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Laboratory evaluation of toxicity of insecticides to populations of tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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Abstract
The tomato pinworm, *Tuta absoluta* (Meyrick), an invasive pest has become a serious threat to world wide tomato production. Depending on the infestation level and cropping system control relies on insecticides. Within few years *Tuta absoluta* has also developed resistance to a wide range of insecticides. Laboratory bioassay showed the LC_{50} values of spinosad are estimated that on larval instars 2^{nd}, 3^{rd} and 4^{th} as 0.37 ppm, 0.45 ppm and 0.61 ppm, for indoxacarb (0.93 ppm, 1.12 ppm and 1.54 ppm), for chlorantraniliprole (0.32 ppm, 0.49 ppm and 0.61 ppm) for chlorpyrifos (1124.65 ppm, 2183.48 ppm and 3455.62 ppm) for flubendiamide (1.56ppm, 1.78 ppm and 2.48 ppm) and imidacloprid (1.15 ppm, 1.23 ppm and 2.16 ppm) respectively. *T. absoluta* has the potential to develop pesticide resistance very rapidly due to short generation time and high reproductive potential. Insecticides are found to be as effective need to be used judiciously with the rotation of various insecticides with different modes of actions.

Keywords: Tomato pin worm, *Tuta absoluta,* insecticides, toxicity

Introduction

In India, tomato is the leading horticultural crop with a production area of 8.1 lakh ha with a production of 20.71 million metric tonnes and an average productivity of 26.00 metric tonnes per ha. Tomato is cultivated both under greenhouse and field conditions. Tomato leaf miner, *Tuta absoluta* is a native microlepidopteran and oligophagous pest of South America (Torres *et al.*, 2009) [25] and it is described at Peru in 1917 by the entomologist E Meyrick (Rojas, 1981) [20] and it is considered to be one of the most destructive pest on tomato crops (EPPO 2005) [8]. Tomato pinworm usually attack tomato plants at any stage from seedling to mature plants and deposit their eggs on leaves, stems and fruits. After hatching the neonates penetrate in to the leaves, stems, fruits and cause damage (EPPO, 2005) [8]. This pest is initially recorded in Brazil between 1979 and 1980 (Muszinski *et al.* 1982) [14] and this pest was detected in Spain (Urbaneja *et al.* 2007) [28] and in Greece and Egypt (Roditakis *et al.* 2010) [16] and Africa, middle East and parts of Asia (Biondi *et al.* 2018). Because of its aggressive nature, multivoltine character, high biotic potential and insecticide resistance are the main reasons for this pest to become key pest in new area (Desneux *et al.* 2011) [7]. In India, *T. absoluta* was first reported during October, 2014 in tomato fields at Pune and then it was recorded in Karnataka, Tamil Nadu, Andhra Pradesh, Telangana, New Delhi, Gujarat, Madhya Pradesh, Punjab, Meghalaya, Himachal Pradesh and Kerala, caused severe damage both yield and fruit quality to tomato (Abdul Rasheed, 2018) [2]. The severe infestation of *T. absoluta* causes 80-100% yield loss (Tropia Garzia *et al.* 2012) [26].

New molecules of insecticides such as indoxacarb, spinosad, imidacloprid,deltamethrin and Bt var *kurstaki* have successfully been used in Spain (Russel, 2009) [21], chlorpyriphos and pyrethrins were frequently used in Italy (Garzia *et al.* 2009) [26], indoxacarb, spinosad, imidacloprid,, abamectin, thiacloprid and *Bt var Kurstaki* were used in Malta (Mallia, 2009) [13]. Currently the pest has developed resistance against insecticides including diamides, spinosyns, and organophosphates (Jallow *et al.* 2018) [11]. Few active ingredients were effective against tomato pinworm and they were selective to beneficials and pollinators. Integration pest management methods like cultural, mechanical, biological and biotechnological tools through a light on formulation of IPM to *T. absoluta.*

Chemical insecticides are also one of the important Components in IPM technology. Indiscriminate and continuous use of same chemicals could lead to insecticides resistance development and also harm beneficial insects (Landgren *et al.* 2009; Silva *et al.* 2011) [12, 23]. Hence, it is necessary to identify the effective insecticide for management of *T. absoluta* and the present paper address solution for the need of the hour.

Material and methods

Test Insects

Populations of *T. absoluta* were collected from commercial tomato fields. Larva of *Tuta absoluta* were collected in infested leaves, stalks and fruits. Infested materials collected were packed in plastic bags and brought to the laboratory. The population was immediately transferred into a larval rearing cage (45 × 45 × 45 cm) with mesh. Adult cages (30 × 30 × 30 cm) were used for oviposition only, where leaves of tomato were provided daily as substrate. Adults of *T. absoluta* were fed with 10% sugar solution, while larvae were fed with tomato leaves, cultivated under greenhouse conditions without any insecticide application. The populations were reared in the laboratory at 25±0.5^{0}C, with a relative humidity of 75±5% and a 12:12 L: D photoperiod.

Bioassay

A toxicological bioassay was conducted using a completely randomized design with three replications, and the whole bioassay was repeated twice. Insecticide concentrations and control (distilled water) were prepared with 0.01% Triton X-100. Tomato leaflets were immersed for 30 seconds in insecticide solution and dried for 2 h. The leaves were subsequently placed in petri dishes (8 cm diameter × 1.5 cm height) containing filter paper moistened with 400 µL of distilled water. Ten 2nd, 3rd and 4th instar larvae were transferred to each petri dish, which were placed into a growth chamber at 25±0.5^{0}C, 75±5% relative humidity and 12 h photoperiod. Larval mortality was assessed after 72 h of exposure. Mortality evaluations were performed with the aid of a light source and magnifying glass. Larvae were withdrawn carefully from galleries of tomato leaves with a fine paint brush, and those larvae were considered dead if unable to move the length of their body.

Data analysis

Mortality data from dose-response bioassays were subjected to probit analysis (Finney, 1971) [9]. Corrected mortality percentages were worked out by using Abbotts' formula (Abbott, 1925) [1]. The mortality response on linearity, slope, lethal concentrations and the confidence limits (CL) of the lethal concentrations were also registered.

Results and discussion

Results of probit regression analysis, median lethal concentrations for second, third and fourth instar larvae of tomato pinworm have been presented in Table 1. The responses of insect population to insecticides were homogenous and fitted the log (dose)/ probit (mortality) model. However, there were great variations in slopes of the insecticides. The slope for insecticides in all populations was higher than two or three chemicals, potentially suggesting higher homogeneity in lab population (Finney, 1971) [9]. The results obtained revealed low to high susceptibility of second, third and fourth instar larva of tomato pinworm to insecticides. However, differences in the susceptibility were observed among larval stage. Second instar larva was most susceptible, while susceptibility was lower in third and fourth instar larvae.

Table 1: Susceptibility of lab population to *T. absoluta* with different chemicals

Larval stage	Slope	SE^a	X^{2b}	LC$_{50}$^{c}	Confidence limits (95%)		LC$_{95}$^{c}	Confidence limits (95%)	
					Lower limit	Upper limit		Lower limit	Upper limit
Chlorantraniliprole									
II INSTAR	3.143	1.436	3.852	0.324	0.298	0.417	0.895	0.852	4.135
III INSTAR	4.045	1.117	0.749	0.497	0.387	0.595	1.269	0.913	3.560
IV INSTAR	2.480	1.106	0.268	0.613	0.428	5.247	2.824	1.171	8.030
Flubendiamide									
II INSTAR	2.724	0.594	2.344	1.564	0.927	1.735	6.873	3.766	17.544
III INSTAR	3.883	0.986	1.370	1.788	1.193	1.193	4.740	3.566	9.869
IV INSTAR	2.516	0.655	1.518	2.485	1.722	3.226	11.191	6.762	48.194
Spinosad									
II INSTAR	1.389	0.224	0.742	0.377	0.248	0.467	4.587	2.324	18.847
III INSTAR	1.825	0.426	1.045	0.451	0.251	0.663	3.587	1.919	16.568
IV INSTAR	1.258	0.452	0.052	0.611	0.265	1.753	12.391	3.091	23.346
Imidacloprid									
II INSTAR	1.285	0.312	2.854	1.154	0.567	1.645	22.344	9.684	158.858
III INSTAR	1.379	0.334	2.757	1.235	0.501	2.087	19.226	8.587	146.716
IV INSTAR	1.772	0.398	1.733	2.169	1.510	3.136	18.368	9.003	105.546
Indoxacarb									
II INSTAR	1.644	0.272	0.913	0.935	0.578	1.358	8.163	5.615	21.576
III INSTAR	1.526	0.260	1.709	1.124	0.728	1.622	13.438	7.085	44.542
IV INSTAR	1.448	0.362	0.171	1.546	0.969	2.699	21.126	7.945	324.370
Clorpyriphos									
II INSTAR	2.567	0.774	3.825	1124.657	762.554	1672.865	6954.376	3826.946	42845.468
III INSTAR	2.920	0.964	0.593	2183.489	1354.059	2951.817	7986.077	4787.752	72686.383
IV INSTAR	4.464	1.698	0.176	3455.623	2675.085	5099.915	8070.969	5321.764	116949.000

a-Standard error, b-Chi Square c- ppm

Diamide group of insecticides are very much promising in controlling tomato pinworm. They have a unique mode of action *viz* they act on ryanodine receptor modulator and cause the channel to open and release calcium ions which leads to death of an insect. The tested population exhibited high slopes in response to diamide exposure. The slope values generated from concentration mortality response to chlorantraniliprole and flubendiamide varied widely among different instars of *T. absoluta*. The slope values generated from concentration mortality response to chlorantraniliprole for 2nd, 3rd and 4thinstar were 3.14, 4.04 and 2.48 and the LC_{50}values were 0.32, 0.49 and 0. 61ppm, respectively. The results of the study are in agreement with the findings of Roditakis *et al.* (2012a) [17]; Roditakis *et al.* (2012b) [17] Silva *et al.* (2016) [24]; Jallow *et al.* (2018) [11]; Roditakis *et al.* (2014), and Campos *et al.* (2014) [4] which reported that the LC50 value of chlorantraniiprole was 0.12 to 0.53ppm, 0.14 to 2.10 ppm, 0.004 to 1262.7 ppm0.29 to 1.13 ppm,0.18 to 435 ppm and 3.17 to 29.64 respectively. For flubendiamide the LC_{50}values for for 2nd, 3rd and 4th instar was1.56, 1.78 and 2.48ppm, and slope values were 2.72, 3.88 and 2.51 respectively. The results of the present study are consistent with the results of Silva *et al.* (2016) [24]; Jallow *et al.* (2018) [11]; Roditakis *et al.* (2012a) [17]; Roditakis *et al.* (2015) [19] and Campos *et al.* (2015) [5] Were 0.03 to 3018 ppm, 0.16 to 12.89 ppm, 0.3 to 1.3 ppm, 0.8 to 1376 ppm and 94 to 230 ppm respectively.

Spinosad showed high efficacy in controlling all instar larvae of pinworm. The slope values generated from concentration mortality response to spinosad for 2nd, 3rd and 4th instar were 1.38 ppm, 1.82 ppm and 1.25ppm and the LC_{50} values for 2nd, 3rd and 4th instar were 0.37, 0.45 and 0.61ppm respectively. Similar results were also found by Dagli *et al.* (2012); Roditakis *et al.* (2012b) [17] and Campos *et al.* (2014) [4] 0.3 to 1.3 ppm, 0.23 to 0.64 ppm and 0.01 to 1717.3 ppm respectively.

The slopes of the response line to imidacloprid, for 2nd, 3rd and 4th instar were 1.28, 1.37 and 1.77 ppm and the LC_{50} values for 2nd, 3rd and 4th instar are1.15, 1.23 and 2.16 ppm respectively. Imidacloprid was the found to be superior toxicant used against third instar larvae (3.11 ppm) of *Tuta absoluta* (Radwan and Taha, 2012) [15]. The toxic effect of imidacloprid to fourth instar larva was very low with LC_{50} 2115.70 ppm (Sallam, 2015) [22].

The susceptibility to indoxacarb was very high for third instar larvae of *T. absoluta* and comparable with emamectin benzoate and methoxyfenzoid. The indoxacarb susceptibility varied in space and time (Gontijo *et al.* 2013) [10]. The slopes of the response line to indoxacarb, for were 1.64, 1.52 and 1.44, and the LC_{50} values for 2nd, 3rd and 4th instar are 0.93, 1.12 and 1.54ppm respectively. These observations agree with the findings of Roditakis *et al.* (2012a) [17]; Silva *et al.* (2011) [23]; Dagli *et al.* (2012) and Roditakis *et al.* (2012b) [17] who recorded 1.73 to 17.50 ppm 0.39 to 10.82 ppm, 0.2 4 ppm and 0.26 to 19.37 ppm respectively.

The slopes of the response line to chlorpyriphos, for 2nd,3rd and 4th instar are 2.56, 2.92 and 4.45 and the LC_{50} values for 2nd,3rd and 4th instar are 1124.65, 2183 and 3455ppm respectively. Our results are in agreement with Roditakis *et al.* (2012b) [17] was 530 to 2038 ppm. The toxic effect of chlorpyriphos to fouth instar larva is very low with LC_{50} 899.71mg/l (Sallam, 2015) [22]. It is suspected that the tested lab population exhibited high tolerance to chlorpyriphos.

The results obtained from this study will pave a way to include above insecticides as one of the component in the development of Integrated Pest Management (IPM) strategies in the management of tomato pinworm in India.

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