemlineata*at caused by three lichen species extracts at different time intervals under laboratory conditions

		Mean mortality different intervals in hours[a]				
Treatments	Concentration (mg/ml)	24[b]	48[b]	72[b]	96[b]	120[b]
Lecanora muralis	2.5	0.00±0.00a	6.66±1.15ab	6.66±1.15a	10±1.00a	13.33±1.52b
	5	3.33±0.57a	20±1.00b	30±1.73b	36.66±1.15b	40±1.00c
	10	20±1.00b	53.33±2.51c	53.33±2.51c	56.66±2.51c	63.33±1.52d
	20	30±1.73b	56.66±1.52c	60±1.73cd	70±1.00c	76.66±0.57e
Letharia vulpina	2.5	76.66±0.57cd	90±0.00d	100±0.00e	100±0.00d	100±0.00f
	5	86.66±1.52de	100±0.00d	100±0.00e	100±0.00d	100±0.00f
	10	100±0.00e	100±0.00d	100±0.00e	100±0.00d	100±0.00f
	20	100±0.00e	100±0.00d	100±0.00e	100±0.00d	100±0.00f
Peltigera rufescens	2.5	63.33±0.57c	66.66±0.57c	73.33±0.57d	93.33±0.57d	100±0.00f
	5	70±1.00c	86.66±0.57d	100±0.00e	100±0.00d	100±0.00f
	10	93.33±0.57e	100±0.00d	100±0.00e	100±0.00d	100±0.00f
	20	93.33±0.57e	100±0.00d	100±0.00e	100±0.00d	100±0.00f
Control	-	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a

[a] Mean ±SE of three replicates, each set-up with 10 larvae. [b] Exposure time (hr)
Values followed by different letters in the same column differ significantly at $p<0.05$

Table (3): Effect of 96 and 120 hrs time intervals and LC_{50} and LC_{95} values (mg/ml) of three lichen species to 4th instar larvae of *Leptinotarsa decemlineata* under laboratory conditions

Treatments	Exposure Time (hr)	LC_{50} (Limits)	LC_{95} (Limits)	Slope ± SE
Lecanora muralis	96	9.111 (6.757-13.130)	67.345 (34.381-306.501)	1.893 ± 0.390
	120	7.549 (5.610-10.277)	50.073 (27.923-173.541)	2.002 ± 0.390
Letharia vulpina	96	[a]	[a]	0.000 ± 0.000
	120	[a]	[a]	0.000 ± 0.000
Peltigera rufescens	96	1.152 ([b])	2.720 ([b])	4.411 ± 4.160
	120	[a]	[a]	0.000 ± 0.000

[a] For this lichen extract no LC values are computed because the ratios of response counts to subject counts are the same, i.e. the slope is zero.
[b] Narrow limit.

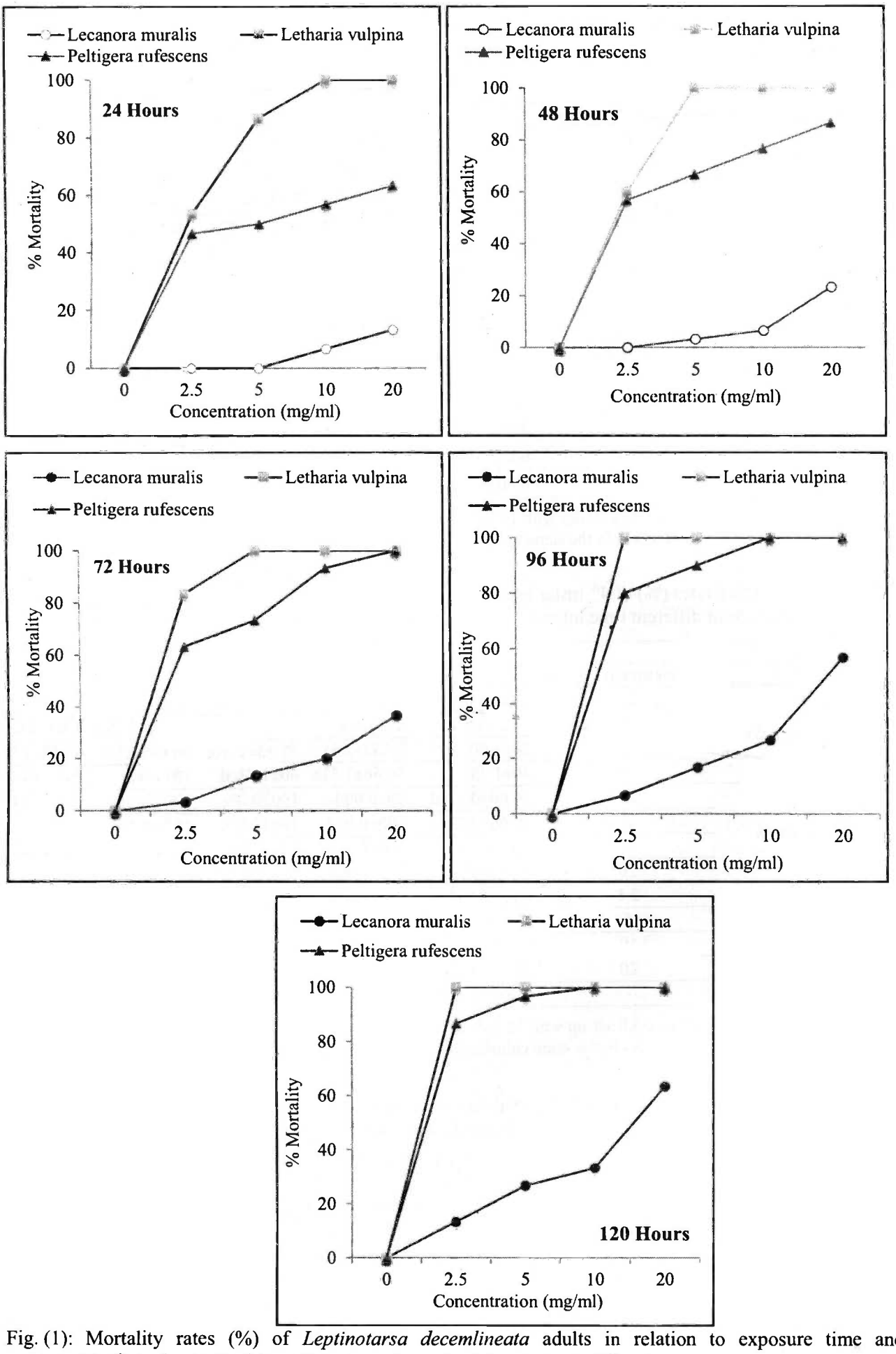

Fig. (1): Mortality rates (%) of *Leptinotarsa decemlineata* adults in relation to exposure time and concentration of extract of three lichen species under laboratory conditions.

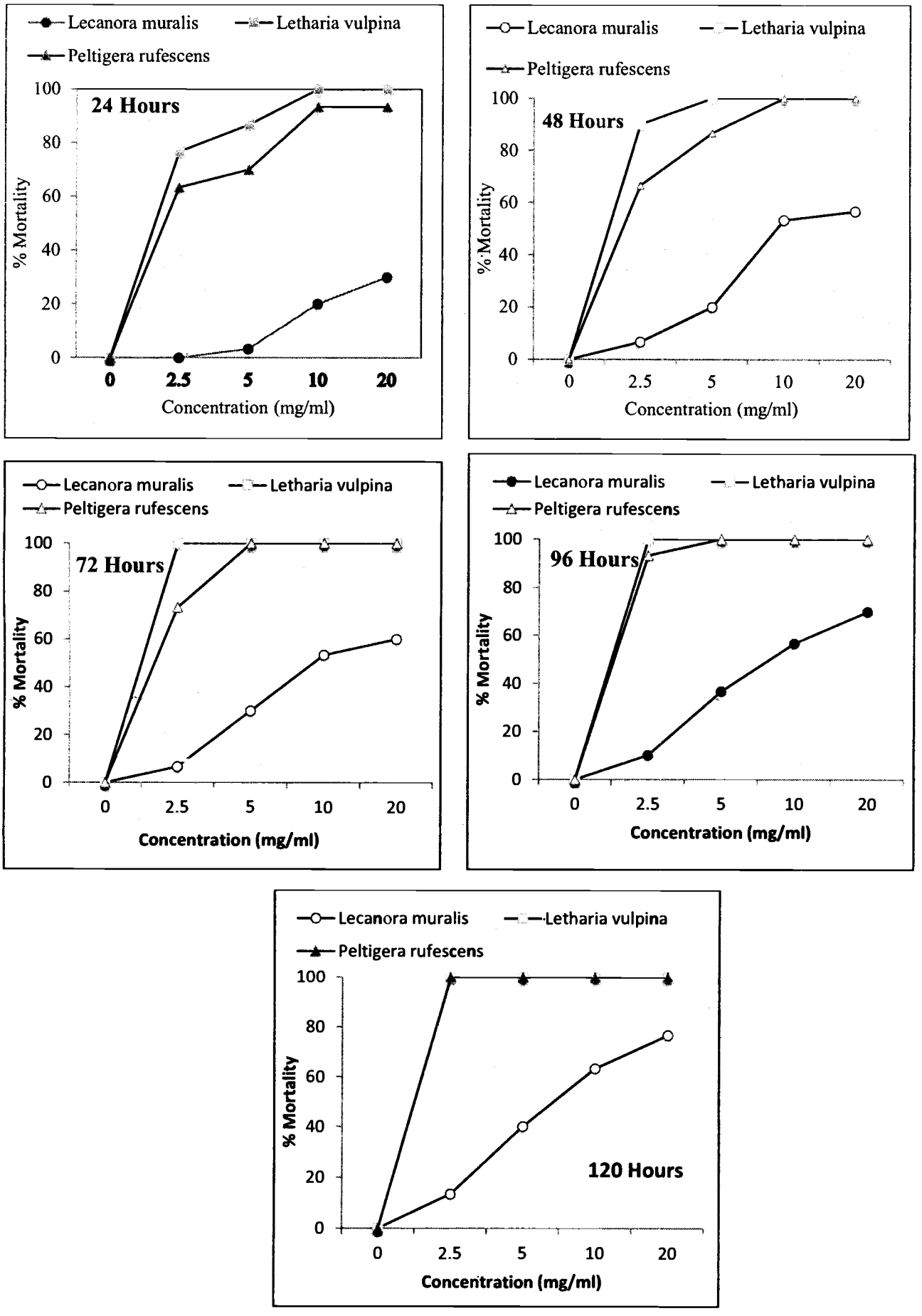

Fig. (2): Mortality rates (%) of 4th instar larvae of *Leptinotarsa decemlineata* in relation to exposure time and concentration of extracts of three lichen species under laboratory conditions.

Table (4): Effect of 96 and 120 hrs time intervals and LC_{50} and LC_{95} values (mg/ml) of three lichen species to adults of *Leptinotarsa decemlineata* under laboratory conditions

Treatments	Exposure time (hr)	LC_{50} (Limits)	LC_{95} (Limits)	Slope ± SE
Lecanora muralis	96	17.978 (12.517-37.352)	143.414 (57.593-1542.767)	1.824 ± 0.425
	120	14.156 (9.697-29.796)	168.824 (59.051-3393.808)	1.528 ± 0.384
Letharia vulpina	96	[a]	[a]	0.000 ± 0.000
	120	[a]	[a]	0.000 ± 0.000
Peltigera rufescens	96	1.239 (0.072-2.097)	5.625 (3.935-20.964)	2.503 ± 0.920
	120	1.034 (0.000-1.919)	3.926 (2.527-18.589)	2.839 ± 1.403

[a] For this lichen extract LC values were not computed because the ratios of response counts to subject counts were the same, i.e. the slope is zero.

and adults of *L. decemlineata*. LC_{50} value of *L. muralis* extract for the 4th larval instar of *L. decemlineata* was 9.111 at 96 hrs and 7.549 at 120 hrs (Table 3) and that for adults was 17.978 at 96 hrs and 14.156 at 120 hrs (Table 4).

Natural products are now being considered as alternatives to arsenal of synthetic pesticides currently available (Dayan *et al.*, 1999), therefore lichens are potential for pest management when proved safe to beneficial fauna and environment. Previous studies demonstrated that, in general, toxicity of extracts isolated from lichen samples against pests is related to their secondary components (Emmerich *et al.*, 1993; Bombuwala, 2001; Kathirgamanathar *et al.*, 2006; Nimis and Skert, 2006; Balaji *et al.*, 2007; Cetin *et al.*, 2008; Silva *et al.*, 2009; Emsen *et al.*, 2012a and Yildirim *et al.*, 2012a, b). Their results suggested that the extracts isolated from different lichen species have different toxicity effects, which can be attributed to their different chemical composition and components. 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*, vary from 2.6 to 4.8% of dry weight (Culberson *et al.*, 1977), in *Cetraria islandica* contents of fumarprotocetraric acid could reach 11% (Gudjonsdottir and Ingolfsdottir, 1977), while *Pertusaria alaianta* contains 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 value. In fact, several lichen species have proved to be endowed with diversified biological activities. In these studies, some lichens showed antifeedant and lethal characteristics on insects. Lichen acids isolated from lichen extracts are functional substances that will be able to cause effect on only the target organisms.

In conclusion, extracts of the lichens *L. vulpina* and *P. rufescens* were found efficient against larvae and adults of *L. decemlineata*. But in use, exposure time has to be considered as *L. vulpina* was effective at short time period. Obtained results suggest that lichen compounds could be useful in the search for new safe bioinsecticides.

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