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Insecticidal properties of neem (*Azadirachta indica*), annona (*Annona squamosa*) and castor (*Ricinus communis*) seed extracts against tomato leaf miner, *Tuta absoluta* (Meyrick*)* (Lepidoptera: Gelechiidae)

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ABSTRACT: Laboratory bioassays were conducted to investigate the insecticidal effects of seed extracts of neem (*Azadirachta indica*), annona (*Annona squamosa*) and castor (*Ricinus communis*) on the larvae of tomato leaf miner, *Tuta absoluta*. Six different concentrations of the seed extracts were tested on the second instar larvae of *T. absoluta*. The mean per cent mortality of the larvae revealed a significant toxic effect of all seed extracts that were tested. The seed extracts of annona showed highest mortality (75.1%) of *T. absoluta* followed by neem (70.1%) and castor (52.5%). Based on bioassay and probit analysis, more toxic effect on *T. absoluta* was from annona (LD_{50} 656.21mg/l), and therfore annona seed extract would be a alternative to chemical insecticides in managing *T. absoluta*.

Keywords: *Tuta absoluta*, botanical, seed extract, bioassay, LD_{50}, tomato

INTRODUCTION

The tomato pin worm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a key pest of tomato and native to the western part of South America. This pest is crossing borders and devastating tomato production in both protected and open fields in South America, Europe, Africa, and Asia (Goudarzvand and Habib, 2017). In India, *T. absoluta* was first reported in 2014 from open-field and green house tomato crops at Bangalore and nearby areas (Sridhar *et al*., 2014). Subsequently, the pest has been noticed from almost all tomato growing regions of the country. The larvae of *T. absoluta* could cause 80-100% yield loss by damaging all stages of tomato crop such as leaf, buds, stems and fruits by feed vigorously and producing large galleries on the leaves (Prasannakumar *et al*., 2020a; 2020b). At present, chemical pesticides are being in use by the farmers to control *T. absoluta* in India (Prasannakumar *et al.,* 2020a). Extensive and indiscriminate use of synthetic pesticides against agricultural pests often creates major deleterious side effects. Therefore botanicals play a crucial role in minimising such repercussions. Some of the botanicals such as Arka neem seed powder pellet, Neem seed kernel extract, neem soap, pongamia soaps are effective against sucking pests like aphids, thrips and whiteflies (Prasannakumar *et al*., 2013; Prasannakumar *et al*., 2014). The efficacy of the botanical extracts on herbivores and pathogens are due to presence of many secondary metabolites such as phenols, tannins, flavoinds, limonoids **(**Khan *et al*., 2017). On *T. absoluta* there were some studies carried out by employing plant extracts like *Piper* spp., *R. communis, Thymus vulgaris, Ononis natrix, Peganum harmala, Argania spinosa, Lawsonia inermis, A. indica and Elettaria cardamomum* (Trindade *et al*., 2000; Moreno *et al*., 2012; Braham and Hajji, 2012; Nilahyane *et al.,* 2012; Ghanim and Abdel, 2014; Kona *et al*., 2014; Brito *et al*., 2015). However, usage of botanicals are highly restricted and limited to a particular region due to various reasons such as availability, pest specificity and lack of awareness. In India, no botanical has been evaluated on *T. absoluta*. Therefore, insecticidal effects of seed extracts of neem (*Azadirachta indica*), annona (*Annona squamosa*) and castor (*Ricinus communis*) were evaluated in the laboratory.

MATERIALS AND METHODS

Preparation of the seed extracts

The extraction of neem, annona and castor seeds was made at the Department of Basic Sciences, ICAR-Indian Institute of Horticultural Research (IIHR), Bangalore, Karnataka, India. Each seed powder of each of the species weighing 100g was macerated for 24h in 100ml of solvent under ambient temperature (26± 2° C). Filtration was carried out using Whatman 1 filter paper and then the solvent was evaporated by using rotary evaporator under vacuums. These extracts were dried until the solid residue was obtained

Table 1. Effect of neem, annona and castor seed extracts on *T. absoluta* larvae

Concentration (mg/l)	N	Neem			Total mortality (%)	Concentration (mg/l)	Annona			Total mortality (%)	Concentration (mg/l)	Castor			Total mortality (%)
		24h	48h	72h			24h	48h	72h			24h	48h	72h	
84.0	20	1.3 (4.6)	1.3 (4.6)	1.3 (4.6)	3.9	135	0.0 (1.8)	2.5 (7.4)	2.5 (7.4)	5	104	0.0 (1.8)	0.0 (1.8)	0.0 (1.8)	0.0
253.0	20	2.5 (7.4)	3.8 (10.2)	3.8 (10.2)	10.1	395	5.0 (11.6)	7.5 (15.8)	6.3 (14.4)	18.8	312	1.3 (4.6)	1.3 (1.4)	1.3 (4.6)	3.9
421.0	20	7.5 (14.1)	6.3 (14.4)	7.5 (15.8)	21.3	656	8.8 (16.9)	8.8 (17.2)	11.3 (19.6)	28.9	520	2.5 (7.4)	1.3 (1.4)	2.5 (7.4)	6.3
589.0	20	8.8 (17.2)	11.3 (19.6)	10.0 (18.2)	30.1	920	8.8 (17.2)	11.3 (19.3)	11.3 (19.6)	31.4	728	5.0 (13.1)	5.0 (5.1)	11.3 (19.4)	21.3
758.0	20	11.3 (19.6)	15.0 (22.7)	12.5 (20.6)	38.9	1183	17.5 (24.2)	16.3 (23.6)	16.3 (23.7)	50.1	936	8.8 (17.2)	11.3 (11.4)	8.8 (17.2)	28.9
926.0	20	17.5 (24.6)	23.8 (28.9)	25.0 (30.0)	66.3	1446	22.5 (28.3)	22.5 (28.3)	23.8 (29.2)	68.8	1144	11.3 (19.6)	18.8 (18.9)	15.0 (20.3)	45.1
1094.0	20	21.3 (27.5)	23.8 (29.0)	25.0 (29.9)	70.1	1700	27.5 (21.7)	23.8 (29.2)	23.8 (29.2)	75.1	1352	15.0 (22.5)	20.0 (20.1)	17.5 (22.1)	52.5
Control	20	0.0 (1.8)	0.0 (1.8)	0.0 (1.8)	0.0	Control	0.0 (1.8)	0.0 (1.8)	0.0 (1.8)	0.0	Control	0.0 (1.8)	0.0 (1.8)	0.0 (1.8)	0.0
SE±		3.316	3.679	3.722	-	-	3.975	2.390	2.464	-	-	2.901	3.680	3.084	
CD (p=0.01)		9.4	9.0	7.6			8.9	7.9	7.6			7.1	8.1	14.3	

N-sample size; Means followed by the same letter(s) are not significantly different at P≤0.05; means between brackets are transformed by Arcsine (× + 0.1)

Bioassay of extracts against *T. absoluta*

The experiments were conducted in the laboratory at temperature of 25 – 27 C° and 50 ±10% RH. Infected leaves were collected from the green house and reared for one generation at vegetable entomology laboratory to obtain the uniform 2nd instar larvae. Susceptible culture was also maintained for many generations without exposure to any insecticide/botanical. Seven concentrations of each of neem (84-1094mg/l), annona (135-1700mg/l) and castor (104-1352mg/l) seed extracts were prepared in distilled water separately and each concentration was replicated four times**.** Healthy tomato plants free from pests and diseases were grown under protected conditions without exposing to any of the insecticides. Leaves from these healthy tomato plants were used for all bioassay studies. The control leaves were dipped in the distilled water without extracts whereas the treatment leaves dipped individually in different solutions containing seed extracts for 3 seconds with agitation. Later the leaves were allowed to dry for 1–2 h at room temperature and subsequently placed adaxially on moist tissue paper in petri plates. Ten second instar larvae were released for each replication and a total of 40 larvae used for each treatment (concentration). All bioassays were incubated at 25 ± 0.5°C and 65 ± 5% relative humidity and the larval mortality was recorded 24, 48 and 72h. Moribund larvae were considered as dead if they were unable to move the length of their bodies after gentle prodding with a camel-hair brush.

Data analysis

The percentage of larval mortality was calculated for each extract are as below. The mortality of each extract was then corrected using an adapted form of Abbott's formula (Abbott, 1925)

Corrected mortality (%) = 100 x [(1- % treatment mortality)/%control mortality]

Probit analysis of the mortality data was conducted using IBM SPSS statistics version 21 to determine the LD_{50} and LD_{90} values

RESULTS AND DISCUSSION

Effect of botanical extracts on larvae of *T. absoluta*

Significant mortality of *T. absoluta* larvae was noticed with increased in concentration of all the three plant extracts. As the concentration of the extracts increased the morality was also increased (Table 1). In neem seed extract 1.3% mortality was recorded at lower concentration (84mg/l) and 70% mortality was observed at higher dose (1094mg/l).Similarly, 5% larval mortality was noticed in lower concentration (1325mg/l) and 75% in higher concentration (1700mg/l) of annona seed extract (Fig 1).

For castor extract, the 0.0 % mortality was observed at lower concentration (104mg/l) and 52.2% at higher concentration (1352mg/l). Among the three seed extracts, the neem and annona found superior over castor resulted in high mortality of *T. absoluta*. From the study it was found that concentration of the dose had significant effect on the larval mortality. The time of exposure of insect to any of the extracts found non significant effect.

The LD_{50} of neem was 542.15 mg/L with lower confidence limit (LCL) 370.77 mg/l and upper confidence limit (UCL) 792.73mg/l. Whereas for annona, it was 656.21 mg/l with LCL 485.23 mg/l and UCL 828.92 mg/l and for castor highest LD_{50} was recorded with 1013.68 mg/l with LCL 562.79mg/l and UCL 8163.17 (Table 2). From both lab bioassay and probit analysis the seed extracts of annona having the strong mortality of second larval stage of T. absoluta followed by neem.

The study investigated the efficacies of annona, neem and castor plant extracts on larvae of *T. absoluta* under laboratory conditions. The results of the study indicated that the insecticidal activity of the three plant extracts increased with concentration but not with time of exposure of insects. Seed extract from annona recorded highest larval mortality (75.1%) followed by neem seed extract (70.0%). Though the neem seed extract had good mortality, however at higher doses (1094mg/l) showed phytotoxic effect on leaf and hence further dilutions were not made and therefore annona extract found superior over other two extracts.

Usage of synthetic pesticides during the past decades and their efficacy against different pests has led to their wide acceptance across the world. However, their extensive usages have resulted in certain drawbacks and hazardous including persistence, toxicity to other organisms, pest resistance and environmental pollution (Kona *et al*., 2014; Prasannakumar *et al*., 2020b). Due to increase in repercussion on environment and insecticide resistance problems researchers have been working on plant based products for management of many agricultural pests over many decades.

The effect of anonnin, azadirachtin, karanjin and their mixtures on larvae of *T. absoluta* found as a good alternative to currently used chemicals under greenhouse and field conditions (Durmusoglu *et al*., 2011). Azadirachtin in neem and sterols and terepene alcohols in Jatropha (*Jatropha curcus*) had insecticidal

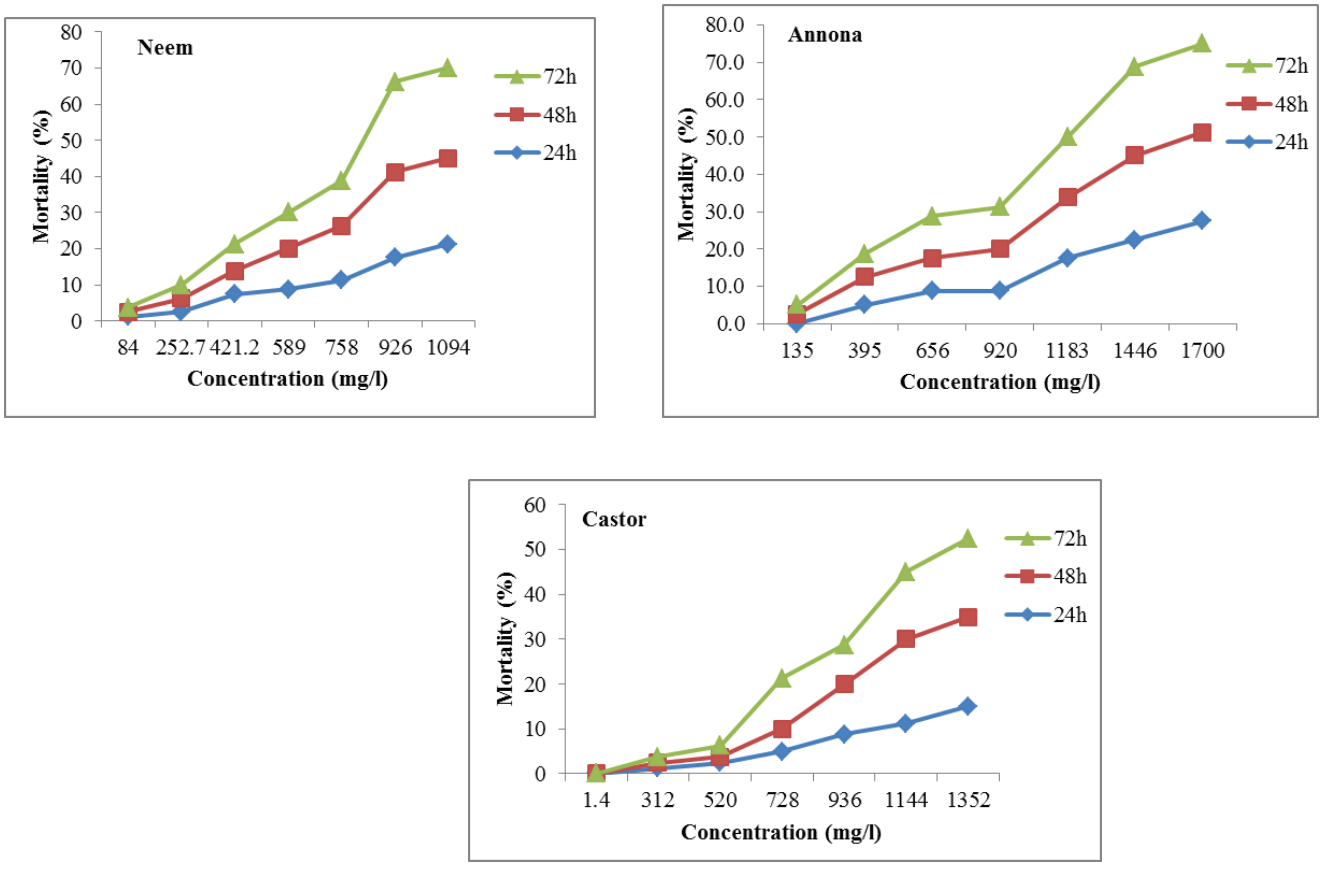

Fig 1: Mortality of *T. absoluta* larvae to different concentrations of botanicals over time

Table 2: Toxicity of neem, annona and castor seed extracts on larvae of *T. absoluta*

Insect	Extracts	LD_{50} LCL-UCL (95% confidence limit)	LD_{90} LCL-UCL (95% confidence limit)	χ^2	df
Tuta absoluta	Neem	542.15 (370.77-792.73)	1689.3 (869.56-2106.3)	1.254	6
	Annona	656.21 (485.23-828.92)	1864.32 (1025.1-2546.6)	2.28	6
	Castor	1013.68 (562.79-2295.76)	8163.17 (1423.08-46826)	0.551	6

LD_{50} -Lethal dose for 50% killing of the exposure larvae; CL.- Confidence Limit (95%); n^a- number of larvae; S.E. - Standard error;χ^2-Chi square; df- degree of freedom

effect on eggs and larvae of *T. absoluta* (Kona *et al*., 2014). Ovicidal and larvicidal effect of the essential oil of cardamom, *Elettaria cardamomum* on *T. absoluta* was due to presence of major components in the oil such as a-terpinyl acetate (36.61%), 1, 8-cineole (30.42%), linalyl acetate (5.79%) and sabinene (4.85%) (Chegini and Abbasipour, 2017)), Likewise, Crude hexane extract of *Acmella. oleraceae* was found effective on *T. absoluta* due to presence of three alkamides viz., spilanthol, (E)-N-isobutylundeca-2-en-8,10-diynamide and (R, E)-N-(2-methylbutyl)undeca-2-en-8,10-diynamide (Moreno *et al*., 2012). The extracts from jojoba, neem, Tossegn (Garden thyme, *Thymus vulgaris*), Chinaberry (*Melia azedarach*) and castorbean plants were reported to be efficient against *T. absoluta* under laboratory condition (Chhetri, 2018)., Significant mortality of *T. absoluta* larvae was also noticed from different *Piper* spp. (*P. mikanianum P. mollicomum P. glabratum and P. Amalago*) extracts on tomato crop (Brito *et al*., 2015). From the present study the extracts from the neem and annona seeds will be promising in terms of their larvicidal activity on *T. absoluta.* However due to phytotoxic effect of neem, annona extract would be a potential botanical for managing *T. absoluta*. Therefore seed extract of annona would be an alternative to chemicals for sustainable pest management.

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