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Levels of insecticide resistance in *Phthorimaea absoluta* (Meyrick) populations collected from South India

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ABSTRACT: *Phthorimaea absoluta* (Meyrick) has become a serious threat to global tomato production. Management of an invasive pest mainly relies on insecticides because of immediate effect. In this study, susceptibility of second instar larvae of *P. absoluta* collected from different locations of south India to seven insecticides belonging to different chemical groups was evaluated to determine the lethal concentration (LC) values. Leaf dip bioassay method revealed that the LC_{50} values in *P. absoluta* varied to different tested insecticides among different populations collected from south India. The median lethal concentrations of all the insecticides against *P. absoluta* ranged from 1.4 to 106.0 ppm. Among tested insecticides, chlorantraniliprole showed more toxicity to second instar larvae of *P. absoluta* which recorded lowest LC_{50} values, followed by cyantraniliprole, spinosad, indoxacarb, flubendiamide and the least susceptibility of *P. absoluta* larvae were recorded to neonicotinoids (acetamiprid and imidacloprid). Regarding resistance ratio, maximum of 7.8-fold resistance to acetamiprid was recorded when compared to remaining six insecticides.

Keywords: *Phthorimaea absoluta*, median lethal concentration, chlorantraniliprole, cyantraniliprole, spinosad, indoxacarb, flubendiamide, acetamiprid, imidacloprid

INTRODUCTION

Insect pest invasions have been rapidly increasing worldwide and with increased movement of people and goods from one country to another, there are high chances for increased numbers of invasive species conquering many regions (Pimentel *et al.,* 2001). These invasive agricultural pest species are widely recognized as a major threat to agro-ecosystems and agricultural production. One such invasive species that have been wreaking havoc to tomato crop worldwide is tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) which is also commonly called as tomato borer or tomato pinworm (Campos *et al.,* 2014a). *T. absoluta* native to South America and was first described in Peru in 1917 as *Phthorimaea absoluta* (Meyrick, 1917) (Desneux *et al.,* 2010). This has other synonyms: *Scrobipalpuloides absoluta* (Povolny, 1987), *Scrobipalpula absoluta* (Povolny, 1964) and (EPPO 2005). *Tuta absoluta* again was renamed as *Phthorimaea absoluta* based on cladistic analysis of morphology (Chang and Metz, 2021)

Phthorimaea absoluta has become a serious threat to global tomato production. In India, incidence of this pest was first recorded at Indian Institute of Horticultural Research (IIHR), Hesaraghatta, Bengaluru (Sridhar *et al.,* 2014) Maharashtra (Shashank *et al.,* 2015) and Malnad region of Karnataka (Kalleshwaraswamy *et. al.,* 2015). Its invasion to India has led to major economic impact on tomato production as its infestation starts from nursery stage of the crop itself and when the infestation pressure is high, besides attacking vegetative stage of the crop, infestation is observed both on developing and mature fruits. Due to its distinctive feeding behavior (larvae mine in the leaf mesophyll forming irregular, transparent mines) makes it a challenging insect pest to manage (Kandil *et al.,* 2020).

While managing invasive pests, insecticides are often the first choice. Though, insecticides are one of the important components in IPM program, indiscriminate and continuous use of same insecticides could lead to development of resistance in insect pests and may also become harmful to the natural enemies of insect pest. Hence, there is a need to generate information on resistance levels of an invasive pest, *P. absoluta* over a broad geographical range of population, which provides information regarding natural variation in identifying those insecticides where resistance is more likely to develop (Kumar *et al.,* 2020).and also will be helpful in developing Insecticide Resistance Management (IRM) strategies. Therefore, the present study was carried out to know the level of resistance to commonly used insecticides *viz.,* chlorantraniliprole, cyantraniliprole, flubendiamide, spinosad, indoxacarb, acetamiprid and imidacloprid which were tested against field collected population of *P. absoluta* from Karnataka, Tamil Nadu, Maharashtra and Andhra Pradesh.

MATERIALS AND METHODS

Insecticides

Commercial formulations of regularly used insecticides for the management of tomato leafminer belonging to different chemical groups with various modes of action as defined by the Insecticide Resistance Action Committee (IRAC) were selected and purchased from local market for bioassay studies *viz.,* chlorantraniliprole (coragen 18.5 SC, DuPont India Ltd. Hyderabad), cyantraniliprole (benevia 10.26 OD, DuPont India Ltd. Hyderabad), flubendiamide (fame 39.35 SC, Bayer crop science Mumbai), indoxacarb (kingdox 14.5 SC, Gharda chemicals Ltd. Mumbai), spinosad (tracer 45 SC, Dow Agro Science India Ltd. Mumbai), acetamiprid (pride 20 SP, Syngenta India Ltd. Mumbai) and imidacloprid (confidor 17.8 SL, Bayer crop science Mumbai) were used in bioassay studies.

Maintenance of insect culture and host plant in the laboratory

Insect population

Population for this study were collected from ten districts representing four states of South India *viz.,* Karnataka (Davanagere, Chamarajnagar, Chikkamagaluru and Raichur), Maharashtra (Kolhapur and Solapur), Tamil Nadu (Coimbatore and Madurai) and Andhra Pradesh (Chittoor and Kurnool). From each sampling site, tomato leaves infested with different instars of *P. absoluta* larvae were collected into large plastic bags (45 cm X 30 cm). Approximately 100-200 larvae were collected from each site. The samples were transferred into a plastic box and covered with muslin cloth to avoid stress on insects. The plastic boxes containing the infested leaves with larvae were opened in insect-proof rearing cages containing fresh tomato seedlings, so that larvae could resume normal development. The rearing cages were maintained at 25 ± 2.5 °C, 65% RH and 16:8 h light: dark photoperiod. The second instar larvae of F_1 generation were used for insecticidal bioassay and the experiment was carried out at Toxicology Laboratory, Department of Agricultural Entomology, College of Agriculture, Shivamogga.

Rearing of *P. absoluta* in laboratory

The field collected *P. absoluta* populations were maintained and reared on healthy tomato leaves separately in boxes with 25 ± 2.5 °C room temperature and 65 ± 5% relative humidity, fully mined leaves were replaced with fresh tomato leaves to the larvae until pupation. The pupae were collected from the tray and placed them in insect breeding boxes and the box was kept in adult emergence cage. Newly emerged adults were provided with 10 per cent honey solution with cotton swab. Thirty-day old tomato seedlings grown in small pots were kept in the adult emergence cage for oviposition and number of eggs laid were observed visually. If an adequate number of eggs were observed (*i.e.,* more than 150–200 eggs/plant), then the plant was carefully removed and new plants were placed in the oviposition area to allow continuation of the oviposition. The seedlings with eggs were kept in separate cages and observed for hatching. The hatched larvae maintained by providing fresh seedlings and the culture was maintained continuously in laboratory and insect proof net house without any insecticide application. Field populations from different locations were collected from unsprayed fields and reared separately for one generation and F_1 populations were used for toxicity studies. Second instar larvae were used for laboratory experiments.

Host Plant material

Tomato plants (JKTH 811- hybrid widely cultivated in university jurisdiction) were maintained in pest free insect-proof cages under laboratory conditions and in polyhouse. No insecticides were used during the plant development phase.

Bioassay method

Laboratory bioassay of insecticides on *P. absoluta* was carried out as per the IRAC test method No. 022. Median lethal concentrations (LC_{50} and LC_{90} values) for these insecticides were estimated through leaf dip bioassay method. Accurate dilutions of the different insecticides from the identified commercial product were prepared. Prior to bioassays, bracketing was done for every insecticide to fix concentrations causing approximately 10 to 90 per cent mortality of *P. absoluta* larvae. Initially stock solutions of maximum concentration were prepared and serially diluted to obtain desired concentrations. Bioassay for every insecticide was conducted at five concentrations in geometric progression and the experiment was conducted under laboratory conditions. Each insecticide concentration was replicated thrice. The use of a wetter/spreader (non-ionic adjuvant) is highly recommended in order to obtain optimal leaf coverage and hence in all cases, the selected surfactant triton X 100 agent was used in the study.

Tender young tomato leaves of uniform size were collected from 30 days seedlings grown in the polyhouse were used for the bioassay. Leaflets were individually dipped in each insecticide concentration for three to five seconds with gentle agitation, ensuring that the entire surface is treated equally. Then the treated leaflets were dried with the leaf axial surface facing upwards at room temperature for 15 min on moist tissue paper cut to fit the insect breeding box. The control leaves were dipped in the distilled water without insecticide. Second-instar *P. absoluta* larvae were carefully removed from the galleries in infested tomato leaves maintained in the cage and starved for a period of four hour. It was also ensured that larvae never been under starvation stress during the study. Twenty second-instar larvae were released for each concentration by using a fine soft brush on the treated leaf in the box as previously described and the box lid was immediately fitted to prevent larvae from escaping. Three boxes were used served as replication for each treatment, resulting in a total of 45 to 60 larvae per concentration. All treatments were placed under laboratory conditions (26 ± 2 ◦ C, 50-60% RH, 16:8 h L:D). A moist cotton plug was attached at the cut end of the leaf and the leaf was then placed in a transparent insect breeding box (9 cm). The same procedure was followed for all the doses and for all the insecticides, starting with the "untreated" control then followed by the more diluted dose and advancing progressively to the higher concentrations. The mortality was assessed after 24 h of exposure at different intervals. The data on final values of mortality (LC_{50}) were represented at 72 hrs. Larvae were recorded as moribund if no coordinated movement or deficient response to external stimulus was observed and were considered dead if they failed to right-back themselves when turned upside down with a brush. Mortality was estimated from the total number of dead and moribund insects. If insect vitality could not be clearly determined (live or moribund), the larvae were carefully extracted from the leaf to observe the responses when undistracted by the leaf epidermis.

Data analysis

The mortality data were corrected by the Abbott's formula (Abbott 1925) and subjected to Probit analysis using the statistical program SPSS version 16.0 software (IBM SPSS, Armonk, New York, USA) to obtain the LC_{50}, fiducial limits (95%), slopes and Chi-square values.

Relative potency

The LC_{50} of the susceptible population among the field collcted population was used for the calculation of relative potency for each insecticide. In the absence of a characterised susceptible reference strain, results are compared with those of the most susceptible population *i.e.,* the population which has a least value of LC_{50} assumed as susceptible population for each insecticide. Relative potency ratio was thus estimated to know the potency of the active ingredients used in the study by the formula LC_{50} of the least susceptible population divided by the LC_{50} of the most susceptible population (Deshmukh *et al.,* 2020).

RESULTS AND DISCUSSION

Bioassay of insecticides on *P. absoluta* collected from different locations of South India. Susceptibility to different insecticides having different mode of action among field collected tomato leaf miner populations during 2017-19 from ten locations from different districts of Karnataka, Maharashtra, Tamil Nadu and Andhra Pradesh was studied by following leaf dip bioassay method. Per cent mortality of *P. absoluta* larvae was checked from 24 h up to 72 h after treatment. The insecticides had a substantial difference in larval mortality at 72 h after treatment. Hence, final values are represented for 72 h after treatment. The ranges of LC_{50} and LC_{99} values calculated for *P. absoluta* populations from various locations are presented in Table 1. The results indicated that populations of *P. absoluta* among ten locations had variable responses to tested insecticides in this experiment.

The slopes of the dose response curves of the tested populations were high and varied among *P. absoluta* populations, but they were not extremely broad. In all cases, the slopes for insecticides were more than two, presumably indicating greater homogeneity/uniformity among *P. absoluta* populations collected from different locations. In response to diamide exposure, the tested populations showed highest slopes. For chlorantraniliprole the slopes ranged from 1.9 to 2.6 with an average of 2.2 (SE = 0.21). The slope values for cyantraniliprole among different population ranged from 1.7 to 2.6. High slopes of the response line to flubendiamide were observed, ranging from 2.2 to 3.6 and resulting in an average of 2.6 (SE = 0.2).

The calculated LC_{50} value of *P. absoluta* for chlorantraniliprole from different locations ranged from 1.4 to 4.7 ppm at 72 h after treatment. Resistance ratio (RR) varied from 1.1-fold to 3.3-fold, which might be considered within the natural variability range or developing low resistance (Table 1). For cyantraniliprole the LC_{50} ranged from 2.7 ppm to 9.2 ppm and resistance ratio (RR) varied from 1.5-fold to 3.4-fold showing a fairly homogeneous response among the populations. Madurai

Table 1. Relative toxicity of insecticides against field population of *Phthorimaea absoluta* from different locations by leaf dip bioassay

Population	n	LC_{50} (ppm)	Fiducial limits (95%)	LC_{95} (ppm)	Fiducial limits (95%)	Slope ± SE	Chi-square	RR ratio	Recommended field dose (ppm)
					Chlorantraniliprole				
Davanagere	360	1.4	1.0-1.9	6.9	4.3-17.3	2.4 ± 0.2	3.0	-	55
Chikkamagaluru	360	1.6	1.3-1.9	8.8	6.3-14.5	2.2 ± 0.2	1.6	1.1	55
Madurai	270	1.7	1.4-2.1	11.0	7.5-19.6	2.0 ± 0.2	2.2	1.2	55
Chittoor	360	1.8	1.5-2.3	13.4	8.8-25.9	1.9 ± 0.2	2.9	1.2	55
Coimbatore	270	2.3	1.9-2.8	14.5	10.0-25.7	2.0 ± 0.2	2.2	1.6	55
Solapur	360	2.4	1.9-2.9	16.6	11.0-30.8	1.9 ± 0.2	2.5	1.7	55
Chamarajanagar	360	2.5	2.1-3.0	10.7	7.5-18.8	2.6 ± 0.3	2.5	1.7	55
Raichur	270	2.6	1.8-3.9	15.3	8.3-65.0	2.1 ± 0.2	4.3	1.8	55
Kurnool	270	3.2	2.7-3.9	17.5	12.5-28.5	2.2 ± 0.2	1.5	2.2	55
Kolhapur	360	4.7	4.0-5.6	21.2	15.8-32.3	2.5 ± 0.2	2.0	3.3	55
					Cyantraniliprole				
Madurai	270	2.7	2.3-3.3	16.3	11.5-27.4	2.1 ± 0.2	2.9	-	184
Kurnool	270	4.1	2.7-6.0	19.6	11.0-85.6	2.4 ± 0.2	5.5	1.5	184
Coimbatore	270	4.2	3.0-5.9	21.7	12.8-66.1	2.3 ±0.2	3.8	1.5	184
Raichur	360	5.4	3.2-9.0	30.3	15.3-198.2	2.2 ±0 .2	6.9	2.0	184
Solapur	360	5.5	4.6-6.7	32.6	22.9-54.7	2.1 ± 0.2	2.9	2.0	184
Chikkamagaluru	270	6.2	4.4-9.2	46.6	24.4-181.9	1.8 ± 0.2	3.2	2.2	184
Chamarajanagar	360	6.3	5.3-7.5	28.2	20.9-43,5	2.5 ± 0.2	1.9	2.3	184
Davanagere	360	6.6	3.5-10.5	27.9	15.4-215.5	2.6 ± 0.3	7.7	2.4	184
Kolhapur	270	8.2	6.8-9.9	49.8	34.8-84.1	2.1 ± 0.2	2.7	3.0	184
Chittoor	270	9.2	5.6-16.2	85.9	36.6-834.8	1.7 ± 0.2	4.9	3.4	184
					Flubendiamide				
Madurai	270	2.3	1.7-3.0	6.6	4.6-12.7	3.6 ± 0.3	4.1	-	100
Solapur	270	2.6	1.8-3.6	10.9	6.8-28.0	2.6 ± 0.2	4.3	1.1	100
Coimbatore	270	2.7	1.9-3.8	9.5	6.1-23.8	3.0 ±0.2	5.4	1.1	100
Kurnool	270	2.9	1.7-4.9	15.4	7.7-110.0	2.2 ± 0.2	7.5	1.2	100
Davanagere	360	5.1	4.3-6.0	21.6	16.5-31.6	2.6 ±0.2	2.9	2.2	100
Chittoor	270	8.1	6.0-10.9	29.0	19.2-63.5	2.9 ±0 .2	4.2	3.5	100
Chamarajanagar	360	9.0	6.5-12.4	40.2	25.0-102.8	2.5 ±0.2	3.7	3.9	100
Chikkamagaluru	360	11.0	6.5-18.4	50.0	26.6-309.4	2.5 ±0.2	8.3	4.7	100
Raichur	360	11.3	9.6-13.3	44.1	33.8-64.9	2.7 ±0.3	2.4	4.9	100
Kolhapur	270	16.2	6.7-35.7	86.6	38.2-4087.8	2.2 ± 0.2	13.1	7.0	100
					Indoxacarb				
Solapur	270	2.8	1.9-4.1	20.0	10.5-78.0	1.9 ± 0.2	3.5	-	145
Coimbatore	270	3.8	3.0-4.8	40.9	24.8-89.7	1.6 ±0.1	2.7	1.3	145
Kurnool	270	4.6	2.6-8.4	25.0	12.1-236.6	2.2 ± 0.2	9.3	1.6	145
Chittoor	270	5.2	3.5-7.8	54.6	26.0-269.9	1.6 ±0.1	3.0	1.8	145
Raichur	360	5.4	3.2-9.0	54.3	24.2-417.4	1.6 ±0.1	4.9	1.9	145
Chikkamagaluru	360	6.1	4.1-9.6	56.0	26.1-319.2	1.1 ±0.1	3.7	2.1	145
Davanagere	360	6.5	3.8-11.3	75.7	30.9-847.6	1.5 ±0.1	4.4	2.3	145
Chamarajanagar	270	6.6	3.9-11.7	103.5	38.2-1481.6	1.3 ± 0.1	3.9	2.3	145
Madurai	270	7.7	6.2-9.8	82.3	49.8-183.6	1.6 ± 0.1	2.8	2.7	145
Kolhapur	270	12.2	8.5-18.7	109.3	53.0-524.1	1.7 ± 0.2	3.3	4.3	145

Spinosad									
Madurai	270	2.1	1.2-3.4	13.4	6.9-75.8	2.0 ± 0.2	6.0	1	144
Kurnool	270	2.6	2.2-3.1	14.5	10.4-23.5	2.2 ± 0.2	1.3	1.2	144
Solapur	270	3.1	1.9-5.3	17.3	8.5-128.7	2.0 ± 0.2	6.6	1.4	144
Chittoor	270	3.9	2.7-5.4	18.2	11.0-55.5	2.4 ± 0.2	4.3	1.8	144
Coimbatore	270	4.7	3.9-5.6	27.0	19.2-44.3	2.1 ± 0.2	1.4	2.2	144
Davanagere	360	5.1	4.4-6.0	21.4	16.4-31.2	2.6 ± 0.2	2.7	2.4	144
Chikkamagaluru	360	5.4	4.5-6.4	28.2	20.4-44.9	2.2 ± 0.2	1.2	2.5	144
Kolhapur	270	6.6	5.5-7.9	36.9	26.4-60.1	2.2 ± 0.2	1.5	3.1	144
Chamarajanagar	360	8.2	6.6-10.2	68.4	44.0-135.1	1.7 ± 0.2	1.0	3.9	144
Raichur	270	9.3	6.7-12.6	51.6	31.6-129.1	2.2 ± 0.2	3.1	4.4	144
Acetamaprid									
Solapur	270	2.9	2.0-4.2	14.2	8.2-43.6	2.3 ± 0.2	4.6	1.0	200
Kurnool	360	6.1	4.1-9.6	56.0	26.1-319.2	1.7 ± 0.1	3.7	2.1	200
Kolhapur	270	7.0	5.8-8.6	45.6	30.6-83.8	2.0 ± 0.2	1.0	2.4	200
Chittoor	270	13.7	11.3-16.8	89.3	59.9-163.	2.0 ± 0.2	1.2	4.7	200
Coimbatore	270	14.2	11.7-17.4	86.8	58.6-158.7	2.0 ± 0.2	1.4	4.8	200
Chamarajanagar	270	14.3	11.9-17.4	91.6	62.8-161.0	2.0 ± 0.2	1.8	4.9	200
Raichur	360	16.6	10.-27.3	135.2	6.0-979.0	1.8 ± 0.2	4.3	5.7	200
Madurai	270	17.2	14.2-21.0	111.7	74.9-204.3	2.0 ± 0.2	1.3	5.9	200
Chikkamagaluru	360	17.4	14.5-21.2	105.8	72.1-188.1	2.1 ± 0.2	1.5	6.0	200
Davanagere	270	22.9	13.9-49.7	121.3	53.8-2518.9	2.7 ± 0.2	7.7	7.8	200
Imidacloprid									
Kurnool	270	26.4	22.0-31.7	149.1	106.5-243.9	2.1 ± 0.2	1.6	-	60
Coimbatore	270	53.0	44.2-63.5	297.6	212.6-486.0	2.1 ± 0.2	1.5	2.0	60
Solapur	360	60.4	49.4-74.5	453.4	296.4-867.8	1.8 ± 0.2	2.8	2.2	60
Raichur	360	62.9	52.9-74.2	294.9	222.9-437.7	2.4 ±0 .2	2.7	2.3	60
Chikkamagaluru	360	66.2	55.2-79.3	372.2	265.9-608.1	2.1 ± 0.2	1.5	2.5	60
Davanagere	360	71.4	60.2-84.7	347.8	255.4-543.0	2.3 ± 0 .2	1.2	2.7	60
Madurai	270	76.8	64.4-91.3	403.1	292.6-643.1	2.3 ± 0.2	1.8	2.9	60
Chamarajanagar	360	81.2	67.1-98.3	515.4	356.6-889.2	2.0 ± 0.2	0.2	3.0	60
Chittoor	270	92.7	77.3-111.1	521.4	372.4-852.0	2.1 ± 0.2	1.5	3.5	60
Kolhapur	270	106.0	88.4-127.0	595.2	425.2-972.1	2.1 ±0 .2	1.5	4.0	60

*n = sample size

*LC_{50} - Lethal concentration which kills 50% of exposed population expressed in parts per million (ppm)

*LC_{95} - Lethal concentration which kills 95% of exposed population expressed in parts per million (ppm)

* SE = Standard Error

*Relative Resistance ratio (RR) = LC_{50} of the least susceptible population /LC_{50} of the most susceptible population

population was most susceptible to cyantraniliprole with LC_{50} value of 2.7 ppm. Population from Kolhapur showed reduced susceptibility to flubendiamide (16. 2 ppm), whereas Madurai population were most susceptible with LC_{50} value of 2.3 ppm. Resistance ratios (RR_{50}) varied from 1.1-fold to 7-fold, indicating slightly more variation among populations than for chlorantraniliprole and cyantraniliprole (Table 1).

The slope values for tomato leaf miner population collected from different location when tested against indoxacarb ranged between 1.1 and 2.2 with an average of 1.61 (SE = 0.1) (Table 1). The most susceptible population among ten different locations to indoxacarb was from Solapur with an LC_{50} value of 2.8 ppm followed by Coimbatore (3.8 ppm), Kurnool (4.6 ppm), Chittoor (5.2 ppm), Raichur (5.4 ppm), Chikkamagaluru (6.1 ppm), Davanagere (6.5 ppm), Chamarajanagar (6.6 ppm) and Madurai (7.7 ppm), while the most tolerant strain of *P. absoluta* to indoxacarb was Kolhapur population (12.2 ppm) resulting in a four-fold increase in the resistance ratio (Table 1).

Indoxacarb affects insects from direct exposure and through ingestion of treated foliage/fruit which leads to immediate feeding cessation. It kills pests by binding to a site on sodium channels and blocking the flow of sodium ions into nerve cells. The result is impaired nerve function, feeding cessation, paralysis, and death. It may take days for insects to die (Brugger, 1997).

The slope of response line to spinosad ranged from 1.7 to 2.6 with an average of 2.16 (SE = 0.2). The LC_{50} value for *P. absoluta* population from different location ranged from 2.1 to 9.3 ppm. Raichur population showed least susceptibility to spinosad with an LC_{50} value of 9.3 ppm resulting in four-fold resistance to the insecticide. Madurai population had an LC_{50} value of 2.1 ppm indicating most susceptibility to Spinosad. To this insecticide LC_{95} values ranged from 13.4 to 51.6 ppm which was lower than the recommended field dose (Table 1).

Spinosad has very low toxicity for mammals, birds, insect predators and the greatest effect of spinosad is on the Lepidoptera, Diptera, Thysanoptera, Coleoptera and Orthoptera (Toews *et al.,* 2003). Spinosad has two unique modes of action, acting primarily on the insect nervous system at the nicotinic acetylcholine receptor and exhibiting activity at the GABA receptor (Watson *et al.,* 2010).

Acetamiprid and imidacloprid belonging to neonicotinoid group were selected to examine the toxicity of these insecticides against second instar larvae of *P. absoluta* population collected from different locations from Karnataka, Maharashtra, Tamil Nadu and Andhra Pradesh.

The slopes of acetamiprid varied from 1.7 to 2.7 with an average of 2.06 (SE = 1.9) (Table 1). Median lethal concentration values tested to acetamiprid among *P. absoluta* population of different locations ranged from 2.9 to 22.9 ppm resulting in eight-fold resistance, highest resistance ratio among all insecticides tested. Solapur field strains showed more susceptibility to acetamiprid with an LC_{50} value of 2.9 ppm followed by Kurnool (6.1 ppm), Kolhapur (7.0 ppm), Chittoor (13.7 ppm), Coimbatore (14.2 ppm), Chamarajanagar (14.3 ppm), Raichur (16.6 ppm), Madurai (17.2 ppm) and Chikkamagaluru (17.4 ppm). Davanagere population showed least susceptibility to tested insecticide with an LC_{50} value of 22.9 ppm (Table 1).

With respect to another neonicotinoid *i.e.,* imidacloprid, the slope of dose response line ranged from 1.8 to 2.4 with an average of 2.13 (SE = 0.2). Among all tested insecticides so far against *P. absoluta* population from various locations, imidacloprid exhibited least toxicity for tomato leafminer with an LC_{50} value ranging from 26.4 to 106.0 ppm resulting in four-fold difference in resistance among different population. Kurnool population were more susceptible with LC_{50} value of 26.4 ppm and the most tolerant one was recorded from Kolhapur (LC_{50} 106.0 ppm) indicating less susceptibility to imidacloprid when compared to other populations and also LC_{95} values of all the populations (149.1 to 595.2 ppm) exceeded the recommended field dose (60 ppm) (Table 1).

Insecticides belonging to different group like diamides, oxadiazines, spinosyns and neonicotinoids having different mode of action exhibited varied level of toxicity across the populations collected from different locations of Karnataka, Maharashtra, Tamil Nadu and Andhra Pradesh. Among diamides, *viz.,* flubendiamide, chlorantraniliprole, cyantraniliprole, variation in relative toxicity against *P. absoluta* populations were observed. The present study revealed that *P. absoluta* population from different locations had high susceptibility to chlorantraniliprole (LC_{50} values 1.4 to 4.7 ppm) followed by cyantraniliprole (LC_{50} values 2.7 to 9.2 ppm) and flubendiamide (LC_{50} values 2.3 to 16.2 ppm). These results are in conformity with findings of Kumar *et al.* (2020) that LC_{50} values of chlorantraniliprole and flubendiamide to *P. absoluta* population from five districts of Tamil Nadu ranged between 0.27 to 0.60 ppm and 1.01 to 2.25 ppm respectively, indicating lower LC_{95} values than the recommended label rate for both chlorantraniliprole and flubendiamide, suggesting that the particular diamide insecticides would provide the expected control of *P. absoluta* infestation. Similar results were also recorded by Prasannakumar *et al.* (2020) LC_{50} values for cyantraniliprole ranged from 10.0 (Bangalore population) to 29.4 ppm (Anantapur population) and for flubendiamide it was 5.1 (Kolar population) to 32.3 ppm (Anantapur population) indicating that, to both the insecticides Anantapur population showed reduced susceptibility compared to populations from other location. which necessitates the judicious use of chemicals for its management. Therefore, regular monitoring the susceptibility of different population exposed to distinct active ingredients is essential. Similarly, Roditakis *et al.* (2012) reported that *P. absoluta* population from Greece showed susceptibility to both chlorantranilliprole and flubendiamide with an LC_{50} values ranging between 0.12 to 0.53 mg/L and 0.31 to 1.31 mg/L respectively. The LC_{50} values varied from 3.17 to 29.64 µg/L and 94 to 230 µg/L for chlorantraniliprole and flubendiamide with nine-fold and three-fold resistance ratios (Campos *et al*., 2014b).

Results of present study and also reports of Kumar *et al.,* 2020 and Prasannakumar *et al.* (2020) from India have demonstrated that development of resistance to diamides have not been reported in India as compared to native place of this pest, resistance ratio ranged between two to ten-fold, which indicates low level of resistance status in *P. absoluta* populations from different location of South India. From our study, it can be concluded that, this pest with 8 to 10 generations per year, combined with wide spread and intensive use of flubendiamide and chlorantraniliprole to control *P. absoluta,* may end up with development of resistance in a relatively shorter period of time in the field where populations are large and selection pressures can be much higher than in the laboratory and also due to continuous spraying of over dosages of insecticides, ignorance or a lack of concern in dealing with usages of insecticides. (Guillemaud *et al*., 2015). Also, inter-regional differences with respect to median lethal concentrations among tested populations may be due to insecticide use patterns wherein during initial days of introduction of this pest, majority of farmers were unaware about nature of damage, economic importance and early identification of the pest and also certain farmers had abandoned infested fields for cattle grazing and hence incurred complete crop loss. Hence, alternative management approaches, such as the preservation of natural enemies, the destruction of crop remains and the application of economic injury levels, would help to delay diamide resistance. More crucially, farmers should be encouraged to adopt insecticide rotation, which is likely one of the most feasible strategies. Silva *et al.* (2016a) found that the majority of *P. absoluta* are susceptible to spinosyns, which might be utilised as a substitute for diamides. Additionally, absence of cross-resistance between spinosyns and diamides have been demonstrated by Campos *et al.* (2014a) which suggests an interesting alternative for managing resistance for this pest and even molecules such as abamectin, chlorfenapyr, indoxacarb, and metaflumizone, may be used as alternative chemicals to manage *P. absoluta* populations.

With respect oxadiazines insecticide indoxacarb, present study revealed that there was variation in median lethal concentration values of *P. absoluta* populations from different locations of South India wherein LC_{50} values ranged between 2.8 (Solapur population) to 7.7 ppm (Madurai population) with a resistance ratio of 1.3-to-4.3-fold resistance. These results are in conformity with the findings of Silva *et al.* (2016) that the LC_{50} values varied among eight field populations of Brazil to indoxacarb, wherein LC_{50} value varied from 0.92 (Tiangu) to 2.89 (Pelotas) mg/L, with resistance ratio values between 1.1 and 3.3 times when compared with susceptible population LC_{50} value which was 0.86 mg /L from Iraquara, showing that all the populations are susceptible to this insecticide. Also, LC_{50} values to indoxacarb was found to be 17.5 ppm, which indicated that Brazilian population is more resistant to indoxacarb than Indian population of *P. absoluta*, implying that the pest was first detected in Brazil before being introduced to India. However, the LC_{95} was always lower than the recommended label rate and no cases of control failures were detected (Roditakis *et al.,* 2012). Variability in responses to indoxacarb have also been reported by Kumar *et al.* (2020) in populations from Tamil Nadu, with LC_{50} ranging from 0.82 to 6.38 ppm resulting in an eight-fold difference; Prasannakumar *et al.* (2020) in populations from Bangalore, Kolar, Madurai, Salem, Coimbatore and Anantapur wherein Kolar (22.8 ppm), Salem (16.00 ppm) and Anantapur (33.21 ppm) population showed reduced susceptibility to indoxacarb than the susceptible population (11.13 ppm).

P. absoluta population exhibited variation in LC_{50} when they were treated with spinosad insecticide with Madurai population showing more susceptibility (LC_{50} value of 2.1 ppm) and Raichur population showing least susceptibility (LC_{50} value of 9.3 ppm). Similar results were observed when relative toxicity of spinosad against second instar larvae of *P. absoluta* from Urmia city in Iran was evaluated by Hosseinzadeh *et al.* (2019). LC_{50} values at 72h after treatment indicated second instar larva was more susceptible to spinosad (1453.0 ppm) but the LC_{50} values recorded in this study are higher than those observed in our results. This is due to the fact that pest was noticed for the first time in the year 2010 in Iran earlier than India and probably pesticide usage pattern and availability of host and number of generations per year may be the reason for having higher LC_{50} values. *P. absoluta* population from nine distinct locations in Greece showed LC_{50} value ranging from 0.08 to 0.26 ppm, resulting in a three-fold difference and also high slopes of the response line to spinosad were observed, ranging from 1.45 to 2.28 and resulting in an average of 1.83 (Roditakis *et al.,* 2012). LC_{50} values were lower than present results which may be due to less cropping area, temperate country and low pesticide usage intensity.

Among seven insecticides tested against *P.* absoluta in the present study Neonicotinoids (imidacloprid and acetamiprid) were found to be least toxic to the test insect with LC_{50} values ranging from 2. 9 to 22.9 ppm for acetamiprid and 26.4 to 106.0 ppm for imidacloprid. These results are in line with findings of Sallam *et al.* (2015) who have repored that imidacloprid was inferior