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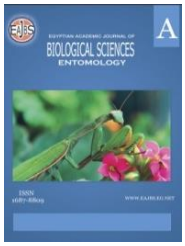

Toxicity of Alone and Combine Application of Botanical Extracts against 2nd Instar Larvae of *Liriomyza trifolli* on Tomato, *Lycopersicum esculentum*

Azhar Mujahid[1*], Hamza Aziz Khan[1], Sanveed Sarwar[2], Junaid Mustafa[1], Hamza Khan[1], Muhammad Sohail Qadir[2] and Zulnorain Sajid[3],

1-Department of Agriculture and Agribusiness Management, University of Karachi, Karachi, Pakistan

2-Department of Entomology, University of Agriculture, Faisalabad

3-Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

Email: azharbravi@gmail.com

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ABSTRACT

Tomato is an important vegetable crop and cultivated all over the world. The production of tomato is reducing due to the attack of various insect pests such as *Liriomyza trifolii*. *L. trifolii* is a very destructive pest and can cause severe economic losses of tomato, *Lycopersicon esculentum*. Botanicals, *Azadirachta indica*, and *Euclyptus camaldulensis* were checked during 2017 under laboratory conditions to check them alone and combine toxicity against second instar larvae of tomato *Liriomyza trifolii*. Significant differences ($P<0.001$) were recorded. Highly significant mortality 11.40±0.533c was recorded by the combine treatment of *A. indica* + *E. camaldulensis* at 10% concentration followed by *A. indica* 7.90±1.302ac at 10% concentration. The study concluded both plant extracts (*Azadirachta indica* and *Euclyptus camaldulensis)* could be used as effective management strategy to control insect pests especially *Liriomyza trifolii* in laboratory as well as field conditions.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most important and extensively consumed vegetables worldwide. It is a rich source of vitamins, minerals, amino acids, sugars, and dietary fiber. It can provide high profits to farmers and source of employment opportunities (Mari *et al.*, 2007) in various countries. The total cultivated area under the tomato crop is 53.4 thousand ha with a production of 561.9 thousand tons (GoP, 2008).

The major pest of tomato crops is fruit borer (*Helicoverpa armigera*) Aphids (*Aphis gossypii*) Jassid (*Amarasca devastans*). Among them, leafminer, *Liriomyza trifolii* is a key pest of tomato crop all over the world including Pakistan. *Liriomyza trifolii* belongs to the order dipteral and family Agromyzidae. Genus *Liriomyza* contains more than 300 species worldwide approximately 24 species are economically important (Rai *et al.*, 2013). Both adult and larval stages can cause damage to the main agricultural and horticultural industry especially tomato in the world. The larvae of *L. trifolii* feed on internal tissues of the host plant and create irregular mines resulting in the reduction of cosmetic appearance and photosynthetic activity of many ornamental and vegetable crops.

The female lays eggs on the leaf surface after puncturing. The photosynthesis and aesthetic value of the host plant can reduce during the severe attack of leafminer (Al-Khateeb and Al Jabr, 2006; Gitonga *et al.*, 2010 and Hallman *et al.*, 2011). It can cause huge crop losses in tomato growing areas of the world.

Integrated pest management approaches such as biological, cultural, physical, and chemicals have been adopted at commercial scale (Ramzan *et al.*, 2019). Botanical extracts of plants i.e *Azadirachta indica* and *Euclayptus camaldulensis* are now playing a key role in controlling insect pests on various crops such as tomato. These plant extracts have repellent and ant-feeding properties that proved the best control of insect pests like leafminer, *L. trifolii* due to this property (Seyoum *et al.*, 2003; Tunc *et al.*, 2000; Zhu *et al.*, 2006). The growth and development of leafminer can reduce by applying botanical extracts (Khorshidi *et al.*, 2018; Lopes *et al.*, 2019). The current study was conducted to check the toxicity alone and combine the application of botanical extracts against *L. trifolii* on tomato.

MATERIALS AND METHODS

Collection of Plant Leaves and Their Extract Preparation:

Leaves of *A. indica* and *E. camaldulensis* were collected from different areas of Karachi and brought to the laboratory for further process. Collected leaves were thoroughly washed with normal tap water and dried under shade for 7-14 days. After shade drying, the leaves colour was changed from green to lighter and crisped. An electric blender was used to grind the crispy leaves until the fine powder was obtained. Grinded leaves were sieved and stored in a clean airtight jar at 8^0C until needed. Each plant sample 10 g was extracted with 100 ml of distilled water by using as soxhlet hot water extractor for 4 h. Obtained extracts were stored in a sealed glass jar and kept at 5^oC till it was used.

Colony Maintenance of *L. trifolii* and Laboratory Bioassay:

L. trifolii colony was maintained in a rearing chamber at Plant protection laboratory on tomato plants under controlled conditions (22^0C±2^0C temperature, 70±5% relative humidity, photoperiod of 14:10 (L:D) h.

Bioassay:

The seeds of the tomato were purchased from nearby seed shop and grown in vials for experimental use. The plants were removed from vials when plants bear four leaves. The removed plants were shifted into plastic pots containing dried goat-sheep manure. Shifted plants were sprinkled with water and allow to adopt the environment in potting soil for four days before the initial of bioassay study. Equal age, ratio (50 male: 50 female), and equal size 100 adults were collected from the colony and released into cages of potted tomatoes. The infested leaves were examined to check the larval instars of *L. trifolii* by using a lens. The bioassay study was conducted at 2^{nd} instar larvae by using foliar applications. The alone and combine plant extract was applied at the rate of 2.5%, 5%, and 10%. Only distilled water was applied in the control treatment. There were five replications and the experiment repeated three times. Data were recorded at 24, 48, 72, and 96 hours, respectively.

Statistical Analysis:

Data were analyzed statistically by analysis of variance (ANOVA). Means were separated by using Tukey's Honestly Significant Difference (HSD). All data results with $P \leq 0.05\%$ were considered statistically significant.

RESULTS

The larval mortalities (93% and 75%) were recorded with combinations of (*A. indica* + *Eucalyptus*) at 10 and 5% concentration, respectively after 96 hours (Table 1). The

application of *E. camaldulensis* 10% was given 33, 46, 53 and 53% mortalities at 24, 48, 72 and 96 hours respectively while 20, 37, 37 and 46%, respectively at *E. camaldulensis* 5%. The application of *A. indica* 10% was given37, 46, 60 and 68% mortalities at 24, 48, 72 and 96 hours respectively. During the study, no mortality of larvae was recorded.

Table 1: Toxicity of alone and combine applications of extracts on 2nd larval instars of *L. trifolii*at different time intervals.

Concentration	Mean (X) and % mortalities of *L. trifolii* 2nd instar at different intervals							
	24 hours		48hours		72hours		94hours	
	(X)	(%)	(X)	(%)	(X)	(%)	(X)	(%)
A. indica 2.5%	2.3	15	5.6	37.7	6.3	42	7	46
A. indica 5%	4	26	7	46	8	53	9	60
A. indica 10%	5.6	37	7	46	9	60	10	68
*E. camaldulensis*2.5%	1	8	5	33	5.6	37	6.3	42
E. camaldulensis 5%	3	20	6	37	6	37	7	46
E. camaldulensis 10%	5	33	7	46	8	53	8	53
A. indica + *E. camaldulensis* 5%	6.3	42	8	53	10	68	11.3	75
A, indica + *E. camaldulensis* 10%	9	60	10.6	71	12	82	14	93
Control	0	0	0	0	0	0	0	0

The combined applications of botanical extracts were proved best control against leafminer larvae. Similar results have been reported by many early researchers in the world (Civelek and Weintraub, 2004; Sharma and Gupta, 2009). It was observed that pupation occurred at 5-7th day in control. The significant differences in the total larval mortalities of 2nd instar larvae were recorded at 2.5%, 5%, and 10% of *A. indica, E. camaldulensis* treatments alone and combinations (Table 3).

In control, adult emergence and larval longevity were significantly high (trials: F=85.04; df=23.00) as compared to other treatments even the most diluted (*A. indica* and *E. camaldulensis* 2.5%) plant extracts (Table 2).

Table 2: Analysis of variance table for Mean (X) and % mortalities of *L. trifolii* 2nd instar after different intervals**.**

ANOVA					
Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*
Concentrations	33.78	5.00	6.76	25.83	0.00
Days	66.72	3.00	22.24	85.04	0.00
Error	3.92	15.00	0.26		
Total	104.43	23.00			

** = Highly significant ($P \leq 0.01$) Coefficient of variation (CV) = 4.31%

The highest concentration of each extract i.e *A. indica* 10%, *E. camaldulensis* 10% applied against larvae was statistically effective in a short time than other applied concentrations. The combine applications of extracts were found most effective and give highest larval mortality in short period of time (trials: F=21.08; df=15.00; $P \leq 0.01$) than alone applications (Table 1).

A. indica active ingredient azadirichtin has antifeedant and growth-regulating actions (Isman *et al.*, 1990). *A. indica* has proved more effective against freshly laid eggs of *L. sativa* (Weintrauband Horowitz, 1997). These botanicals can change the physiological like molting (Silva *et al.*, 2016) and behavioral activity of insect pests (Isman *et al.*, 1990; Sharma and Gupta, 2009). Similar observations are recorded in the current study. Our findings are in agreement with other findings (Bernardes *et al.*, 2017; Ganapathy *et al.*, 2010). The larval mortality percentage was increased with an increase in chemical concentration.

Costa *et al* (2018) had reported similar findings. It was observed that alone and combine application proved the best strategy to control leafminers. The applied botanicals were given 80-90% larval mortality of *L. trifolii* (Mishra and Shantipriya, 2008). Toxicity of *A. indica* extracts against tomato leaf miner *Tuta absoluta* significantly affects egg-laying, deterrent larval mining, and adult emergence (Tomé *et al.*, 2013). Azadirachtin foliar application can disrupt molting (Devkota *et al.*, 2020).

The combined application of *A. indica* + *E. camaldulensis* 10% extracts was given maximum mortality of *L. trifolii* larvae (11.40±0.533c) after 96 hours of post-treatment followed by *A. indica* 10% (7.90±1.302ac) and *A. indica* 5 % (7.00±0.623c). While the lowest mortality was noticed in *E. camaldulensis* at 5% (5.50±0.422ab) followed by *E. camaldulensis* at 2.5% (4.48±1.053b) (Table 3).

Table 3: Toxicity of alone and combine the application of botanical extracts on 2nd instars of *L. trifolii* using HSD

Concentration	Mean Comparison Test (HSD)
A. indica 2.5 %	5.30±0.535bc
A. indica 5%	7.00±0.623c
A. indica 10%	7.90±1.302ac
E. camaldulensis 2.5%	4.48±1.053b
E. camaldulensis 5%	5.50±0.422ab
E. camaldulensis 10 %	7.00±0.942c
A. indica + *E. camaldulensis* 5%	8.90±1.432ac
A. indica + *E. camaldulensis* 10%	11.40±0.533c
Control	4.57±0.394ac

CONCLUSION

The experiment results revealed that pest is adapting all types of climates, especially in the study area. There is a need to adopt quick action concerning the management of *Liriomyza* leafminer. Amongst all tested botanicals extracts like *A. indica* and *E. camaldulensis* were not significantly different from the standard check. *A. indica* with a combination of *E. camaldulensis* at 10% was significantly reduced feeding and give maximum larval mortality (53-93%) on 7th day. It was also recorded combined application increased the insecticide action in a short period. *A. indica* with *Eucalyptus* at 10 % concentration was given 60% mortality of 2nd instar larvae after 24 hours of post-treatment.

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