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Evaluation of plant extracts for the management of citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae)

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Abstract

Citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is a major pest of citrus, which feeds on almost all citrus cultivars, and some related Rutaceae spp. We evaluated the efficacy of aqueous and alcoholic extracts of selected plant species (neem *Azadirachta indica* A. Juss, banana *Musa acuminate* Colla, eucalyptus *Eucalyptus camaldulensis* (Dehn.), mint *Mentha arvensis* L., datura *Datura stramonium* L., tumma *Citrullus colocynthis* (L.) and lemon *Citrus limon* (L.) against *P. citrella larvae* by two different application methods: leaf dip and topical applications. The highest *P. citrella* mortality was observed in the aqueous (61.17%) and alcoholic (58.3%) extracts of *A. indica* compared to rest of the plant extracts after 24 hours of exposure. Among two treatment application methods, higher *P. citrella* mortality was obtained in the topical application of *A. indica* extract than leaf dip application. Furthermore, the LC_{50} value of *A. indica* aqueous extracts was 6.8% in leaf dip bioassay and 4.55% in topical application which was lower compared to all other extracts. *Musa acuminate* and C. *limon* aqueous and alcoholic extracts were found least effective against *P. citrella* larvae. When the combined efficacy of plant extracts with abamectin was evaluated, the aqueous and alcoholic extract of *A. indica* combination treatments provided the highest mortality (62.25% and 66.25% respectively) than the rest of the treatments. Our findings indicate that *A. indica* extract has the potential to be tested as a botanical insecticide for the management of *P. citrella* larvae as a stand-alone (for organic growers) or in an integrated approach with abamectin. It would reduce the input cost of the growers and also help reduce the negative impacts of synthetic chemical insecticides.

Keywords: Citrus mandarins; leaf dip bioassay; mortality percentage; plant extracts; topical application.

1. Introduction

Endemic to Asia, citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is a major insect pest of citrus in almost all the citrus-producing countries including India, Pakistan, China, Phillipines, Sri Lanka and Bangladesh (CABI, 2017). During the early 1990's, it rapidly spread to the citrus growing areas of America and the Mediterranean basin (Beattie, 1993). Now it is considered one of the most destructive citrus pests worldwide, causing growers to have economic losses in both crop damage and pest management. In Pakistan, it is distributed in Punjab, Sindh and Khyber Pakhtunkhwa provinces. It is known for direct and indirect damage to citrus plants (Arshad *et al.*, 2018).

Repeated applications of insecticides are often required for effective control of *P. citrella* due to their high reproduction rate, multivoltine life history (Yumruktepe *et al.*, 1996), protection of larvae within the mines, evading topical sprays, and pupal protection by the rolled leaf margins (Beattie, 2004). Together all these factors may reduce suppression of the pest in the growing season and/or development of insecticide resistance in the target populations (Amiri-Besheli, 2008, 2009; Farooq & Freed, 2018). Considering the importance of alternative management practices, in the past decade, several researchers suggested the need of integrating biopesticides in the conventional pest control programs (Broderick *et al.*, 2000; Lacey *et al.*, 2001). Among different approaches, the utility of plant extracts as a broad-spectrum pesticide (insecticidal, antiviral, antifungal, antibacterial, anti-feedant, insect growth regulators) have been widely investigated and recommended for their use against multiple pest of economic importance (Belmain *et al.*, 2001; Carlini & Grossi-de-Sá, 2002; Grzywacz *et al.*, 2014; Manzoor *et al.*, 2016).

The multiple active ingredients in insecticidal plant extracts act synergistically and exhibit various modes of action that prevent resistance development in insect pests (Belmain *et al.*, 2001). In addition, these natural insecticides are relatively harmless to non-target organisms (Isman, 2006).

Neem [*Azadirachta indica* A. Juss (family: Meliaceae)], a plant native to the Indian Subcontinent, is extensively grown in Pakistan and India for its medicinal and pesticidal properties. Neem products such as bark, seed, leaves and neem oil have been reported to suppress over 200 species of insect pests, five nematodes, and three mite species, and they are also considered benign to non-target organisms (Raguraman & Singh, 1999; Ukeh *et al.*, 2007). It acts as a repellent and affects an insect's growth by inhibiting the release of prothoracic hormones (Isman, 2006; Khattak & Rashid, 2006). In addition, the plant extracts of a perennial herb, *Datura stramonium* L. have been documented as a repellent to many agricultural insect pests in Asia (Zhang *et al.*, 2006; Kumral *et al.*, 2010). Furthermore, in agricultural pest management, natural insecticides are safer to use in organic food production in industrialized countries. They also play an important role in the production and post-harvest protection of food in developing countries (Isman, 2006).

Considering the threat that *P. citrella* poses to citrus production in Pakistan, there is a serious need to develop sustainable management system that involves the environment friendly approaches to control this pest. Furthermore, number of plants having insecticidal properties is available in nature, but a limited number of studies have dealt with the use of botanicals against *P. citrella*. Thus, in this study our objective was to extract and assess the comparative efficacy of aqueous and alcoholic extracts of multiple plants with known pesticidal properties; [leaves of *A. indica*, eucalyptus *Eucalyptus camaldulensis* (family: Myrtaceae), mint *Mentha arvensis* (family: Lamiaceae), tumma *Citrullus colocynthis* (family: Cucurbitaceae), lemon *Citrus limon* (family: Rutaceae), peel of banana *Musa acuminate* (family: Musaceae), and leaves and seed of datura *Datura stramonium* (family: Solanaceae)] when applied alone and in combination with abamectin (grower standard) against *P. citrella* larvae.

2. Materials and methods

2.1. Plant extracts preparation

The experiment was conducted in the Entomology Laboratory of the University of Sargodha, Pakistan (32°08'01.5"N 72°41'11.4"E). Plant materials were collected in and around the vicinity of the University from the natural area and were thoroughly washed with tap water to remove dust and other unwanted materials from the environment which may have accumulated on them. The dust free materials were allowed to dry away from direct sunlight in the laboratory for 24h. This was followed by oven drying at 50°C for 2 days. Then the dried samples were turned into a powder by using an electric blender to further use for extraction. Twenty grams powder of each plant material were added into a 200-ml conical flask, and 100 ml of a solvent such as water and methanol were separately poured into different flasks. The conical flasks were covered with aluminum foil and kept in an electrical shaker for 24h for continuous agitation at 150 rev/min in the laboratory. After that, the sample was filtered by using a muslin cloth followed by Whatman no 1 filter paper. The solvent was evaporated by using rotary vacuum evaporator (LABOROTA 4001, Heidolph, USA) with a water bath temperature of 50°C. Finally, the samples were transferred to glass vials and stored in the refrigerator at 4°C for further experimentation. Further dilution of each plant extract was carried out to prepare three concentrations, 2.5%, 5% and 7%.

This was accomplished by mixing water, and methanol individually to check the effect of each aqueous and alcoholic extract against *P. citrella* larvae.

2.2. Bioassay

Two separate bioassay methods were used to evaluate toxicity of the different plant extracts against *P. citrella* larvae, and the experiment was repeated once under laboratory conditions. For bioassays, the leaves with actively feeding third instar *P. citrella* larvae were collected from *C. mandarins* field (32°07'53.0"N 72°41'02.5"E) in the research area of the University of Sargodha. One larva was ensured on each leaf. If others were found, they were removed using a soft hair-brush. Leaves in the plastic paper were placed in the ice box and immediately brought into the laboratory. After cleaning the leaves, the petiole of leaves was wrapped with cotton to keep the leaves turgescent. For leaf dip bioassay, leaves were separately dipped for 10 seconds in tested plant extracts 1) *A. indica,* 2) *M. acuminate*, 3) *E. camaldulensis,* 4) *M. arvensis,* 5) *D. stramonium*, 6) *C. colocynthis and* 7) *C. limon.* Next, leaves were air dried for 2h and placed in clean Petri plates (60mm x 15mm). Similarly, leaves with actively feeding third instar *P. citrella* larvae were also treated through the topical application method described by Shapiro *et al.* (1998). One drop (about $4\mu l$) of each extract was applied on each *P. citrella* larval body using a sterilized micro-syringe (Hamilton Co., Bonaduz, Switzerland). Distilled water and methanol were used as a control treatment. Each treatment was replicated five times in both bioassays and five leaves were treated for each replicate. Leaves were examined under a stereo-microscope (MZ1280, Micros, Austria) to check for movement of *P. citrella* larvae. In addition, the mortality of larvae was considered when it displayed a lack of external or peristaltic movement when probed. The mortality data were recorded after 3, 6, 12 and 24h of treatment exposure.

2.3. Combination of plant extracts with abamectin

A preliminary experiment was conducted to check the efficacy of three synthetic insecticides (spinosad 45% SC, abamectin 1.8% EC, and thiamethoxam 25% WG) against *P. citrella* larvae. Among the tested chemicals, abamectin showed significantly higher mortality (63.5%) of *P. citrella* larvae, compared to spinosad (49.3%) and thiamethoxam (32%). Similarly, all the plant extracts at 7% concentration showed greater mortality of *P. citrella* as compared to the lower concentration (2.5% and 5%) of each. Therefore, abamectin at field recommended dose (0.4 ml/l) were mixed with all plant extracts at higher concentration, to check the combinatorial effect of plant extracts with insecticide. Two ratios of each plant extracts with abamectin were prepared. For ratio 1 (1:2), one part of the insecticide was mixed with 2 parts of each plant extract. Similarly, for ratio 2 (2:1), two parts of insecticide were mixed with one part of extract.

2.4. Statistical analysis

The data were analyzed by completely randomized design using factorial arrangements of treatments, time interval, type of bioassay and concentration for the percent mortality of *P. citrella* larvae. The variable measured per replicate of each treatment was larval mortality (number of dead larvae). Percent mortality data were transformed by arcsin transformation and subjected to ANOVA. The percentage mortality data was calculated and corrected by Abbott's (1925) formula. The lethal concentration LC_{50}, of each treatment, was also calculated by probit analysis. Means separation were tested by a Tukey HSD pairwise comparison test and all the analyses were performed using Minitab 16.1 and SPSS 20.0 software.

3. Results

Table 1 shows the efficacy of aqueous and alcoholic plant extracts against *P. citrella* larvae by means of percent mortality at the different time of exposure. The aqueous plant extracts showed significant ($F_{7,232}$ = 4.13, P<0.001) variations in percent mortality of *P. citrella* larvae at a different time of exposure. The results revealed that aqueous extract of *A. indica* induced greater mortality of *P. citrella* larvae (61.1%) as followed by *D. stramonium* seed extract (40.7%) after 24h of exposure. Similarly, the alcoholic plant extracts also showed the significant impact ($F_{7, 232}$ = 3.79, P<0.001) at different time intervals after treatment exposure. A higher *P. citrella* mortality was observed at 24h post exposure relative to 3h in all tested plant extracts. On the other hand, the least effective plant extracts were *M. acuminate* and *C. limon* (Table 1).

There was a significant variation in the percent mortality for *P. citrella* larvae after the application of aqueous plant extracts in the leaf dip ($F_{7, 472}$ = 8.97, P<0.001) and topical bioassays ($F_{7, 472}$ = 24.4, P<0.001). The aqueous extract of *A. indica* showed greater mortality of *P. citrella* in both application methods (35.0% and 52.6% respectively), whereas the aqueous extract of *M. acuminate* and *C. limon* showed the least mortality (16-19%) (Table 2). Similarly, in alcoholic extracts, both leaf dip bioassay ($F_{7, 472}$ =16.4, P<0.001) and topical bioassay ($F_{7, 472}$ =18.6, P<0.001) showed significant variations in percent mortality of *P. citrella*. The alcoholic extract of *A. indica* also showed greater mortality (46.8% and 37.8%) compared to other extracts through topical application and leaf dip bioassay respectively. The alcoholic extract of *M. acuminate* and *C. limon* showed least mortality of *P. citrella* (Table 2). The highest mortality was observed after topical application of *A. indica* in both aqueous and alcoholic nature (Table 2). As shown in Table 3, both aqueous ($F_{7, 312}$ = 27.2, P<0.001) and alcoholic extracts ($F_{7, 312}$ = 29.4, P<0.001) showed significant mortality of *P. citrella* larvae at different concentrations. Among the plant extracts, *A. indica* showed better performance at 7% concentration in both aqueous and alcoholic extracts. The least affected plant extracts were *M. acuminate* and *C. limon*.

As for the aqueous extracts, the results showed that *A. indica* extracts had higher toxicity against *P. citrella* with the lowest LC_{50} value (6.81%) in the leaf

Table 1. Effect of exposure time of aqueous and alcoholic extracts of different plants on percent mortality (mean±SE) of *Phyllocnistis citrella* larvae

	3HAT	6HAT	12HAT	24HAT
Aqueous extracts				
A. indica	23.33±2.97^{a}	40.67±5.57^{a}	50.0±6.84^{a}	61.17±6.35^{a}
M. acuminate	6.00±2.44^{c}	13.3±2.78^{c}	22.0±3.46^{c}	25.8±4.43^{c}
E. camaldulensis	15.3±3.75^{abc}	23.3±4.41^{bc}	28.7±4.75^{bc}	32.0±5.12^{bc}
M. arvensis	16.0±3.45^{ab}	19.3±3.18^{bc}	22.7±3.56^{c}	25.3±4.48^{c}
D. stramonium leaf	16.7±3.06^{ab}	22.7±3.56^{bc}	31.3±4.47^{bc}	31.7±4.24^{bc}
D. stramonium seed	22.7±3.41^{a}	28.0±3.98^{b}	36.7±3.39^{ab}	40.7±4.25^{b}
C. colocynthis	16.7±3.36^{ab}	22.0±3.95^{bc}	28.0±4.66^{bc}	30.7±5.02^{bc}
C. limon	10.0±2.94^{bc}	16.7±2.78^{bc}	19.3±3.09^{c}	24.0±4.28^{c}
Alcoholic extracts				
A. indica	25.3±3.18^{a}	36.7±4.15^{a}	48.7±5.42^{a}	58.3±6.32^{a}
M. acuminate	5.30±2.21^{d}	14.7±2.70^{c}	14.7±2.70^{d}	18.8±3.77^{c}
E. camaldulensis	14.0±3.08^{bc}	20.0±3.97^{bc}	26.0±4.83^{bcd}	30.0±5.06^{bc}
M. arvensis	16.0±2.51^{bc}	18.7±2.71^{bc}	24.0±3.73^{bcd}	26.2±4.29^{bc}
D. stramonium leaf	16.0±2.09^{bc}	22.7±3.12^{bc}	28.0±3.98^{bc}	29.8±4.44^{bc}
D. stramonium seed	20.7±2.87^{ab}	26.7±3.81^{ab}	34.0±2.79^{b}	38.3±5.18^{b}
C. colocynthis	17.3±2.79^{abc}	20.0±3.12^{bc}	27.3±4.90^{bc}	31.5±5.12^{bc}
C. limon	11.3±2.84^{cd}	18.0±2.44^{bc}	20.7±3.18^{cd}	25.2±4.44^{bc}

Means sharing similar letters within columns are not significantly different from one another (*P>0.05*). The experiment was repeated once with similar results
HAT= Hours after treatment

Table 2. Effect of application method of aqueous and alcoholic extracts of different plants on percent mortality (mean±SE) of *Phyllocnistis citrella* larvae

	Aqueous extracts		Alcoholic extracts	
Treatments	Leaf dip	Topical	Leaf dip	Topical
A. indica	35.0±4.99[a]	52.6±5.86[a]	37.8±4.76[a]	46.8±5.28[a]
M. acuminate	17.1±3.65[b]	16.5±2.91[d]	11.8±2.55[d]	14.9±3.14[d]
E. camaldulensis	21.8±4.70[b]	27.8±4.31[bc]	18.4±4.04[cd]	26.6±4.43[bc]
M. arvensis	21.8±3.70[b]	19.8±3.63[cd]	21.8±3.45[bc]	20.7±3.17[cd]
D. stramonium leaf	26.3±3.75[ab]	24.9±3.92[bcd]	22.2±3.32[bc]	26.1±3.49[bc]
D. stramonium seed	33.0±3.311[a]	31.0±4.21[b]	28.6±2.98[b]	31.3±4.34[b]
C. colocynthis	23.2±3.91[b]	25.5±4.58[bcd]	18.8±3.76[cd]	29.3±4.20[bc]
C. limon	18.5.0±3.22[b]	16.5±3.33[d]	16.2±2.98[cd]	21.4±3.46[cd]

Means sharing similar letters within columns are not significantly different (*P>0.05*). LDB=leaf dip bioassay, TB=topical bioassay

Table 3. Influence of different concentration (using topical/leaf dip application method) of aqueous and alcoholic extracts of different plants on percent mortality (mean±SE) of *Phyllocnistis citrella* larvae

	Aqueous			Alcoholic		
Treatments	**2.5%**	**5%**	**7%**	**2.5%**	**5%**	**7%**
A. indica	24.6±3.53[a]	42.0±4.93[a]	64.8±6.07[a]	22.7±3.01[a]	42.9±4.39[a]	61.1±5.46[a]
M. acuminate	8.00±2.59[c]	18.6±3.20[e]	23.8±4.51[e]	2.50±1.63[d]	16.8±2.36[d]	20.9±2.95[e]
E. camaldulensis	5.50±1.90[c]	30.8±3.48[bc]	38.3±3.53[bc]	4.00±1.84[cd]	26.4±3.04[bc]	37.0±3.07[bcd]
M. arvensis	8.50±2.53[c]	21.8±2.71[cde]	32.3±2.98[cde]	10.0±2.87[bc]	23.3±2.69[cd]	30.4±2.52[cd]
D. stramonium l	11.6±3.26[bc]	27.8±2.75[b-e]	37.4±3.24[bcd]	11.0±2.82[bc]	27.4±2.47[bc]	34.0±3.03[bcd]
D. stramonium s	17.8±2.71[ab]	32.8±2.74[b]	45.5±3.24[b]	16.6±3.03[ab]	31.4±2.74[b]	41.8±3.58[b]
C. colocynthis	6.50±2.47[c]	29.3±2.57[bcd]	37.3±3.49[bcd]	7.10±1.71[cd]	27.4±2.45[bc]	37.6±3.14[bc]
C. limon	5.00±2.21[c]	20.3±2.89[de]	27.3±2.74[de]	7.50±2.47[cd]	20.9±2.86[cd]	28.0±2.89[de]

Means sharing similar letters within columns are not significantly different (*P>0.05*)

Table 4. LC_{50} values of different aqueous and alcoholic extracts (7%) of different plant against *Phyllocnistis citrella* larvae at 24h after treatment

	Aqueous			Alcoholic		
	LC_{50}±SE	**95% CI**	**Y=a+bx**	**LC_{50}±SE**	**95% CI**	**Y=a+bx**
Leaf Dip Bioassay						
A. indica	6.81±0.542	5.75-7.88	-1.35+0.198x	6.39±0.464	5.47-7.29	-1.31+0.21x
M. acuminate	11.72±2.398	7.02-16.42	-1.61+0.137x	10.48±1.414	7.71-13.25	-2.29+0.22x
E. camaldulensis	7.67±0.464	6.76-8.58	-2.37+0.309x	8.09±0.541	7.035-9.154	-2.50+0.31x
M. arvensis	9.30±1.172	7.002-11.59	-1.644+0.177x	9.64±1.364	6.97-12.32	-1.57+0.25x
D. stramonium leaves	8.73±1.088	6.6-10.86	-1.437+0.164x	9.24±1.151	6.99-11.49	-1.66+0.18x
D. stramonium seed	8.028±1.084	5.902-10.15	-1.093+0.136x	9.51±1.733	6.11-12.91	-1.13+0.23x
C. colocynthis	8.37±0.781	6.84-9.903	-1.809+0.262x	8.10±0.553	7.02-9.19	-2.46+0.30x
C. limon	9.73±1.242	7.29-12.16	-1.85+0.276x	9.84±1.217	7.46-12.23	-2.07+0.20x
Topical bioassay						
A. indica	4.55±0.279	3.98-5.08	-1.24+0.216x	5.11±0.313	4.49-5.722	-1.23+0.22x
M. acuminate	11.48±2.178	7.21-15.75	-1.68+0.147x	10.30±1.445	7.47-13.13	-1.93+0.19x
E. camaldulensis	7.59±0.606	6.4-8.8	-1.68+0.222x	7.42±0.514	6.41-8.43	-1.86+0.26x
M. arvensis	9.05±0.973	7.15-10.96	-1.88+0.274x	9.77±1.410	7.02-12.54	-1.59+0.26x
D. stramonium leaves	8.21±0.767	6.70-9.71	-1.71+0.208x	8.54±1.019	6.55-10.54	-1.43+0.17x
D. stramonium seed	7.176±0.537	6.12-8.23	-1.59+0.221x	7.21±0.589	6.05-8.36	-1.46+0.23x
C. colocynthis	7.53±0.508	6.54-8.53	-1.98+0.264x	7.54±0.66	6.25-8.84	-1.51+0.19x
C. limon	8.91±0.803	7.35-10.49	-2.324+0.261x	9.99±1.594	6.87-13.12	-1.48+0.15x

CI= confidence interval, (Y=a+bx is regression equation in which Y denote percent mortality of CLM and x denote respected chemical), LC_{50} = lethal concertation

dip bioassay method. Similar values were recorded for the topical bioassay, in which the lowest LC_{50} value of 4.55% was observed for *A. indica* (Table 4). The minimum toxicity against *P. citrella* was observed for *M. acuminate* peel, having an LC_{50} value of 11.7% in the leaf dip bioassay and 11.48% when applied topically. Similar results were observed for the alcoholic extracts that showed *A. indica* extracts had greater toxicity against *P. citrella* as compared to other extracts. The LC_{50} value for *A. indica* alcoholic extract was 6.39% for the leaf dip bioassay method. For the topical bioassay, the LC_{50} value was 5.11%. After *A. indica* extract, *D. stramonium* seed extract also showed toxicity, having LC_{50} values 9.5%, 7.2% in the leaf dip bioassay and the topical bioassay, respectively. Similar results for an alcoholic extract of *M. acuminate* peel were found to have a greater LC_{50} value of 10.30% (Table 4).

The combined efficacy of plant aqueous and alcoholic extracts with abamectin is presented in Figure 1 and 2. At ratio 1 (1:2), the mortality was significantly ($F_{7, 256}$ = 22.38, P<0.001) different among treatments for different solvent nature of botanicals. Abamectin plus the aqueous extract of *A. indica* showed a greater mortality of 48.25% as well as 34.38% in case

Table 5. LC_{50} values of abamectin + plant extracts (7%) against *Phyllocnistis citrella* larvae at 24h after treatment

	I+AQ			I+AL		
	LC_{50}±SE	95% CI	Y=a+bx	LC_{50}±SE	95% CI	Y=a+bx
Leaf Dip Bioassay						
A. indica	1.74±0.19	1.36-2.12	-0.84+0.48x	1.47±0.15	1.18-1.75	-0.96+0.65x
M. acuminate	3.46±0.79	1.91-5.02	-.188+0.54x	4.39±2.98	1.45-10.2	-0.84+0.36x
E. camaldulensis	3.5±0.81	1.86-5.04	-1.79+0.52x	2.59±0.59	1.44-3.75	-0.92+0.35x
M. arvensis	3.58±1.19	1.24-5.93	-1.26+0.35x	2.94±0.91	1.15-4.72	-0.88+0.35x
D. stramonium leaf	2.84±0.63	1.61-4.08	-1.16+0.36x	2.72±0.71	1.33-4.11	-0.89+0.33x
D. stramonium seed	3.63±1.21	1.27-6.00	-1.31+0.36x	3.21±1.19	0.88-5.54	-0.88+0.28x
C. colocynthis	3.08±0.78	1.54-4.62	-1.21+0.39x	3.16±1.33	0.58-5.77	-0.75+0.24x
C. limon	4.07±1.36	1.39-6.73	-1.73+0.42x	2.99±0.84	1.35-4.64	-1.02+0.34x
Topical bioassay						
A. indica	1.61±0.09	1.41-1.79	-1.52+0.94x	0.77±0.56	0.32-186	-0.27+0.35x
M. acuminate	3.44±0.83	1.82-5.06	-1.73+0.50x	3.06±1.26	0.59-5.52	-0.72+0.24x
E. camaldulensis	2.99±0.47	2.06-3.91	-1.96+0.66x	2.17±0.36	1.45-2.88	-0.80+0.37x
M. arvensis	3.09±0.70	1.71-4.46	-1.37+0.45x	3.09±1.48	0.19-5.98	-0.63+0.20x
D. stramonium leaf	2.56±0.41	1.76-3.36	-1.27+0.49x	2.43±0.79	0.89-3.97	-0.55+0.23x
D. stramonium seed	2.72±0.41	1.91-3.53	-1.57+0.58x	2.94±0.91	1.15-4.73	-0.88+0.30x
C. colocynthis	3.29±1.09	1.16-5.44	-1.06+0.32x	2.01±0.41	1.22-2.80	-0.56+0.28x
C. limon	4.73±2.41	0.003-9.4	-1.39+0.29x	2.49±0.59	1.33-3.66	-0.79+0.32x

I=insecticides, AQ=aqueous extracts, AL=alcoholic extracts, CI=confidence interval, (Y=a+bx is regression equation in which Y denote percent mortality of CLM and x denote respected chemical)

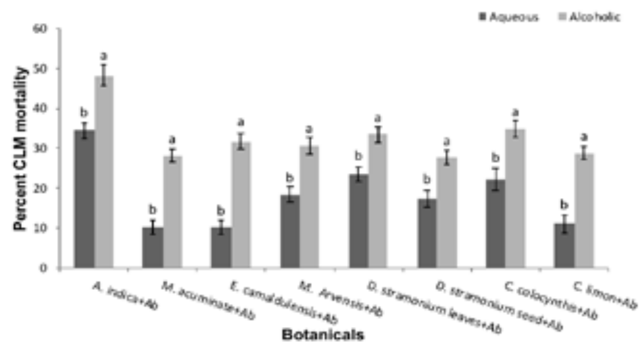

Fig. 1. Percent mortality (mean±SE) of *Phyllocnistis citrella* larvae using aqueous and alcoholic plant extracts mixing with abamectin (Ab) at ratio 1 (1:2 of abamectin with extracts), Means sharing similar letters are not significantly different at P>0.05

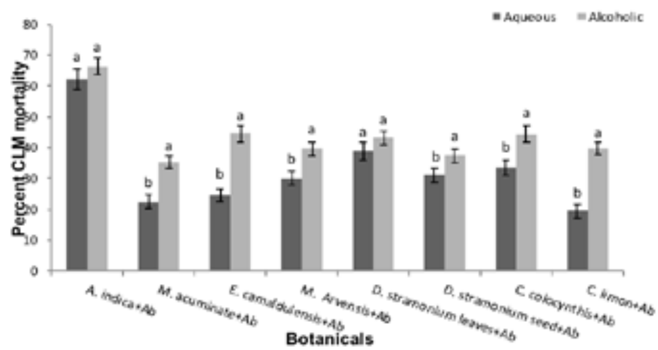

Fig. 2. Percent mortality (mean±SE) of *Phyllocnistis citrella* larvae using aqueous and alcoholic plant extracts mixing with abamectin (Ab) at ratio 2 (2:1 of abamectin with extracts), Means sharing similar letters are not significantly different at P>0.05

of alcoholic extracts (Figure 1). When abamectin was mixed with the aqueous extract of *A. indica* for ratio 2 (2:1), the mortality reached to 62.3% and 66.3% when mixed with an alcoholic extract of *A. indica* (Figure 2).

Probit analysis also showed the greater toxicity of alcoholic extract of *A. indica* extracts mixed with abamectin compared to aqueous extracts. The LC_{50} value for the joint action of abamectin with *A. indica* extract was 1.74% and 1.47%, in aqueous and alcoholic extract, respectively, using leaf dip bioassay methods. As for the topical bioassay, the combined efficacy of abamectin with *A. indica* showed similar results against *P. citrella*. The regression equation showed the negative role of all plant extracts in all combinations against *P. citrella* mortality (Table 5).

4. Discussion

Many plants with insecticidal properties should be considered worthy as insect control strategies because of the excellent availability of plant resources, viability and cost effectiveness (Kumar *et al.*, 2012). Plant extracts are also an eco-friendly approach to pest control, considering their biodegradable nature.

In our study, *A. indica* leaf extract showed that in both types of solvent (aqueous and alcoholic), there was greater mortality of *P. citrella* compared to other plant extracts. The superior efficacy may be due to the triterpenoid compound azadirachtin which interferes with the ecdysis process and affects normal growth and development of larvae (Nisbet, 2000). Prior studies confirmed the effectiveness of azadirachtin against different insect pests, i.e., *Toxoptera citricida* (Kirklady) (Hemiptera: Aphididae), *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae), *Acyrthosiphon pisum Harris*, (Hemiptera: Aphididae) *Ceratitus capitata* (Wiedemann) (Diptera: Tephritidae), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and *Aulacophora foveicollis Lucas* (Chrysomelidae: Coleoptera) [Pandao *et al.* (1992); Raveendran *et al.* (1998); Ukey *et al.* (1999); Charleston *et al.* (2005); Kraiss & Cullen (2008); Italo *et al.* (2009); Kumral *et al.* (2010) and Ali *et al.* (2011)]. According to Sarvanan (2000), azadirachtin 5ml/L showed 80% mortality of *P. citrella*. *Melia azedarach* L. extract, a close relative of *A. indica* was also proven effective against *P. citrella* (Mckenna *et al.*, 2013).

The mortality rate of *P. citrella* larvae showed significant variations in respect to different botanical extracts, concentration and time interval. Our results showed greater mortality of *P. citrella* at a higher concentration and a higher post treatment interval. It may be due to different exposure durations of botanicals and rates of persistence at different concentration (Khan *et al.*, 2015). It is possible that the easy metabolism of diluted extracts, as compared to a more concentrated extract, may lead to slow metabolism and persists for a longer period (Bashir *et al.*, 2013). Khan *et al.* (2015) documented that greater mortality of *A. foveicollis* at 96h is due to a longer exposure to a treated surface that increases the ingestion of a quantity of toxicants in their digestive tracts. Similarly, Osman *et al.* (2013) also confirmed higher mortality of *A. foveicollis* using neem extract at 7.5% concentration. According to Yankanchi *et al.* (2014), sluggish behavior, loss of equilibrium and inability get the lead out on an *A. aegypti* larval body with contact to high concentrations of plant extracts. It has also been observed that *P. citrella* larval mortality fluctuates significantly at higher concentrations which act as an oviposition deterrent (Liu *et al.*, 2001).

In our study, two different application methods were tested to check the efficacy of botanicals against *P. citrella* larvae. The results showed that topical application was a better application method for selected botanicals as compared to the leaf dip method. Pascual-Villalobos & Fernández (1999) also showed that squill bulbs (*Urginea maritima* (L.) Baker) caused greater mortality when applied topically when compared to those that were mixed in the diet. The choice of tested insect and type of bioassay may also affect the outcome of a screening (Cole, 1994). According to Mafi & Ohbayashi (2006), the percentage mortality of *P. citrella* eggs exposed to different insecticides using the dip method ranged between 3 to 44%, and almost over 90% mortality was observed in the first instar larvae of *P. citrella*. However, during the new flush emergence of citrus, the neem formulations can be useful by spraying as prophylactic sprays to manage *P. citrella* (Jayanthi & Verghese, 2007). Prior studies have espoused different assumptions about the efficacy of insecticides against different insect pests using different bioassay techniques (Leibee & Savage, 1992; Dennehy *et al.*, 1983). Two different solvents were used to prepare plant extracts and to check their effectiveness against *P. citrella* larvae. The polarity of the solvent is an important factor in a toxicity study (Khan *et al.*, 2015). In general, the aqueous extract showed promising result against *P. citrella* larvae. However, the variations in percent mortality of *P. citrella* after application of plant extracts having a different solvent nature could be due to the difference in the dissolving nature of extracts (Rizvi *et al.*, 2012; Koubala *et al.*, 2013). The dissolving nature of the active ingredients of plant extracts in a particular solvent can differ, and it may have a synergistic effect with a respective solvent when considering their efficacy against a pest (Oyedokun *et al.*, 2011).

Results indicated that *P. citrella* mortality was higher when the combination treatment (abamectin with plant extracts) was applied as compared to the extracts alone. The combination of abamectin with *A. indica* extracts showed greater mortality of *P. citrella* as compared to other plant extracts but the mortality was not satisfactory, i.e., 50% in ratio 1 (1:2). By mixing abamectin with *A. indica* at the 2:1 ratio, a higher *P. citrella* mortality (>60%) was observed. Previous studies confirmed higher combined efficacies of an insecticide and botanicals against different insect pests (Caraballo, 2000; Seyoum *et al.*, 2002; Kalayanasundaram & Das, 1985; Thangam & Kathiresan, 1990; Mohan *et al.*, 2006, 2007; Shaalan *et al.*, 2005a,b).

Our results were also in line with Jayanthi & Verghes (2007), who reported that botanicals showed synergism with cypermethrin against *P. citrell*a. The problem of resistance in insect pest populations can be minimized using such strategies (Mesbah *et al.*, 2006, 2007; Mahmoud, 2007). Other useful aspects of this mixture could be cost effectiveness, easy preparation and availability of insecticide as part of integrated management techniques. Similarly, the efficiency of chemicals can be enhanced by mixing with plant extract and mineral oils for better coverage of plants and for the penetration of chemicals into the surface of leaves (Bográn *et al.*, 2006). The current results showed neem extract as a potential botanical insecticide against *P. citrella larvae*, and it should be further investigated to classify as an IPM-compatible insecticide.

5. Conclusion

In summary, this work showed that *A. indica* and other plant extracts have variable degrees of effectiveness against *P. citrella* larvae. The aqueous plant extracts at their higher concentrations cause higher mortalities of *P. citrella* larvae by topical application. *A. indica* is compatible with abamectin and can be used alone or in combination against the *P. citrella* in an integrated management program for this pest. However, such combinations are effective in controlled conditions. Further investigations are needed to explore their efficacy against this pest in field conditions in order to assess their compatibility with natural enemies before they could be recommended to the growers. Future study would focus on the abundance of the biological control agents of *P. citrella* in Pakistan and the effect of the botanicals on *P. citrella* and its associated natural enemies in the field.

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تقييم مستخلصات نباتية لمكافحة حشرة حافرة أنفاق الحمضيات *Phyllocnistis citrella* (Lepidoptera: Gracillariidae)

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الملخص

حافرة أنفاق الحمضيات(CLM) ، *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) هي آفة رئيسية تُصيب الحمضيات، وتتغذى على جميع أصنافها تقريباً، وكذلك بعض الفصائل الشبيهة بها. لقد قمنا بتقييم فعالية المستخلصات المائية والكحولية لبعض أنواع النباتات المختارة (neem *Azadirachta indica* A. Juss, banana *Musa acuminate* Colla, eucalyptus *Eucalyptus camaldulensis* (Dehn.), mint *Mentha arvensis* L., datura *Datura stramonium* L., tumma *Citrullus colocynthis* (L.) and lemon *Citrus limon* (L.) ضد يرقات *P. citrella*؛ بطريقتين مختلفتين من طرق التطبيق؛ وهم: غمس الأوراق والتطبيقات الموضعية. ولاحظنا أعلى نسبة وفيات لـ *P. citrella* في المستخلص المائي من نبات A. indica بمعدل (61.17٪) وفي المستخلصات الكحولية منه بمعدل (58.3٪) مقارنةً بباقي المستخلصات النباتية الأخرى وذلك بعد 24 ساعة من التعرض. من بين طريقتي المعالجة، كانت نسبة وفيات *P. citrella* أعلى في التطبيق الموضعي لمستخلص *A. indica* المائية 6.8٪ في الاختبار الحيوي لغمس *A. Indica* لمستخلصات LC_{50} من تطبيق غمس الأوراق. علاوة على ذلك، كانت قيمة الأوراق وكانت 4.55٪ في التطبيق الموضعي الذي كان أقل مقارنة مع جميع المستخلصات الأخرى. وجدنا أن مستخلصات *Musa acuminate* and *C. limon* (المائية والكحولية) أقل فعالية ضد يرقات *P. citrella*.عندما تم تقييم فعالية المستخلصات النباتية المركبة على أعلى معدل وفيات من بقية المعالجات *A. Indica* مع أبامكتين، حصل المستخلص المائي والكحولي من معالجات *A. indica* الأخرى بنسبة (62.25٪ و 66.25٪ على التوالي). وتشير النتائج التي توصلنا إليها إلى أن مستخرج يمكن اختباره *P. citrella* كمبيد حشري نباتي لمكافحة يرقات باعتبارها قائمة بذاتها (للمزارعين العضويين) أو في نهج متكامل مع أبامكتين. فهو لن يقلل فقط من تكاليف مدخلات المزارعين، بل إنه يساعد أيضاً في الحد من الآثار السلبية للمبيدات الكيمائية الصناعية