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Article

Monitoring the current status of insecticide resistance of field and greenhouse-collected populations of tomato leaf miner *Tuta absoluta Meyrick* (Lepidoptera: *Gelechiidae*) in Egypt

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Abstract: In order to track changes in developments and the degree of insecticide resistance, some common insecticides used to control *T. absoluta* in Egyptian fields were examined for seasonal variations in resistance. Tests were designed and tested for a few years at various field sample and greenhouse locations. Ten pesticides were selected for testing, and insects were sampled from eight agricultural fields for the laboratory bioassay. The identification and measurement of resistance in various field populations, seasons, and greenhouses revealed that insects were highly vulnerable to the IGR insecticide tested and only mildly susceptible to OP, Py, carbamate, and nicotinoids. Kalubia and Menufia showed more resistance in terms of both distribution and intensity than Dakahlia, Sharkia, and Giza, which showed lower resistance levels, possibly due to less effective insecticide applications. However, lufenuron was the most effective insecticide, followed by chlorfenapyr (IGRs), methoxyfenzoid, chlorfluazuron, indoxacarb, imidacloprid, cypermethrin, and chlorpyrifos. Dimethoate and spinoteram were the least effective insecticides.

Keywords: insecticide resistance; *Tuta absoluta*; seasonal variation; Egyptian governorates

1. Introduction

The capacity of an insect to withstand exposure to the appropriate dosage of a pesticide due to environmental adaptation brought about by physiological and behavioral traits is known as insecticide resistance, according to Taskin et al., Tayeb et al. and Mansour et al. [1–3]. The tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is primarily responsible for the greatest levels of tomato pine worm population infection in Egypt. This insect is now well-known for limiting tomato production globally and for requiring quarantines in North America and Asia. In a short period of time, the *T. absoluta* outbreak significantly damaged the economy. Various plant components, including leaves, flowers, buds, stems, and fruits, are attacked by larvae [4–6].

In order to protect the environment and the economy, the primary management strategy has historically involved the use of natural enemies or insecticides derived from natural compounds [7]. Accordingly, the most challenging situations include when growers lose valuable crops like tomatoes due to control failures caused by pest resistance to certain insecticide applications [8–13]. Therefore, the lesson from this circumstance is that, in addition to previously studied population dynamics, it is necessary to investigate the factor of sustainable progress in pest control programs, as well as levels of pesticide resistance and insecticide efficiency. In order to support decision-making processes and planning preparations, it is important to periodically monitor the intensity of the insecticides commonly used against this species in cultivated areas. This is because rapid selection pressure can result in high levels of resistance and the potential for spread to new areas [14]. Insecticide susceptibility testing encompasses a wide range of assays and designs, primarily conducted in laboratories with optimal standardization aspects or as field efficacy techniques under varying seasonal conditions. Diploid, locus definition, heterozygosity, or phenotypic resistance detection using standardized bioassays through evaluating the proportion of mortality in precise populations of area survey (monitoring and prevalence) are urgently needed in addition to survival problem encounters.

Outstandingly treatments preparations and organization by certain diagnostic concentrations that predefined using susceptible strain bioassay under controlled laboratory conditions is a distinguishable implement [15]. Performing susceptibility test led to data vary over time because of environmental factors and geographic distance that prevent gene flow over variation in insecticide exposure [16]. The role of the susceptibility tests is the bioassay for resistance including many classes of insecticide as conventional insecticide-resistance survey provide evidence of resistance genotype and phenotype existence in a population with long exposure history to those insecticides [17]. The newer insecticide in bioassay reveals recent information about changes in population changes in resistance allele addition in a specific population, which can be used to predict levels of control failure and classify resistance extent by mortality scoring. An important factor for the efficient monitoring of resistance dynamics in space and time is the large number of monitoring sites at many times. In order to determine fluctuation levels and count the frequency of resistance in numerous cultivated sites from the south and north of Egypt governorate at four seasons, as well as to learn more about the availability of insecticide compounds that are still effective and able to control this pest, *T. absoluta* from field and greenhouse population samples were tested in this study for the development of insecticide resistance to the most widely recommended and available insecticides using a straightforward laboratory bioassay method.

2. Materials and methods

2.1. Insect sampling and rearing

In August of 2015 and 2016, samples of approximately 100 tomato plant leaves infested with *T. absoluta* were randomly collected from all plant parts in cultivated open fields of Kalubia, Dakahlia, Sharkia, and Menufia. These samples annually represent the maximum population total of insect infestation. Tomato leaves are collected annually at a consistent frequency and are carefully transported to the lab for use in biological tests in plastic bags. Similar to the first collection, 100 infected leaves per site from the Giza Governorate’s commercial tomato greenhouses at the Dokki-Giza tomato production station that were severely infested with *T. absoluta* were gathered in August of 2015, 2016, 2017, 2018, 2020, and 2023. The samples were moved to the lab and maintained in rearing rooms with lab conditions at 25 ℃ ± 2 ℃, 65% ± 5% R.H., and a photoperiod of 16 L: 8 D. The samples in bags contained *T. absoluta* larvae in various developmental stages inside mines, as well as larvae extracted from leaves and used in the bioassay. Typically, larvae feed on the original leaves, and if necessary, bull larvae from the old leaves replace them with clean ones.

The leaves of tomato plants are kept inside large glass containers until the pupae emerge, and the pupae are then kept in a clean container until the adult emerges. This susceptible strain was developed from tomato seedlings that were repeatedly infected by *T. absoluta* during the nursery stage and were not exposed to pesticides. The source of the strain was the greenhouse nursery of the Giza agriculture workstation for tomato production.

2.2. Insecticides used

Organophosphates (OP) insecticides were used in the biological assay study. They included dimethoate (Ares 40% EC), chlorpyrifos (Dora 48% EC), carbamates as indoxacarb (Pret 15% EC), nematodes as imidacloprid (Imidachem 35% SP), spinoteram (Radient 20% SC), and IGRs as lufenuron (Match 5% EC), chlorfluazuron (Efcoron 5% SC), methoxyfenzoid (Runner 24% SC), and chlorfenapyr (Exmite 24% SC). For the purpose of conducting pest bioassay studies and product variety analysis studies, the Central Agricultural Pesticide Laboratory received all products, which originated in China.

2.3. Insecticide bioassays

Leaf dip method bioassays, as reported by Reyes et al. [18]. Tomato leaves that were not infected were individually dipped for five seconds to a fine coating in freshly made insecticide solutions, then allowed to dry. Only the controls dipped in water. Then, each treated leaf was placed in a 9-diameter petri dish. Subsequently, 10 larvae in their third instar were placed in each dish containing three replicates and five concentrations. The conditions were carefully monitored at 25 ℃ ± 2 ℃ and 16:8 hours of light to dark. Mortality was noted 24 h following the application of conventional insecticides and 48 hours following the application of insect growth regulator insecticides. When a larva could not move normally, it was deemed dead. Following concentration bioassay, resistance is monitored at various governorates at various times (Both temporally and spatially) through the computation of resistance intensity and phenotypic resistance.

2.4. Resistance detection and quantifications

For resistance frequency and intensity calculation, the diagnostic concentration method of contract percent of population mortality when treated by the unique susceptible 90% mortality concentration predefined that refer to resistance status and the proper phenotypic resistance, with mortality classification as susceptible if mortality were ≥ 98%, possible resistance if mortality were 90%–97% and established resistance if mortality were = 90% [17,19]. In order to identify low, moderate, and high resistance intensity locations using the same insecticides over a 4-year period, the percentage of mortality of exposure to 1 × 0 point 5 × or 10 × of the discriminating concentration was recorded for 8 sites for representative samples in four field and numerous greenhouse samples. This method was used to quantify the intensity of resistance that mean spreads in all places of sample collections.

2.5. Statistical analysis

According to Finney [20], toxicity results obtained by computing $LC_{50,90}$ with confidence intervals, slope, and mortality correction using formula were incorporated into the statistical data of the Polo software program [21,22]. Using data from Robertson and Preisler [23], the resistance ratio (RR) is computed by dividing the LC_{50} of the susceptible population by the LC_{50} of the insecticide treatment at the appropriate site and time. ANOVA significant differences test computations under all conditions, along with multiple linear regression analysis, are used for monitoring differentiation across seasons and sites using SPSS v20 software. Tables and additional statistical analysis produced by an Excel spreadsheet.

3. Results and discussion

3.1. Insecticide susceptibility results

The distribution and trends of insecticide resistance in *T. absoluta* were investigated. Through tests of variation within and between seasons and regions, absolute and the continuous development situation were finished. The bioassay results' toxicity parameters, such as the LC_{50} and LC_{90} in ppm and the confidence intervals, are shown in **Tables 1–4** for the samples that were taken in greenhouses and open fields. Additionally, **Tables 5** and **6** contained slope values along with standard error for every test. Results from bioassays indicate the amount of insecticide needed to kill half of all insects tested in a population under study as well as the populations' susceptibility to these pesticides; the lower the number, the more effective the method. Seasons and sample sites tested in this data showed such variation and fluctuation. According to the results, lufenuron and chlorfluazuron were the most effective compounds in open field samples from 2015 and 2016 (**Tables 1** and **3**) and greenhouse samples from 2015, 2016, 2017, 2018, 2020, and 2023 (**Tables 2** and **4**).

However, in all open field and greenhouse sample tests, chlorpyrifos was the most ineffective, followed by spinoteram and dimethoate. If the LC_{50} does not meet the recommended field dose mentioned on the insecticide Powel label, the insecticide will typically maintain its effectiveness. Based on these findings, it can be said that the insecticide classes that lost efficiency were OP and Py, while the insecticide class that remained effective was IGRs, and resistance to these compounds was extremely uncommon. Data on the history of applying these common classes of chemical compounds to control this pest at the locations where samples were collected was either unavailable or insufficient. The variations between sampling times and sample locations displayed in **Figures 1** and **2** demonstrated that Menufia was more resistant than other species and that chlorpyrifos was useless against *T.absoluta* during the course of the inquiry. According to the ANOVA results, the coefficient of variation (Cv) was 0.655, and there were no significant differences between the insecticide groups at the level of the various seasons and locations (F = 22, df = 136, P = 0.0). However, Cv was 1.14 between sample seasons (F = 6.9, df = 59, p = 1.34) and between sample locations (F = 1.04, df = 134, p = 0.41). Furthermore, all cases were accepted, and Adjusted R-Squared were all around 0.99. The multiple linear regression *p* value ranged between 0.396 and 0.81, the *t* stat from −0.8 to 0.70, and the CV from −1.2 to 0.94. Of these, $F = 121$, df = 7, $p = 0.069$ and H0 (5 percent) were also included.

Table 1. Responses (LC_{50}) and confidence intervals of open field *T. absoluta* larvae tested in 2015 and 2016 by some insecticides.

Insecticide	LC_{50} Fields of 2015				LC_{50} Fields of 2016			
	Dakahlia	**Sharkia**	**Kalubia**	**Menufia**	**Dakahlia**	**Sharkia**	**Kalubia**	**Menufia**
Dimethoate	2.6 (0.9–7.8)	7 (4.6–11.4)	9 (5.5–14.3)	16.3 (8.7–27)	14.3 (8.7–23.7)	15.26 (9.3–25)	12.5 (6.5–24)	8.2 (5–13.7)
Chlorpyrifos	11.9 (7–19.7)	14 (8.8–22)	15.5 (9.3–26)	16.5 (8.6–32)	14.3 (8.3–24.3)	12.3 (7.5–22)	13.8 (8–23.6)	12.9 (7.5–22)
Imidacloprid	3.3 (1.9–5.6)	3.8 (2.3–6.4)	6.3 (4–9.8)	7 (4.3–12)	8.9 (5–15.7)	8.3 (4.7–14.7)	9.9 (6–17)	7.8 (4.4–13.7)
Cypermethrin	2.3 (1.2–4.5)	4.9 (3.2–7.7)	4.1 (2.4–7)	5.7 (3–11)	3.8 (2.2–6.8)	5.2 (3.5–9.2)	5 (2.8–8.8)	4.5 (2.5–8)
Spinoteram	8 (5.3–12.7)	7 (4.2–11.6)	15 (10.3–23)	16 (10.8–24)	9.6 (5.3–17.6)	10 (5.5–18.3)	11.3 (6.2–21)	12 (6.4–21.5)
Chlorfenapyr	1.3 (0.7–2.3)	1.1 (0.54–0.2)	1.7 (0.9–3.3)	2.3 (1.3–4.1)	3. (1.6–5.6)	3.7 (2.5–7.0)	2.8 (1.5–5.2)	2.3 (1.2–4.4)
Indoxacarb	2.0 (1.0–4)	2.4 (1.3–4.8)	4.4 (2.3–8.4)	3.2 (1.7–5.7)	2.4 (1.2–4.4)	3.8 (2–7.3)	3 (1.6–5.7)	2.5 (1.4–4.8)
Lufenuron	0.27 (0.2–0.4)	0.27 (0.16–0.5)	0.2 (0.14–0.4)	0.24 (0.2–0.4)	0.62 (0.34–1)	0.59 (0.33–1)	0.57 (0.3–1.1)	0.59 (0.33–1)
Chlorfluazuron	0.67 (0.39–1)	0.55 (0.3–1)	0.9 (0.6–1.4)	0.83 (0.53–1)	2 (1.2–3.4)	1.4 (0.83–2.3)	2.5 (1.5–4.2)	2.6 (1.5–4.4)
Methoxyfenzoid	2.9 (1.8–4.6)	3.0 (1.9–5)	2.7 (1.6–4.6)	10.2 (6.5–16)	2.7 (1.7–4.3)	11 (7–17.4)	9.4 (6–14.7)	8.4 (5.3–13)

Table 2. Responses (LC50) and confidence intervals of greenhouses *T. absoluta* larvae tested from 2015 to 2023 to some insecticides.

Insecticide	Giza 2015	Giza 2016	Giza 2017	Giza 2018	Giza 2020	Giza 2023
Dimethoate	10.3 (5.9–18)	8.4 (5.4–13)	3.5 (1.5–8)	7.9 (5.2–11.9)	17 (11–28)	10.7 (6.7–17)
Chlorpyrifos	26 (15–43.7)	11.8 (6.5–21)	27 (16–46)	12 (6.4–24.7)	24 (15–38)	28.4 (18–45)
Imidacloprid	8.8 (6–13.6)	15 (8.3–27)	6.4 (3.9–10)	12 (6–23.5)	5.5 (3.4–9)	4.9 (3–8)
Cypermethrin	5.8 (3–10.5)	7 (3.5–14.5)	7.3 (3.3–16)	6.9 (4–12)	6.6 (4–10)	5 (3–8)
Spinoteram	22 (15–32.6)	19.7 (13–30)	17 (13–25)	18 (12–26)	0	0
Chlorfenapyr	3.3 (2–5.4)	0.8 (0.35–1.8)	2.4 (1.3–4)	3.3 (1.9–5)	3.7 (2.3–6)	4.5 (3–7.3)
Indoxacarb	2.9 (1.4–5.9)	2.7 (1.2–6)	3.9 (2.3–7)	2.7 (2–5)	4 (2.4–7)	5 (3–8.7)
Lufenuron	0.26 (0.1–0.43)	0.27 (0.16–0.5)	0.17 (0.1–0.3)	0.19 (0.1–0.4)	0.77 (0.47–1.25)	0.96 (0.58–1.57)
Chlorfluazuron	0.94 (0.6–1.5)	0.99 (0.64–2)	0.84 (0.5–2)	0.61 (0.32–1)	0	0
Methoxyfenzoid	3.6 (2–6.3)	3.8 (2.3–6.6)	3.0 (1.5–6)	4 (2.3–7.3)	0	0

These findings align with certain literature that highlights the effectiveness of specific insecticides against *T. absoluta* during the period of this investigation, achieving moderately favorable results. For instance, Larraín et al. [24] reported that the toxicity of cyantraniliprole resulted in a reduction of fruit damage ranging from 75% to 85% with foliar applications and 82% with soil applications. In the Behaira Governorate of Egypt, Tayeb et al. [2] discovered that insecticide sprays applied at 10-day intervals were highly effective when used individually at field doses. However, combinations of chlorantraniliprole with thiamethoxam, or lufenuron with thiamethoxam at half the recommended dose, achieved a complete reduction of 100%.

Additionally, recent advancements in insecticide alternatives, such as Nano-formulated materials, have emerged as a novel technological approach in the field of plant protection, demonstrating high mortality rates against larvae and eggs, as noted by De Smedt et al. [25].

Table 3. Responses (LC_{90}) and confidence intervals of open field *T. absoluta* larvae tested in 2015 and 2016 by some insecticides.

Insecticide	**LC_{90} Fields of 2015**				**LC_{90} Fields of 2016**			
	Dakahlia	**Sharkia**	**Kalubia**	**Menufia**	**Dakahlia**	**Sharkia**	**Kalubia**	**Menufia**
Dimethoate	38 (33–299)	27.5 (17.4–43.4)	39 (24–62)	101.2 (57.4–179)	71.4 (43.4–117.3)	76.5 (46.5–126)	114 (59.5–219.4)	40 (24–66.4)
Chlorpyrifos	53.3 (32–88)	25.5 (33.4–82)	79.4 (47.5–133)	146.4 (77–279)	74.6 (43.6–127.5)	60.5 (35.4–103.5)	69.7 (40.5–119)	64.6 (38–110.4)
Imidacloprid	17.8 (10–30)	20 (12–34)	26 (17–41.2)	38 (23–62.7)	51.5 (30–90)	41.6 (23.7–73)	67 (38–117.3)	39 (22.3–68.5)
Cypermethrin	17.8 (9–34.8)	19.5 (12.5–30)	22 (13–37.7)	48.4 (26–90)	20.5 (11.6–36.3)	36.4 (20.6–64.3)	33.4 (19–59)	26.5 (15–47)
Spinoteram	29 (18.4–46)	30 (18–50.4)	50 (33.7–73)	54.7 (37–81.3)	60.8 (33–111)	70.55 (39–128.6)	83.2 (45.6–152)	91.3 (50–166.5)
Chlorfenapyr	6.7 (3.7–12.3)	0.3 (4.5–19)	13.4 (7–25.8)	15 (8.4–27)	22.7 (15–51.6)	60 (32.3–112)	22.6 (12–42.2)	16.3 (8.8–30.4)
Indoxacarb	13.9 (6.8–28.6)	15.9 (8.1–31)	34.5 (18–66)	16.7 (9.2–30.3)	15.4 (8.2–29)	53 (28–100)	24.7 (13.1–46.5)	17.8 (9.4–33.4)
Lufenuron	1 (0.66–1.6)	1.7 (0.96–2.96	0.8 (0.5–1.8)	0.84 (0.54–1.3)	4.8 (2.7–8)	6 (3.4–11)	4 (2.2–7.2)	4.4 (2.4–8)
Chlorfluazuron	3 (1.8–5)	3 (1.6–5.3)	3.2 (2–5)	2.9 (1.9–4.6)	9.3 (5.5–15.6)	5.6 (3.3–9.4)	13.6 (8–23)	14.6 (8.7–24.7)
Methoxyfenzoid	10 (6.4–16)	14.4 (8.6–24)	50.4 (32–79)	9.7 (6–16)	12 (7–20)	80 (51–125.5)	40.4 (26–63.4)	32 (20.4–50.4)

Table 4. Responses (LC_{90}) and confidence intervals of greenhouses *T. absoluta* larvae tested from 2015 to 2023 to some insecticides.

Insecticide	**Giza 2015**	**Giza 2016**	**Giza 2017**	**Giza 2018**	**Giza 2020**	**Giza 2023**
Dimethoate	63.5 (37–110)	31.3 (20–48.8)	44.7 (20–100)	25.5 (16.9–38.7)	78 (49–124)	35.2 (22–56)
Chlorpyrifos	157 (92–266)	76.6 (42.4–138)	173 (101–295.6)	124 (61–249)	106 (67–167)	212 (135–334)
Imidacloprid	50 (32.5–77.5)	111.6 (62–200)	30 (18.8–48)	121 (63–232)	26 (16–42)	20.4 (13–32.6)
Cypermethrin	43.7 (24–79)	84.8 (42–171)	126 (56.6–279.6)	46 (26.4–80)	30 (19–47.3)	18 (11.3–28)
Spinoteram	76.6 (52–113)	73 (48.4–110)	52.5 (36–76)	59 (40–87)	0	0
Chlorfenapyr	17 (10–28)	8.8 (3.9–20)	15.3 (8.6–27)	15 (9–24.5)	19 (11.5–30.4)	40 (24.4–64.5)
Indoxacarb	23.9 (11.9–48)	33.7 (15–75.6)	19 (11–32.8)	20 (10.8–36.5)	24.4 (14.3–12)	47 (27.4–80)
Lufenuron	1.3 (0.78–2.3)	1.4 (0.85–2.4)	1.125 (0.6–2.1)	1.5 (0.79–2.8)	3.9 (2.4–6.4)	10.5 (6.4–17)
Chlorfluazuron	3.8 (2.4–6.1)	3.6 (2.3–5.7)	4.78 (2.7–8.3)	4.1 (2.2–7.8)	0	0
Methoxyfenzoid	20 (11.5–34)	20 (12–34.5)	20 (11–36.5)	26 (15–46)	0	0

The application of bioinsecticides, such as those discussed by Nozad-Bonab et al. [11] and Mikhail et al. [26], including Bacillus thuringiensis (Bt), Beauveria, and

Metarhizium, remains effective when compared to conventional insecticides like spinosad, abamectin, indoxacarb, imidacloprid, and chlorantraniliprole. Therefore, implementing insecticide rotations can help mitigate resistance, and utilizing alternatives with varying modes of action in appropriate regions may prove beneficial, as noted by Jin et al. [27]. Overall, the susceptibility status of this pest can potentially be managed through the use of novel chemical compounds or bioactive agents. Furthermore, it is essential to conduct periodic surveillance at the same locations to assess the baseline susceptibility levels of resistance and to monitor any changes in resistance levels and frequency over time, which is crucial for prompt intervention to prevent the spread of resistance, as highlighted by Devillers et al. [28].

Tables 5 and **6** shows the slope values of the toxicity lines for each test. The values, which varied from 0.81 to 2.3, indicate the homogeneity or heterogeneity status of the populations that were tested. The insects that were tested here demonstrated that while some seasons and locations had the steepest line, indicating high susceptibility, others tended to be resistant to certain pesticides. On the one hand, the steepest toxicity line indicates the homogeneity of the sample individuals, while shallow lines (dose response relationships) indicate excessive heterogeneity of the tested sample. Individuals in heterogeneous populations have varying toxicity effects and appear to contain more or less resistant individuals than susceptible ones, whereas homogeneous populations have equal insecticide effects or are adjacent to one another [29].

Table 5. Slope values and standard errors for toxicity lines in all north governorate tests.

Insecticide	**(Slope) Fields of 2015**				**(Slope) Fields of 2016**			
	Dakahlia	**Sharkia**	**Kalubia**	**Menufia**	**Dakahlia**	**Sharkia**	**Kalubia**	**Menufia**
Dimethoate	0.81 + 0.84	2.2 + 0.11	2.0 + 0.10	1.56 ± 0.126	1.85 ± 0.11	1.85 ± 0.11	1.35 ± 0.145	1.8 + 0.11
Chlorpyrifos	1.9 + 0.11	2.2 + 0.11	1.8 + 0.11	1.3 + 0.14	1.83 ± 0.119	1.8 ± 0.117	1.82 ± 0.11	1.81 ± 0.11
Imidacloprid	1.7 ± 0.11	1.78 ± 0.12	2.0 − 0.09	1.79 − 0.11	1.69 ± 0.124	1.69 ± 0.012	1.69 ± 0.12	1.99 ± 0.12
Cypermethrin	1.4 ± 0.14	2.2 ± 0.09	1.75 − 0.11	1.39 − 0.137	1.56 ± 0.121	1.55 ± 0.1	1.56 ± 0.121	1.52 ± 0.11
Spinoteram	2.3 ± 0.10	2.0 ± 0.11	2.5 − 0.086	2.4 − 0.088	1.74 ± 0.13	1.2 ± 0.12	1.32 ± 0.12	1.72 ± 0.13
Chlorfenapyr	1.7 ± 0.13	1.3 ± 0.15	1.43 − 0.14	1.57 − 0.13	1.38 ± 0.12	1.42 ± 0.123	1.4 ± 0.12	1.4 ± 0.134
Indoxacarb	1.52 ± 0.15	1.6 ± 0.148	1.4 ± 0.14	1.78 ± 0.13	1.23 ± 0.14	1.4 ± 0.126	1.38 ± 0.104	1.39 ± 0.123
Lufenuron	2.27 ± 0.09	1.63 ± 0.12	2.3 ± 0.10	2.4 ± 0.42	1.44 ± 1.31	1.36 ± 0.11	1.51 ± 0.13	1.49 ± 0.123
Chlorfluazuron	1.97 ± 1.1	1.8 ± 0.13	2.3 ± 0.09	2.3 ± 0.1	1.2 ± 0.13	1.14 ± 0.13	1.75 ± 0.11	1.57 ± 0.15
Methoxyfenzoid	2.4 ± 0.1	2.0 ± 0.11	0.189 ± 0.13	2.3 ± 0.10	2.0 ± 0.11	2.0 ± 0.162	2.0 ± 0.11	1.99 ± 0.12

Table 6. Slope values and standard errors for toxicity lines in all Giza tests.

Insecticide	**G2015**	**G2016**	**G2017**	**G2018**	**G2020**	**G2023**
Dimethoate	1.6 ± 0.12	2.2 ± 0.09	1.1 ± 0.18	2.5 ± 0.09	1.94 ± 0.103	1.91 ± 0.13
Chlorpyrifos	1.6 ± 0.11	1.58 ± 0.13	1.6 ± 0.12	1.2 ± 0.15	2.0 ± 0.10	2.1 ± 0.12
Imidacloprid	2.2 ± 0.09	1.4 ± 0.12	1.9 ± 0.104	1.3 ± 0.14	1.94 ± 0.101	1.81 ± 0.14
Cypermethrin	1.4 ± 0.13	1.2 ± 0.15	1.0 ± 0.17	1.5 ± 0.12	2.0 ± 0.102	0.19 ± 0.103
Spinoteram	2.4 ± 0.08	2.2 ± 0.09	2.6 ± 0.08	2.4 ± 0.08	0	0
Chlorfenapyr	1.84 ± 0.1	1.2 ± 0.18	1.5 ± 0.13	1.88 ± 0.108	1.88 ± 0.108	0.19 ± 0.11

Table 6. (*Continued*).

Insecticide	**G2015**	**G2016**	**G2017**	**G2018**	**G2020**	**G2023**
Indoxacarb	1.4 ± 0.15	1.16 ± 0.179	1.9 ± 0.12	1.5 ± 0.13	1.72 ± 0.118	1.88 ± 0.12
Lufenuron	1.89 ± 0.1	1.78 ± 0.1	1.56 ± 0.138	1.45 ± 0.14	1.86 ± 0.109	1.76 ± 0.82
Chlorfluazuron	2.1 ± 0.1	2.3 ± 0.097	1.7 ± 0.12	1.5 ± 0.14	0	0
Methoxyfenzoid	1.7 ± 0.12	1.78 ± 0.11	1.56 ± 0.13	1.6 ± 0.12	0	0

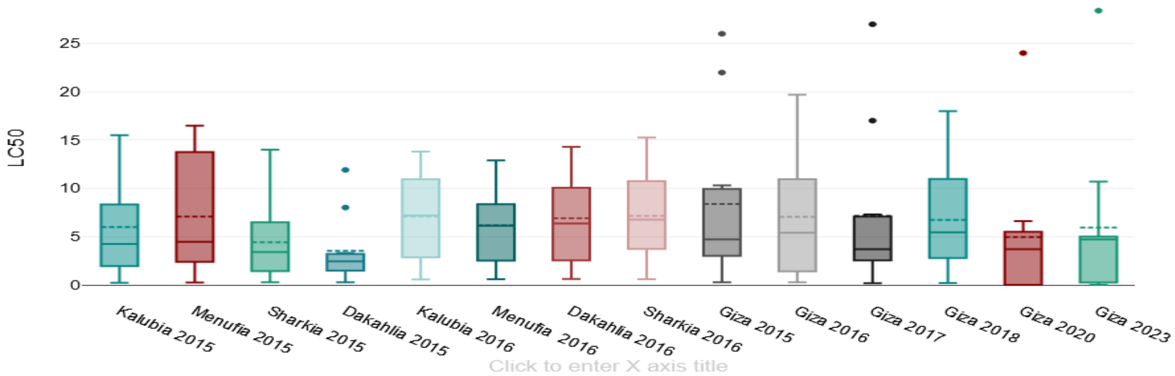

Figure 1. Toxicity responses (LC_{50}) for each open fields and greenhouse larvae tested to all insecticide under investigations. The figure showed the amount of LC_{50} of each governorate to facilitate differentiation between places; the higher value is the higher resistant place to the most insecticide application was the Menufia 2015 samples.

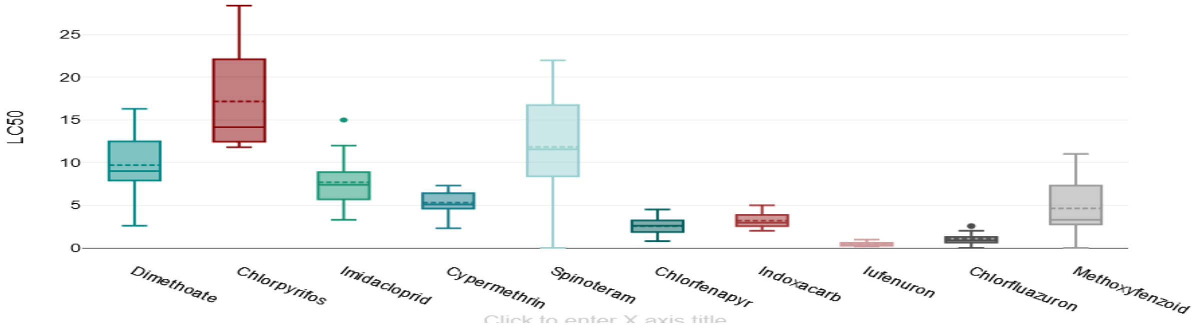

Figure 2. Toxicity responses (LC_{50}) for each insecticide individually. The figure showed the high value is the high control failure possibility of Chlorpyrifos insecticide.

3.2. Resistance ratio for all established cases

Using LC_{50} and LC_{90}, the resistance ratio (RR) was determined for open field data in **Tables 7** and **8** and greenhouse data in **Tables 9** and **10** and **Figures 3** and **4**. Data indicated that greenhouse samples had a lower RR (fold of resistance value) than open field samples, indicating that they were less resistant to the majority of the tested insecticides. For greenhouses, it varied from 46.5 to 1.69, while varied from 70.86 to 2.24 for open field samples. The values for dimethoate in Menufia 2015 and 2016 were generally higher than those for spinoteram in all collection sites at open fields and greenhouses, respectively. Indoxacarb ranged from 13.5 to 25 and from 12.0 to 28.97 fold, respectively, after chlorpyrifos. Furthermore, the lower values for lufenuron and chlofluazuron ranged from 1.69 to 9.7 fold and from 3.8 to 6.12 fold, respectively.

By that time, it was convenient to see that resistance to all insecticides was steadily declining. In contrast to the first season of 2015, the open field RR data for

2018 was lower. Kalubia is thought to have a higher overriding insecticide content. Lietti et al. [30] achieved similar outcomes. Where two greenhouses used topical applications and some insecticide toxicity on susceptible larvae. Deltamethrin and abamectin were detected after *T. absoluta* displayed RR > 68.38 fold of resistance for deltamethrin, 3.49 for abamectin, and 0.86 for methamidophos. Campos et al. [12] discovered in 2014 that spinosad resistance selections had increased by more than 180,000 times in *T. absoluta* over seven generations in Brazil.

Table 7. Resistance ratio at LC_{50} detected for field larvae to the tested insecticides for two seasons of the north governorate**.**

Insecticide	**2015**				**2016**			
	Kalubia	**Menufia**	**Sharkia**	**Dakahlia**	**Kalubia**	**Dakahlia**	**Sharkia**	**Menufia**
Dimethoate	39.13	70.86	30.43	11.30	54.34	35.65	62.17	66.34
Chlorpyrifos	15.81	16.83	14.28	12.14	14.08	13.16	14.59	12.55
Imidacloprid	7.97	8.86	4.81	4.17	12.53	9.87	11.26	10.50
Cypermethrin	11.38	15.83	13.61	6.38	13.88	12.50	10.55	14.44
Spinoteram	51.72	55.17	24.13	27.58	38.96	40.68	33.10	34.48
Chlorfenapyr	4.47	6.05	2.89	3.42	7.36	6.05	7.89	9.73
Indoxacarb	22.00	16.00	12.00	10.00	15.00	12.50	12.00	19.00
Lufenuron	2.24	2.44	2.75	2.75	5.81	6.02	6.32	6.02
Chlorfluazuron	5.62	5.18	3.43	4.18	15.62	16.25	12.50	8.75
Methoxyfenzoid	7.50	7.50	8.33	8.05	26.11	23.33	28.33	30.55

Table 8. Resistance ratio at LC_{50} detected for the tested insecticides for all seasons of Giza as south governorate**.**

Insecticide	**G2015**	**G2016**	**G2017**	**G2018**	**G2020**	**G2023**
Dimethoate	44.78	36.52	15.21	34.34	73.91	46.52
Chlorpyrifos	26.53	12.04	27.55	12.24	24.48	28.97
Imidacloprid	11.13	18.98	8.10	15.18	6.96	6.20
Cypermethrin	16.11	19.44	20.27	19.16	18.33	13.88
Spinoteram	75.86	67.93	58.62	62.06	0.00	0.00
Chlorfenapyr	8.68	2.10	6.31	7.89	9.73	11.84
Indoxacarb	14.50	13.50	19.50	13.50	20.00	25.00
Lufenuron	2.65	2.75	1.69	1.93	7.85	9.79
Chlorfluazuron	5.87	6.18	5.25	3.81	0.00	0.00
Methoxyfenzoid	10.00	10.55	8.33	11.11	0.00	0.00

Table 9. Resistance ratio at LC_{50} detected for field larvae to the tested insecticides for two seasons of the north governorate**.**

Insecticide	2015				2016			
	Kalubia	**Menufia**	**Sharkia**	**Dakahlia**	**Kalubia**	**Dakahlia**	**Sharkia**	**Menufia**
Dimethoate	165.22	119.57	169.57	434.78	310.43	332.61	495.65	173.91
Chlorpyrifos	54.39	26.02	81.02	148.98	75.51	61.73	71.43	65.31
Imidacloprid	22.53	25.32	32.91	48.10	64.56	52.66	84.81	49.37
Cypermethrin	49.44	54.17	61.11	134.44	56.94	101.67	91.67	72.22
Spinoteram	100.00	103.45	172.41	187.59	209.66	243.10	286.21	313.79
Chlorfenapyr	17.63	0.79	35.26	42.11	59.74	157.89	59.47	42.89
Indoxacarb	66.50	79.50	170.00	83.50	77.00	265.00	123.50	85.00
Lufenuron	10.20	17.35	89.80	9.08	48.98	61.22	40.82	44.90
Chlorfluazuron	18.75	18.75	20.00	18.13	58.13	35.00	85.00	91.25
Methoxyfenzoid	27.78	40.00	140.00	26.94	33.33	222.22	111.11	89.72

Table 10. Resistance ratio at LC_{50} detected for the tested insecticides for all seasons of Giza as south governorate**.**

Insecticide	G2015	G2016	G2017	G2018	G2020	G2023
Dimethoate	276.09	136.09	194.35	110.87	339.13	154.35
Chlorpyrifos	160.20	78.16	176.53	126.53	108.16	216.33
Imidacloprid	63.29	141.27	37.97	153.16	32.91	25.82
Cypermethrin	121.39	235.56	350.00	127.78	83.33	50.00
Spinoteram	264.14	251.72	183.79	203.45	0.00	0.00
Chlorfenapyr	44.74	23.16	40.26	39.47	50.00	105.26
Indoxacarb	119.50	168.50	90.00	100.00	122.00	235.00
Lufenuron	13.27	14.29	11.48	15.31	39.80	107.14
Chlorfluazuron	23.75	22.50	29.88	25.63	0.00	0.00
Methoxyfenzoid	55.56	55.56	55.56	72.22	0.00	0.00

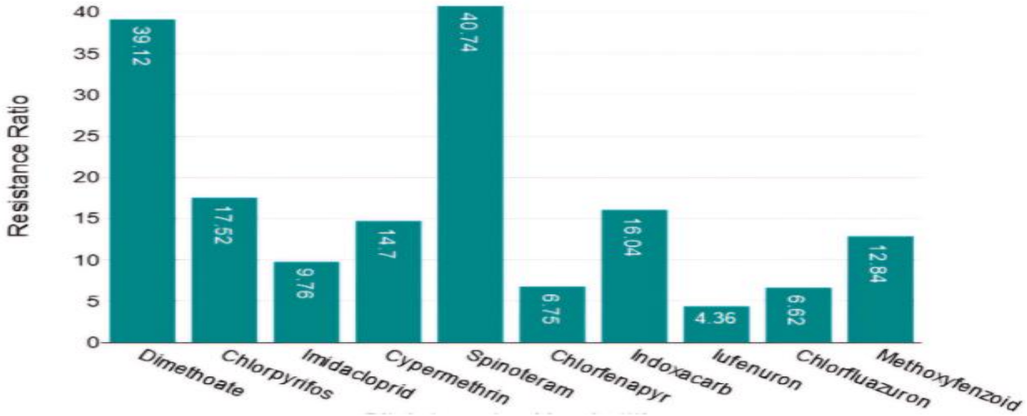

Figure 3. Resistance ratio detected for tested insecticides after subsequent seasons of *T. absoluta* attacking tomato, the figure showed that dimethoate and spinoteram was the higher fold of resistance, while lufenuron was the lower followed by chlorfluazuron, chlorfenapyr and imidacloprid.

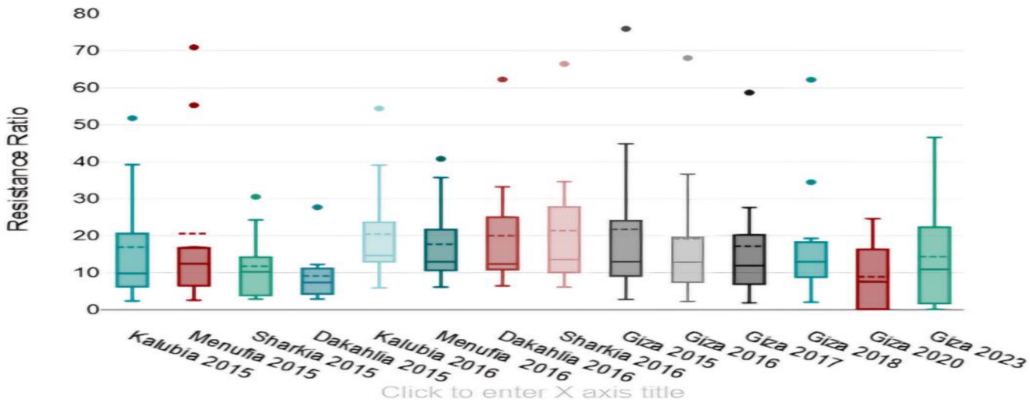

Figure 4. Resistance ratio detected for the tested regions (Governorates) of tomato production. Figure showed higher values was for Sharkia 2016 followed by Dakahlia 2016, and the lower values was for Dakahlia 2015 samples.

3.3. Resistance frequency over time

Tables 11 and **12** and **Figures 5** and **6** contained all of the insecticide resistance detection frequency data, demonstrating the phenotypic resistance status of larvae treated with the appropriate insecticide discriminating concentration that corresponds to 90% mortality of the susceptible population. It separates the larvae based on its mortality percentage, taking into account whether the mortality was ≥ 98%, > 90%, or = 90% to be susceptible, mildly resistant, and actually resistant, respectively. **Table 13**, which shows the mortality percentage of discriminative concentration equal to 1x of susceptible LC_{90} treatment. Mortality percentages for 29 out of 140 treatments (22 from IGRs and the remaining occasionally for dimethoate, imidacloprid, and indoxacarb) are greater than 90%, indicating mild resistance and status; the remaining treatments have percentages ranging from 8 to 88.7, indicating actual resistance phenotype and severe resistance. Additionally, this bioassay can be used to determine the frequency of resistance, measure variation within and between regions being studied, and demonstrate the severity of resistance development in collection sites.

Table 11. The status of frequency of resistance to selected insecticides represent the severity of resistant alleles in *T. absoluta* individuals in Kalubia, Menufia, Sharkia and Dakahlia fields during 2015 and 2016.

Insecticide	**2015**				**2016**			
	Kalubia	**Menufia**	**Sharkia**	**Dakahlia**	**Kalubia**	**Dakahlia**	**Sharkia**	**Menufia**
Dimethoate	1.79	1.63	1.39	0.52	2.49	1.63	1.39	0.52
Chlorpyrifos	1.04	1.1	0.94	0.8	0.92	0.86	0.94	0.8
Imidacloprid	0.65	0.72	0.39	0.34	1.01	0.8	0.39	0.34
Cypermethrin	1.7	2.36	2.03	0.95	2.07	1.86	2.03	0.95
Spinoteram	4.28	4.56	2.0	2.28	3.22	3.37	2.0	2.28
Chlorfenapyr	0.39	0.53	0.25	0.3	0.64	0.53	0.25	0.3
Indoxacarb	0.99	0.72	0.54	0.45	0.68	0.56	0.54	0.45
Lufenuron	0.19	0.21	0.23	0.23	0.49	0.51	0.23	0.23
Chlorfluazuron	0.73	0.67	0.45	0.54	2.03	2.11	0.45	0.54
Methoxyfenzoid	0.66	0.66	0.73	0.7	2.28	2.04	0.73	0.7

Table 12. The status of frequency of resistance to selected insecticides represent severity of resistant alleles in *T. absoluta* individuals in Giza greenhouses from 2015 to 2023.

Insecticide	**G2015**	**G2016**	**G2017**	**G2018**	**G2020**	**G2023**
Dimethoate	2.05	1.67	0.70	1.57	3.39	2.13
Chlorpyrifos	1.74	0.79	1.80	0.80	1.60	1.90
Imidacloprid	0.90	1.54	0.66	1.23	0.56	0.50
Cypermethrin	2.40	2.90	3.02	2.86	2.73	2.07
Spinoteram	6.28	5.62	4.85	5.14	0.00	0.00
Chlorfenapyr	0.76	0.18	0.55	0.69	0.85	1.03
Indoxacarb	0.65	0.61	0.88	0.61	0.90	1.13
Lufenuron	0.22	0.23	0.14	0.16	0.66	0.82
Chlorfluazuron	0.76	0.80	0.68	0.50	0.00	0.00
Methoxyfenzoid	0.87	0.92	0.73	0.97	0.00	0.00

Table 13. Mortality percentage of discriminative concentration equal 1x of susceptible LC_{90} treatment for tested insecticides at subsequent seasons and tested field sites of *T. absoluta* investigation, that considered resistance quantification as a final result lead to take a decision.

Insecticide	**Kalubia 2015**	**Menufia 2015**	**Sharkia 2015**	**Dakahlia 2015**	**Kalubia 2016**	**Menufia 2016**	**Dakahlia 2016**	**Sharkia 2016**	**Giza 2015**	**Giza 2016**	**Giza 2017**	**Giza 2018**	**Giza 2020**	**Giza 2023**
Dimethoate	27.9	15.4	35.9	96.6	20.1	30.6	17.6	16.5	24.4	29.9	71.7	31.8	14.8	23.5
Chlorpyrifos	48.3	45.4	53.5	62.9	54.2	58	52.3	60.8	28.8	63.4	27.7	62.4	31.2	26.4
Imidacloprid	77.4	69.7	128.4	147.8	49.3	62.6	54.8	58.8	55.4	32.5	76.2	40.7	88.7	99.6
Cypermethrin	29.5	21.2	24.7	52.5	24.2	26.8	31.8	23.2	20.8	17.3	16.5	17.5	18.3	24.2
Spinoteram	11.7	11	25	21.9	15.5	14.9	18.3	17.5	8	8.9	10.3	9.7	0	0
Chlorfenapyr	128.2	94.7	198.1	167.6	77.8	94.7	72.6	58.9	66	272.4	90.8	72.6	58.9	48.4
Indoxacarb	50.4	69.3	92.4	110.8	73.9	88.7	92.4	58.3	76.4	82.1	56.8	82.1	55.4	44.3
lufenuron	264.8	242.8	215.8	215.8	102.2	98.8	94	98.8	224.1	215.8	351	306.6	75.7	60.7
Chlorfluazuron	68.4	74.2	112	91.9	24.6	23.7	30.8	44	65.5	62.2	73.3	100.9	0	0
Methoxyfenzoid	76.3	76.3	68.7	71.1	21.9	24.5	20.2	18.7	57.2	54.2	68.7	51.5	0	0

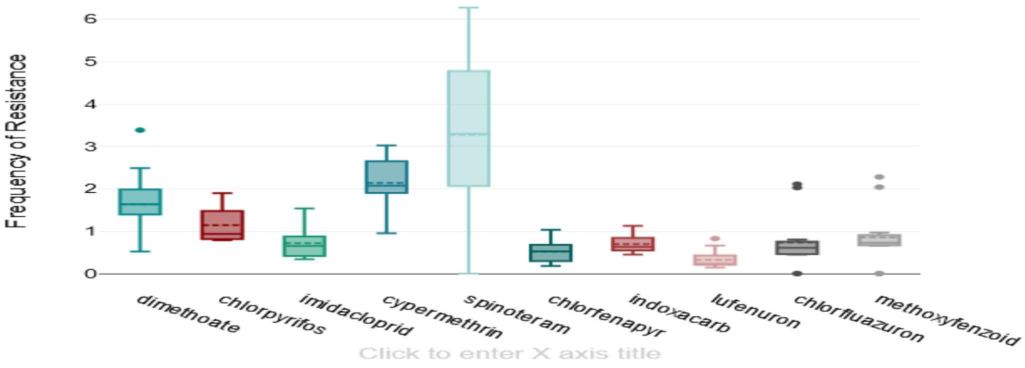

Figure 5. Resistance frequency calculated for insecticide bioassays of *T. absoluta*, and showed how strongest insecticide resistance existence or spreads in fields and greenhouses populations was Spinoteram followed by cypermethrin insecticide.

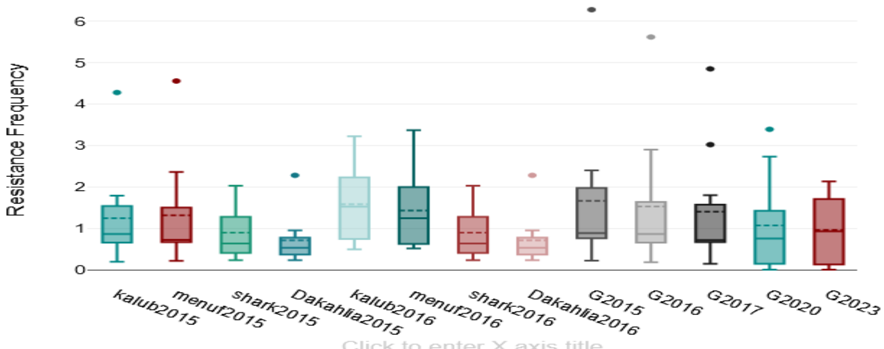

Figure 6. Resistance frequency calculated for insecticide bioassays of *T. absoluta*, and showed the most resistant place and the most resistant season was Kalubia, Menufia 2016 and Giza 2015 samples.

3.4. Resistance intensity and stability testing

Figures 5 and **6** illustrate the data on resistance intensity across sites and seasons and describe how different discriminative concentrations (0.5x, 1x, and 10x) affect larvae mortality. Naturally, treating the various tested field populations with 0.5x only resulted in a very low percentage of mortality, which supported the notion that all populations were resistant. However, **Table 13**, which included the treatment of 1x percentage mortality data, clarified this. The fact that the mortality rates from the 10x treatment exceeded 100% (which was amazing to see) and occasionally doubled or tripled that of 100% demonstrated that all populations were vulnerable to the tested pesticides mostly. *T. Absoluta* greenhouses and field populations showed high susceptibility to the tested IGR insecticide and low susceptibility to OP, Py, carbamate, and nicotinoids. Kalubia and Menufia showed greater resistance than Dakahlia, Sharkia, and Giza, according to the main findings of resistance distribution and intensity. The lower resistance values found may indicate less effective insecticide applications. However, the most effective insecticide was lufenuron, which was followed by chlorfenapyr (IGRs), methoxyfenzoid, chlorfluazuron, indoxacarb, imidacloprid, cypermethrin, and chlorpyrifos. Dimethoate and spinoteram were the least effective.

Selection pressure from widespread, repeated application of the right compound on insect populations resulted in the development of mechanisms that allow insects to withstand the harmful effects of insecticides, which ultimately caused the pest control failure described by Guillemaud et al. [31] as well as Erdogan et al. [29]. Since *Culex pipiens* resistance-associated target-site mutations in potential seasonal variations resulted in wing morphometric character mutations, many insects have reported seasonal fluctuations in frequencies of resistance alleles [1]. Additionally, according to Santos et al. [32], phenotypic resistance also known as technical and practical resistance is the result of interactions between genotype and environment. On the other hand, a heritable alteration in the organism that results in control failure is known as practical resistance, as well as Han et al. reported on Keys for *T.absoluta* IPM manipulation [33,34]. And De Smedt et al. [25] reported on “zeolite”, an insecticide substitute, in 2016. Using bioassay and additional research, such as Piperonyl butoxide synergist assays, biochemical analysis of population structure and genetic diversity, target-site insecticide resistance loci analysis, and PCR varieties of search subjects as [5,27,35]. Some researchers attempt to monitor the status of pests and insecticides in fields [36–38]. Also, Van Damme et al. [39] reported on the overwinter potential under insecticide.

4. Conclusion

Through a lengthy test period of many years of insecticide resistance monitoring techniques that produce valuable data and provide a decent indication of the distribution of pest-resistant populations to various insecticides under applications recommendation schemes in a closed geographical area, the study generally tested the probability of an increase in resistance frequency and development. The resistance status of tomato leafminer *T. absoluta* (Meyrick), was the main focus of this study's investigation, and offer useful information for making decisions about pest control. In order to demonstrate the variation in LC_{50} (lethal concentration that can kill 50% of the population attacking tomato crop), the study began with the collection of larvae and the application of bioassay using the most advised insecticide. $LC_{50,90,}$ and slops were obtained after statistical analysis of the toxicity lines. Data indicated that certain insecticides exhibited a significant increase in LC_{50} and needed attention because levels above this indicate a problem where a high dose is needed to kill the pest but is prohibited by human health authorities due to high toxicity to crop consumers. However, some other insecticides showed higher LC_{50} values than in previous monitoring periods, but they are still effective against the pest and pose no threat to humans.

Conflict of interest: The author declares no conflict of interest.

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