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Studies on outbreak of tomato pinworm, *Tuta absoluta* (Meyrick) in South India and its differential susceptibility to insecticides

N.R. PRASANNAKUMAR*, N. JYOTHI, G. RAM KUMAR, S. SAROJA and V. SRIDHAR

Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake , Bengaluru-560089, India

E-mail*: prasannaent@gmail.com

ABSTRACT: Tomato fields in different locations of south India from where outbreaks of *Tuta absoluta* (Meyrick) occured were surveyed during 2019 -20 to assess the farmer's adopted management strategies. The severity of *T. absoluta* resulted in the crop losses ranging from 20-90 per cent. The main reasons for the outbreak of *T. absoluta* were lack of knowledge on IPM among the farmers, delay in implementing management strategies, over reliance on insecticide sprays and usage of spurious and un-recommended insecticides. Bioassays of field collected *T. absoluta* populations shown resistance to a few insecticides *viz.*, indoxacarb14.5SC, flubendiamide 39.5SC, cyantraniliprole 10.25SC and emamectin bezoate 5SG which were used for management of pest by the farmers. The present study thus analyses the recent outbreak of *T. absoluta* and offers Insecticide Resistance Management (IRM) strategy to avoid future loss to tomato growers.

Keywords: Tomato pin worm, insecticide resistance, bioassay, tomato, outbreak, *Tuta absoluta*

INTRODUCTION

Tomato, *Solanum lycopersicum* is one the most important vegetable crops widely cultivated in India. The major production constraints in tomato are biotic stresses such as insect pests and diseases. Tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), a new invasive pest was initially reported from India at Bengaluru during 2014 (Sridhar *et al.,* 2014). Since its first report, the pest has spread to almost all tomato growing regions of the country. *Tuta absoluta* attacks the tomato leaves, buds, stems, and fruits. The larvae feed vigorously producing large galleries in leaves, and capable of causing up to 100% yield losses (Ashok *et al.,* 2016). It has maximum lifetime fecundity of 260 eggs per female and the whole life cycle of the *T. absoluta* from egg to adult emergence under laboratory conditions ranged from 21.0-27.50 days with an average of 24.5±2.15 days (Kalleshwaraswamy, 2015).

Currently, selected insecticides, pheromone traps, biorational insecticides, egg parasitoids are being used for suppression of *T. absoluta* population (Zappala *et al.*, 2013), but main control practice by farmers is spraying of broad-spectrum insecticides viz., indoxacarb 14.5 SC, emamectin benzoate 5 SG, spinosad 45 SC, spinetoram 11.5 SC, flubendiamide 39.5 SC. The outbreaks of the pest were already reported in many countries where it was noticed. However, outbreaks of the pest causing enormous damage with a yield loss up to 70-80% have been reported by farmers in certain locations in South India during 2019-20. Keeping in view the importance of the pest and recent outbreaks, a survey was carried out in selected parts of Karnataka, Tamil Nadu and Andhra Pradesh to investigate reasons for the pest flare-up and educate the farmers for its successful management.

MATERIALS AND METHODS

Surveys were carried out during 2019- 2020 in tomato growing fields covering a few major vegetable growing districts viz., Bengaluru rural and Kolar district of Karnataka, Anantapur district in Andhra Pradesh, Coimbatore, Salem, Oddanchatram and Madurai in Tamil Nadu. The *T. absoluta* damage was recorded on five randomly selected plants at regular interval (20m interval) in each field by following zig zag manner and the per cent pest incidence and damage were worked out. Insecticide usage pattern was also obtained from respective farmers of the field. The *T. absoluta* population were collected in polythene bags and labeled them according to field and location.

Rearing of the field collected *T. absoluta*

In order to get uniform pest population, the field collected *T. absoluta* populations were maintained and reared on healthy tomato plants separately in bug -dorm cages with 25 ± 2.5^{o}C and 65 ± 5% relative humidity, at the Division of Crop Protection, ICAR-Indian Institute of Horticulture Research, Bengaluru. The second instar larvae of F_1 generation were used for insecticidal bioassay.

Table 1: Occurrence and damage of *Tuta absoluta* in different tomato growing areas

District	Location	Date of Observation	Latitude	Longitude	Variety/hybrid	Plant parts damaged	Incidence of *T. absoluta* (%)	List of Insecticides sprayed
Bengaluru	Kodagitirumalapura	18/07/2019	13.5788	77.48° E	501	Leaves	15.0	Sagarika. Indoxacarb14.5SC, Flubendiamide39.35SC
	Huralickikkanahalli	18/07/2019	13.58° N	77.49° E	Arkarakshak	Leaves and fruits	12.5	Sagarika. Indoxacarb14.5SC, Flubendiamide39.35SC
Kolar	Thimmasandra	20/08/ 2019	13.59° N	78.00° E	Rishik	leaves	5.1	Indoxacarb14.5SC , deltamethrin
	Chintamani	20/08/2019	13.20 ° N	78.10° E	Rishik	Leaves and fruits	10.6	Indoxacarb14.5SC, Isabion Syngenta
	Dhanamittanahalli	20/08/ 2019	13.21° N	77.19° E	Rishik	Older leaves	10.1	Isabion Syngenta, Larvin, Sagarika, Chlorantraniliprole18.5SC
	Anakallu	20/082019	13.13° N	78.12° E	44-81	Fruits	20.0	-
	Thipannahalli	31/11/2019	13.17° N	77.99 ° E	Rishik	Leaves and fruits	90.5	Isabion Syngenta, Lambda Cyhalothrin, Phenthoate 50EC, Acetamiprid 20%SP, Floronil40%+imidacloprid40% . Deltamethrin 2.8EC, Pegasis
	Betta hosapura grama	31/11/2019	13.08° N	78.07° E	Rishik	Leaves and fruits	70.5	Isabion Syngenta, Larvin, Sagarika, Indoxacarb 14.5SC
	Byrasandra	31/11/2019	12.9180° N	77.9582° N	Kowshik	Leaves and fruits	45.0	-
Anantapur	Garladinne	18/02/2020	14.8256° N	77.5935° E	Sahoo	Leaves and fruits	66.5	Emamectin benzoate 5SG, Spinosad 44.33SC, Indoxacarb14.5SC, Chlorantraniliprole18.5SC, Flubendiamide39.35SC
	Marthadu	18/02/2020	14.7914° N	77.5447° E	Sahoo	Leaves and fruits	60.80	Emamectin benzoate5SG, spinosad44.03SC, Indoxacarb14.5SC, Chlorantraniliprole18.5SC, Flubendiamide39.35SC
Coimbatore	Madampatti	1/02/2019	10.9698° N	76.8598° E	Sivam	Leaves and fruits	35.2	Thiodiocarb75%WP, Chlorantraniliprole18.5SC ,Encounter,Indoxacarb14.5SC
	Poolvapatti	1/02/2019	10.9621° N	76.8151° E	Sarvi	Leaves	40.1	Thiodiocarb75%WP, Encounter
	Viraliur	1/02/2019	10.9951657° N	76.7843° E	916	Leaves and fruits	25.0	Thiodiocarb75%WP, Chlorantraniliprole18.5SC, Encounter
	Perumalpatti	1/02/2019	9.053° N	77.62997° N	Sivam	Leaves and fruits	30.0	-
Madurai	Nadupati	03/02/2019	10.00° N	77.79° E	Sivam	Leaves and fruits	55.2	Chlorantraniliprole18.5SC, , Encounter
	Jathigondapatti	03/02/2019	10.10° N	77.78° E	Sivam	Leaves and fruits	30.0	Chlorantraniliprole18.5SC, Spinetoram 11.7SC, Encounter
Dindigal	Kallimandam	02/02/2019	10.5912° N	77.6864° E	Sivam	Leaves and fruits	16.0	Thiodiocarb75%WP, Chlorantraniliprole18.5SC, Encounter
	Oddanchatram	03/02/2019	10.4897° N	77.7544° E	Sivam	Leaves and fruits	45.0	Thiodiocarb75%WP, Chlorantraniliprole18.5SC, Encounter
Salem	Salem	26/02/2019	11.6643° N	78.1460° E	916	Leaves and fruits	36.2	Indoxacarb14.5SC, Chlorantraniliprole18.5SC, Thiodiocarb75%WP , Encounter

Susceptible culture

Since *T. absoluta* was first recorded at Bengaluru and hence population from the same location was used as susceptible culture to compare the resistance levels of other locations. In a separate room, *T. absoluta* Bengaluru population was maintained which was never exposed to any of the insecticides from 10-12 generation.

Insecticides for Bioassay

For all bioassays and insecticide treatment, commercial formulations of the diamide insecticides- cyantraniliprole 10.25 SC (DuPont, France) and flubendiamide (Belt® 24WG, Bayer Crop Science AG, Germany); oxadiazine- indoxacarb (Kingdox) Avermectins group- emamectin benzoate (proclaim 5SG) were used.

Bioassay

Infected leaves were collected from laboratory reared field population to collect uniform second instar larvae. Subsequently, the IRAC method leaf dip was adopted for the toxicological assays. All the insecticides are prepared in five different concentrations ranging from 1-60 ppm/l with four replications. The control leaves dipped in the distilled water without insecticide and other leaves were dipped individually in the different solutions for 3 seconds with agitation. Treated leaves were allowed to dry for 1–2 h at room temperature and subsequently placed adaxially on moist tissue paper in a petri plates. Ten second instar larvae were released for each replication. All bioassays were incubated at 25 ± 0.5°C and 65 ± 5% relative humidity. Larval mortality was recorded 24, 48, 72 and 96 h. Moribund larvae were considered as dead if they were unable to move the length of their body after gentle prodding with a camel-hair brush. To calculate resistance ratios, LC 50 values obtained from different field population were compared to that of susceptible population maintained in laboratory for 10-12 generation without exposure to any insecticides.

Biochemical assay

Preparation of cytosols from *T. absoluta* larvae for MFO and esterase assays

The enzyme sample was prepared from whole body of 3rd instar larvae (n=20) from both susceptible and resistance population. Tissues were homogenized in 1.5 ml of 0.1M ice cold sodium phosphate buffer, pH-7.2. Homogenates were centrifuged at 12,000 rpm for 30 min at 4ºC, after centrifugation the supernatant again centrifuged at 10,000 rpm for 15 min. The enzyme supernatant was collected in a sterile vial and used as enzyme source for activity of MFO and esterase enzymes. The protein concentrations in the prepared crude extracts were determined by the method of Lowry *et al.* (1951) using bovine serum albumin standard (BSA).

Determination of Mixed function oxidase activity

Mixed function oxidase activity was measured by according to Brogdon *et al.* (1989) using peroxidation of 3, 3', 5, 5'- Tetramethyl-benzidine (TMBZ). The total reaction mixture contains 50µl of enzyme sample, 500µl of TMBZ (dissolved in 0.05M TrisHCl pH-7.0 containing 1.5% Kcl), 250µl Potassium phosphate buffer 0.05M, and 200µl of 3%H_2O_2. The absorbance was read in the UV-visible double beam spectrophotometer (Cary 60 UV-VIS, Agilent) at 630nm for 10 min. The Total activity expressed as n mol equivalent cytochrome P450/ mg protein using cytochrome C for the standard curve.

Determination of esterase activity towards α-naphthylacetate (α-NA)

Substrate **α** -naphtyl acetate (β-NA) was used to determine esterase activity according to Kranthi (2005). For each test tube, 10µl of enzyme sample, 990µl of phosphate buffer saline along with 5ml of substrate solution was added. All the components except enzyme solution served as blank/control. Then the tubes were incubated 30^0 C at dark for 10min. Then 1ml of staining (Fast blue BB Salt) solution was added and the tubes were incubated at room temperature for 20min. A change in absorbance was measured spectrophotometrically at 450nm.

Data analysis

Probit analysis of the moratlity data was conducted using IBM SPSS statistics version 21 to determine the LC_{50} and LC_{90} values. Resistance ratios (RRs) were calculated by dividing the LC50 value of each field population by the LC50 value of the susceptible population of *T. absoluta*. The difference or similarity in enzyme activity obtained from spectrophotometer was estimated by student T-test.

RESULTS AND DISCUSSION

Field Survey results

During the surveys, infestation of *T. absoluta* was noticed in all the tomato fields. Higher incidence of the pest was observed on leaves than on fruits. Severe incidence of *T. absoluta* (90.50%) was recorded at Thipannahalli village of Kolar district followed by Marthadu and Garladinne villages of Anantapur district

Table 2. Bioassay results of different field populations of *T. absoluta*

Population	Insecticide	n[a]	LC_{50} LCL-UCL (95% confidence limit)	LC_{90} LCL-UCL (95% confidence limit)	χ^2	df	RR Ratio
Susceptible population	Indoxacarb 14.5 SC	200	11.036 (9.805-12.054)	20.906 (18.860-24.705)	3.312	3	
	Flubendiamide 39.5 SC	200	6.969 (4.756-10.9333)	19.090 (16.990-21.275)	3.645	3	
	Emamectin benzoate	200	7.598 (6.856-9.546)	15.735 (16.543-19.236)	3.084	3	
	Spinetoram 11.5 SC	200	11.886 (10.916-16.021)	18.996 (21.797-31.629)	8.229	3	
	Cyantraniliprole 10.25 SC	200	9.026 (8.085-9.845)	12.639 (14.365-17.540)	1.768	3	
Kolar field population	Indoxacarb 14.5 SC	200	22.85 (21.840-31.201)	83.301 (62.82-140.30)	5.05	3	2.07
	Flubendiamide 39.5 SC	200	5.104 (3.013-8.152)	9.955 (6.860-10.331)	5.12	3	0.732
	Emamectin benzoate	200	8.427 (5.995-8.426)	11.472 (10.765-13.124)	6.832	3	1.077
	Spinetoram 11.5 SC	200	12.071 (8.833-12.907)	15.44 (13.389-23.004)	1.487	3	1.031
	Cyantraniliprole 10.25 SC	200	20.22 (16.574-25.404)	32.344 (26.553-40.396)	1.944	3	2.24
Anantapur Field population	Indoxacarb 14.5 SC	200	33.216 (30.842-36.156)	58.523 (53.036-66.236)	4.944	5	3.001
	Flubendiamide 39.5 SC	200	32.343 (30.515-34.508)	55.444 (50.779-62.130)	1.590	5	4.640
	Emamectin benzoate5SC	200	29.270 (28.114-32.398)	54.688 (49.771-61.642)	7.481	5	3.458
	Spinetoram 11.5 SC	200	25.618 (21.345-33.824)	56.169 (46.826-74.245)	8.226	5	2.13
	Cyantraniliprole 10.25 SC	200	29.495 (25.805-35.438)	50.050 (41-949-67.201)	1.2160	5	3.267

LC50 lethal concentration that kills 50% of the exposed larvae, Confidence Limit , $\chi 2$ chi-square,n – Sample number, LCL-lower confidence limit, UCL- upper confidence limit.RR = Resistance ratio, determined by dividing the LC50 of resistant strain by LC50 of susceptible strain.

(66.50%) and in Madurai (50%). Lowest *T. absoluta* incidence (5.1%) was observed in Tippasandra village, Chintamani (10.60%) in Kolar and Huralichikkanahalli (12.5%) in Bangaluru district of Karnataka (Table 1; Fig. 1).). Severe fruit damage was also observed in Kolar (85%), Coimbatore (50%) and Anantapur (46%) districts (Table 1). The farmers were unaware about the type of damage caused by the pest, management initiated at higher incidence of the pest, indiscriminative and repeated usage of same insecticides, spurious chemicals and lack of IPM practices were the few reasons for the outbreaks In addition, the congenial weather, particularly higher temperatures above 32C during the cropping period was further supplemented for flare up of the pest. Most of the fields surveyed were unattended and abandoned due to outbreak of the pest. The farmers expressed that the money going to incur towards harvest of the crop itself could not be recovered from the produce.

When the pest was noticed for the first time the damage on leaf was 15-35 % at Pune, (Shashank *et al.,* 2015), 5-97.9% at Telangana (Anitha *et al.* 2015), Dharmapuri 20-25% (Shashank *et al.,* 2016). Whereas on fruits, the loss was 0.5 to 13.5 per cent in Tamil Nadu, 2.0 to 100 per cent in Karnataka and 5 to 12 per cent in Gujarat (Ballal *et al.*, 2016). Sudden increase in pest incidence from a very low incidence to outbreaks may be due to its rapid multiplication, short life cycle, favored high temperatures during February-May.

To ascertain development of insecticide resistance by the pest, lab bioassay was carried out with different insecticides for the *T. absoluta* population from each location viz., Karnataka, Andhra Pradesh and Tamil Nadu (Table 2). Reduced susceptibility was found in Bengaluru *T. absoluta* field population from Bengaluru to flubendiamide (1.34 folds). Whereas, Kolar population showed reduced susceptibility to cyantraniliprole (2.241-folds) and indoxacarb (2.07 -folds). Similarly, reduced susceptibility of Madurai population was 1.213-fold to cyantraniliprole. Likewise, Salem *T. absoluta* population showed reduced susceptibility to indoxacarb (1.44-folds) and cyantraniliprole (1.452 folds). Population from Anantapur district also showed reduced susceptability to flubendiamide (4.640 –folds), emamectin benzoate (3.458- folds), cyantraniliprole (3.20-folds) and to indoxacarb (3.00-folds).

Through continuous monitoring it was found that reduced susceptibility of Bengaluru population to flubendiamide might be due to continuous usage of same chemical from the past two years. Though in Kolar no usage of cyantranilprole and indoxacarb on tomato crop from where samples were collected but reduced susceptibility to these insecticides was observed. This type of results sometimes happens when usage of chemicals (here cyantranilprole and indoxacarb) by the growers on other tomato fields that might have affected on the insects which are high migratory in nature. These chemicals are being widely used on other vegetable crops of the district as it is known as one of the vegetable belts in Karnataka.

The Salem and Madurai populations showed slightly less susceptible and hence cannot be consider under development of resistance by the pest to the chemicals. However, frequent monitoring of resistance may provide clear picture from these locations. Recent outbreaks of *T. absoluta* from Anantapur resulted in abandon of fields to cattle grazing. Though, the farmers of Anantapur used newer molecules viz., flubendiamide, emamectin benzoate, cyantranilirpole and indoxacarb, which have

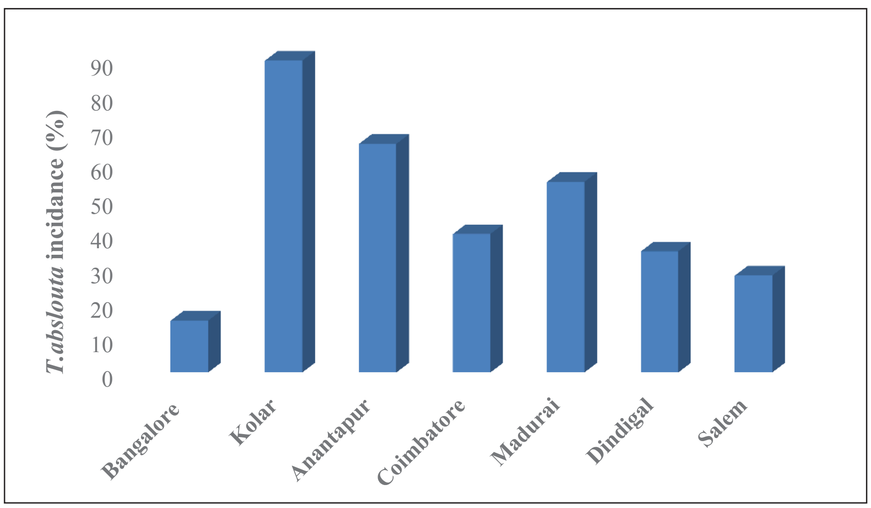

Fig1. Incidence *Tuta absoluta* on tomato in different districts of South India

also been recommended on tomato crop, but control failures were observed. Farmers expressed unaware about nature damage of the pest, economic importance of the pest, early identification of the pest and IPM. The recommended insecticides were used when the crop incurred significant loss from the pest and hence farmers and traders left the crop from harvest to avoid further loss from engaging labors for harvesting. Further, the insecticidal bioassay clearly showed an indicative trend of developing resistance by the pest to flubendiamide, emamectin benzoate, cyantranilirpole and indoxacarb. This trend was due to repeated application of the chemicals and indiscriminate usage of the chemicals without rotating at right time.

The results from biochemical assay indicate the significant ($P<0.005$) increase in MFOs – cytochrome p450 and esterase's activity. The MFO-CYP450 activities were notably increased in Bengaluru (1.2 folds), Kolar (1.4 folds), Madurai (1.6 folds), Salem (1.3 folds) and in Anantapur (1.3-folds) compared to susceptible population ($p<0.05$). Enzymatic activity analysis of esterases from *T. absoluta* was done with EST-α-NA assay. EST-α-NA activities were also increased significantly in Bengaluru (2.21folds), Kolar (1.2 folds), Madurai (1.4 folds), Salem (1.3folds) and in Anantapur (1.26 folds) ($p<0.05$) compared to susceptible population. Cytochrome P450 monooxygenases and esterase's generally take a role in direct metabolism of several insecticides (Scott *et.al.* 2001, Heidari *et. al.,* 2005 and Huang *et. al.,* 2004). A study in 2019 (Silva *et al.*2019,*)* reported the activity of esterases, glutathione S-transferases, and cytochrome P450-dependent monooxygenases are apparently involved in the chlorantraniliprole resistance in *Tuta absoluta*.

Outbreak and resistance development of the pest was observed in other countries from where the pest was previously reported. Development of resistance to indoxacarb was observed in Greece (1794-fold resistance) due to putative target resistance mutation in F18454 and V18481 gene (Emmanouil *et al.,* 2017). Resistance of *T. absoluta* to flubendiamide (750-folds) and chlorantraniliprole (860-folds) was also recorded in Kuwait the resistance level indicates presence of differential selection pressures such as use of different amount of insecticide in different areas (Jallow *et al.*, 2019).

In the present study, awareness was spread among farmers about the pest, nature of damage and also provided technical know-how to tackle the pest in future by adopting IPM practices. The pinworm is attaining most serious pest status by causing significant damage, developing resistance to insecticides, spreading to many areas due to its rapid multiplication. Though meaningful resistance was not noticed in present investigation, results show that in future, *T. absoluta* may develop resistance to most of the insecticides which are being used by the farmers. Therefore focus on educating the farmers and practicing IPM and Insecticide Resistance Management (IRM) strategies is required for management of this invasive pest.

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