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Insecticidal Effects of Two Plant Aqueous Extracts against Second Instar Larvae of *Lycoriella Auripila* (Diptera: Sciaridae)

Najmeh Shirvani Farsani, Abbas Ali Zamani, Saeed Abbasi, Katayoon Kheradmand

***Abstract*—**The toxicity of aqueous extracts of two plants, *Nicotiana tobacum* and *Eucalyptus globulus* were investigated against second instar larvae of *Lycoriella auripila*, one of the most important pests of button mushroom, using agar dilution technique. Seven concentrations of aqueous extracts of both plants were applied on second instar larvae and their mortality were evaluated after 24, 48 and 72 h. The obtained results revealed that aqueous extracts of *N. tabacum* and *E. globulus* caused 77.55 and 72.5% mortality of larvae of *L. auripila* at concentration of 4000 ppm after 72h, respectively. Toxicities of tobacco extract after 24, 48 and 72 h were 1.52, 1.85 and 1.70 times greather than eucalyptus, respectively. The estimated LC_{50} after 24, 48 and 72 h were 7316.5, 2468.5 and 2013.1 ppm for tobacco and 64870.0, 6839.5 and 3326.4 ppm for eucalyptus, respectively. These plants merit further study as potential insecticides for the control of *L. auripila*.

***Keywords*—**LC_{50}, *Lycoriella auripila*, plants extracts, Toxicity

I. Introduction

CULTIVATION of the button mushroom, *Agaricus bisporus* (Lange) Imbach,s is commonly affected by *Lycoriella* spp. (Diptera: Sciaridae) [3]. The sciarid fly *L. auripila* is the major pest of cultivated mushrooms. Its larvae, which are capable of damaging the crop at all instars of production, may cause severe yield losses [5]. Secondary metabolites of plants have been suggested as an alternative source for insect control because they constitute a rich source of bioactive chemicals. They act in many ways on various types of pest complex and can be applied to mushroom houses and cellars in the same manner as the insecticides currently used. Many plant preparations and their constituents exhibit biological activities, such as ovicidal, repellent, and insecticidal activities against various insect species [2]. Additionally, some plant preparations and their constituents are found to be highly effective against insecticide-resistant insect pests [4]. Because of this, much effort has been focused on plants or their constituents as potential sources of commercial insect control products [2].In this study, we assessed the potential of two plants extracts, tobacco and eucalyptus, for use as commercial insecticides. The insecticidal activity of water extracts from two plant samples was assessed against second instar larvae of *L. auripila*.

N. Shirvani Farsani, is with the Dep. of Plant Protection, Campus of Agriculture and natural Resources, Razi University, Kermanshah, Iran; (e-mail: nj.shirvani@yahoo.com).

A. A. Zamani, is with the Dep. of Plant Protection, Campus of Agriculture and natural Resources, Razi University, Kermanshah, Iran; (corresponding author phone: +98-831-8323734, cell phone: +98-913-112-4190, e-mail: zamany55@gmail.com).

S. Abbasi, is with the Dep. of Plant Protection, Campus of Agriculture and natural Resources, Razi University, Kermanshah, Iran; (e-mail: abbasikhs@yahoo.com).

K. Kheradmand, is with the Dep. of Entomology and Plant Pathology, College of Abouraihan, University of Tehran, Iran; (email: kkheradmand@yahoo.com)

II. Materials and Methods

A. Preparation of crude extracts

Two plant species, *N. tabacum* and *E. globulus* were selected for extraction. The powdered form of plant materials were extracted using distilled water. Aqueous extraction was achieved by adding 100 ml distilled water to 5g powdered tissues. The mixture was shaked continuously for 48 hours using a rotary shaker at room temperature and filtered through Whatman filter paper. Then, the solvent was evaporated using rotary evaporator.

B. Insects

Some adults of *L. auripila* were put into glass petri dishes filled with *A. bisporus* mycelia grown on (PDA) medium at 25±2°C, 65±5% relative humidity under a photoperiod of 12:12 (L:D) h. Hatched larvae were reared on *A. bisporus* under the same conditions until emergence of second instar larvae. Thereafter, 10 second instar larvae were used for each concentration. Larvae were checked daily for mortality until 3 days and the numbers of dead ones were counted after 25, 48 and 72 h. These experiments were replicated four times for all treatments.

C. Bioassays

The insecticidal effect of the extracts against second instar larvae of L. auripila was studied using agar dilution technique. Seven concentrations of 2, 20, 80, 200, 800, 2000 and 4000 ppm were prepared by mixing both plant extracts with potato dextrose agar (PDA) in flasks and poured into sterile Petri dishes (10cm diameter×1cm). Control was treated by mixing of one ml sterile distilled water with 20 ml PDA media.

D. Statistical analysis

The experiment was conducted using a completely randomized design. Concentration- responses relationship was determined by Probit analysis [6]. The Abbott formula [1] was used to corrected mortality rates:

$$\% CM = P\text{-}P_0 / 100\text{-}P_0$$

where *CM* is corrected mortality rates, *P* is mortality rates in treatment and P_0 is mortality rates in control. A parallelism test for regression line slopes were run to compare relative potency of the same extracts at different times or different extracts at the same times.

III. Results and Discussion

The estimated LC_{10}, LC_{50}, slope and chi-square of tobacco and eucalyptus at 24, 48 and 72 hours are presented in Table I. The mortality caused using different concentrations of tobacco and eucalyptus extracts showed a linear relationship between the log of concentrations and mortality probit. The chi-square values are all non-significant which are consistent with a homogenous population. The contact insecticidal effects of tobacco and eucalyptus extracts on second instar larvae of *L. auripila* increased with exposure time, due to reducing amounts of LC_{50} over time. The corrected percentage of mortalities of second instar larvae were increase with incrasing both plant extract concentration and exposure time (Fig. 1). The highest mortalities by both plant extracts were occurred at concentration of 100000 ppm and after 72 h. The results revealed that there were significant differences among lines slopes (X^2=8.581; *df*=2; P_{value}=0.014) at 24, 48 and 72 hours in tobacco extract (Table II), while, no significant differences were observed for eucalyptus extract according parallelism test (X^2=3.647; *df*=2; P_{value}=0.161),therefore, we can compare relative potency of eucalyptus extract on the second instar larvae of *L. auripila* among 24, 48 and 72 h. According to results, the contact toxicity of eucalyptus increased with increasing exposure time and the lowest LC_{50} were occurred for 24 h (Table III). In addition, relative potency of two plants extracts at the same times is showed in Table IV. Accordingly, toxicity of tobacco extract after 24, 48 and 72 h were 1.52, 1.85 and 1.70 times greater than eucalyptus, respectively. Aqueous extracts of tobacco and eucalyptus caused 77.55 and 72.5% mortality on second instar larvae of *L. auripila* at concentration of 4000 ppm after 72 h, respectively. The obtained results show that tobacco and eucalyptus could be considered as potential organic insecticide in control of sciarid flies in mushroom cultivation farm.

Acknowledgment

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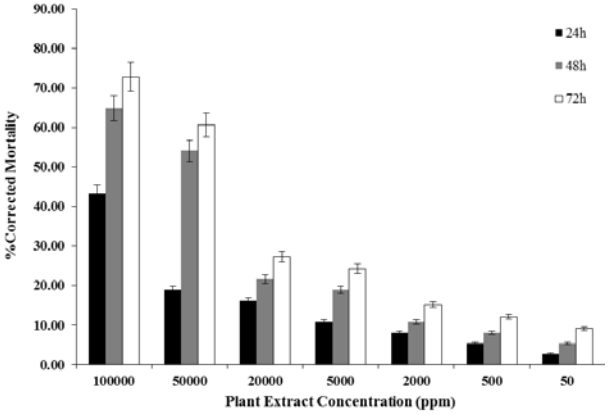
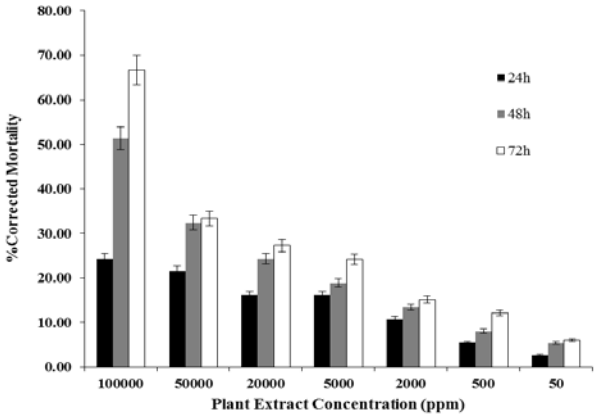


(A)



(B)

Fig. 1 The corrected mortality percentages (±SE) of second larvae of *Lycoriella auripila* exposed to different concentrations of (A) *Nicotiana tabacum* and (B) *Eucalyptus globulus* after 24, 48 and 72 h

TABLE I
Contact Insecticidal Effects Of Tobacco And Eucalyptus Extracts On Second Instar Larvae Of *Lycoriella Auripila* After Different Exposure Times

Plant species	Time interval (h)	Lethal concentration (*PPM*) LC_{10}	Lethal concentration (*PPM*) LC_{50}	Slope ± SE	X^2	P_{value}
N. tabacum	24	954	7316	1.45±1.5	7.2	1.00
	48	327	2468	1.46±0.6	36.4	0.06
	72	352	2013	1.69±0.8	28.6	0.28
E. globulus	24	7	64870	0.26±0.4	8.4	0.99
	48	173	6839	0.80±0.4	18.3	0.83
	72	839	3326	2.14±1.7	10.8	0.99

TABLE II
COMPARISON RELATIVE POTENCY OF TOBACCO ON SECOND INSTAR LARVAE *LYCORIELLA AURIPILA* AT DIFFERENT EXPOSURE TIMES

Time	LC_{50} ratio	Confidence limits (95%)	Parallelism test		
			X^2	*df*	P_{value}
24:48	4.568	1.526-50.866			
24:72	11.475	3.101-288.434	8.58	2	0.01
48:72	2.512	1.179-9.774			

TABLE III
COMPARISON RELATIVE POTENCY OF EUCALYPTUS ON SECOND INSTAR LARVAE *LYCORIELL AURIPILA* AT DIFFERENT EXPOSURE TIMES

Time	LC_{50} ratio	Confidence limits (95%)	Parallelism test		
			X^2	*df*	P_{value}
24:48	6.747	0.919-12073.054			
24:72	42.757	2.889-6615285.296	3.65	2	0.16
48:72	6.337	1.489-1157.412			

TABLE IV
COMPARISON RELATIVE POTENCY OF TOBACCO AND EUCALYPTUS ON SECOND INSTAR LARVAE *LYCORIELL AURIPILA* AT THE SAME EXPOSURE TIMES

Plant species	Time (h)	LC_{50} ratio	Confidence limits (95%)	Parallelism test		
				X^2	*df*	P_{value}
E. globulus: N. tabacum	24	1.52	-	1.97	1	0.16
E. globulus: N. tabacum	48	1.85	0.85-27.62	0.27	1	0.60
E. globulus: N. tabacum	72	1.70	0.89-247.29	0.01	1	0.95

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