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Preliminary Observations and Domestic Management of The Polyphagous Fall Armyworm, *(Spodoptera frugiperda)* in Sharqia Governorate, Egypt

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ABSTRACT

Purpose: *In mid-2019, fall armyworm, Spodoptera frugiperda (FAW), a highly destructive and polyphagous pest was first discovered in Egypt. It is now a candidate to be a major economic threat to crop production. This study is the first report of this pest at Sharqia Governorate, northern-east Egypt. The available chemical control that is reviewed and reported which is mostly the first assessment in Egypt regarding the extreme shortage in any local data*

Research Method: *Compare the morphological features and description of the detected insect stages with the ideal published data. Through leaf dip bioassay in second instar larvae; the lethal concentrations (LC values) of various conventional and newer insecticides were detected in FAW. Four different insecticides (indoxacarb, methomyl, chlorpyrifos, and metaflumizone were evaluated against both strains (laboratory and field) of FAW.*

Findings: *Generally, the field strain showed resistance to the tested insecticides as compared to the laboratory one; however, indoxacarb (sodium channel blocker) was recorded as the most toxicant one, recording the lowest LC_{50} and LC_{90} values (48.79 and 208.47%) for field strain and (16.47 and 103.78%) for laboratory one, respectively. The field strain's observed insecticide resistance was attributed to resistance mechanisms including a noteworthy rise in the activities of detoxification enzymes. indoxacarb showed the lowest resistance significant values in the field strain as compared to laboratory one. We can conclude that sodium channel blocker i.e. indoxicarb is a promising choice in IPM strategies of FAW in Egypt.*

Research Limitations: *More field studies are required to examine the tested insecticides on different plant species to confirm their effectiveness under field circumstances and to make conclusive recommendations.*
Originality / Value: *Findings can confirm the presence of the invasive FAW in this specific area, register it for the first time, and provide detailed descriptions, also the candidate insecticides proved to be promising control agents to combat FAW larvae under local efforts and domestic circumstances.*

Keywords: *Biochemical parameters, Chemical control, Egypt, Invasive alien, Resistance, Spodoptera frugiperda.*

INTRODUCTION

In recent years, the Earth's planet has witnessed many environmental phenomena and climatic changes that have greatly contributed to changing the insect and plant composition in most regions of the globe, and the invasion of many insect species to new areas that were not suitable for them before. This exacerbated the danger of these species as a result of their settlement in new places without being accompanied by the natural biological enemies that dominate them in their original environments.

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The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctudiae), is a polyphagous herbivorous pest that attacks approximately 350 plant species, feeding in enormous numbers on most parts of the shoot system, seriously harming a wide range of field crops, such as wheat, sugarcane, rice, corn, and sorghum, as well as fibers such as cotton and many other vegetables (FAO and CABI, 2019). Originally from the Americas, this organism has been repeatedly placed under quarantine in Europe and has rapidly spread across the Old World due to its enormous ability to migrate; the first report of its presence in Africa was back in 2016, where it caused significant damage to certain crops such as maize and sorghum (Goergen *et al*., 2016). In 2018, Ganiger *et al*. (2018) reported FAW for the first time in the Indian subcontinent. Since then, it has settled in Sri Lanka, Bangladesh, Thailand, Myanmar, and China (IPPC, 2018; 2019; FAO, 2019). The presence of acceptable host plants and the abundance of favorable climatic circumstances for FAW in many regions of Africa and Asia suggest the pest is capable of producing many generations in the same season, which qualifies it to become epidemic. Recent data supported by (EPPO, 2015; 2022) listed FAW as A2 (pests are locally present in the EPPO region) within the European and Mediterranean regions.

By November 2019, the pest was also confirmed in Sudan and Egypt, as the Central Administration of Plant Quarantine, Ministry of Agriculture and Land Reclamation, Egypt, has officially declared the recognition of FAW in two specific locations, Kom Ombo and Edfu in Aswan Governorate, on maize plants (IPPC, 2019), and then it spread to most of the rest of the governorates, as well as in many Asian countries. Between February and May 2020, its existence was confirmed in the United Arab Emirates and Australia. It was also identified in Syria, Jordan, and the East Mediterranean in late 2020. By April 2021, it had occupied the Spanish Canary Islands. As of now, FAW has spread out of the Americas to over 70 countries.

Despite all the risks and warnings associated with the handling and application of pesticides in the field of pest control, which have been covered by many researchers, pesticides have some advantages that are rarely found in other control methods, and which make their use in some cases the ideal solution in many situations. These include their relative cheapness, convenient usage, quick effectiveness, and the ideal solution to confront sudden outbreaks and pest pressure or to combat insect invasions of new areas free of their natural enemies (Kongming, 2018).

Therefore, many countries around the world resorted to the use of insecticides belonging to different chemical groups as a decisive measure in controlling FAW, particularly remarkably efficient ones and fundamental to the IPM of this dangerous pest. Owing to FAW developing resistance to synthetic insecticides, this has necessitated addressing various mechanisms and modes of action such as organophosphorus, carbamate, insect growth regulators, and sodium channel blockers. Consequently, in South Africa, FAO registered the insecticides and mode of action groups including the oldest insecticides commercial groups (Acetylcholine esterase inhibitors), either organophosphorus or carbamates, and sodium channel blockers (Indoxicarb and Metaflumizone) (IRAC, 2021).

Also, in China, Zhang *et al*. (2021) and in South American countries (Garlet *et al*., 2021) applied chlorpyrifos (organophosphorus) to control FAW. Similarly, Soares *et al*. (2021) utilized methomyl (organophosphorus) in reducing the emergence rate of FAW in Brazil. The toxicity of Metaflumizone (a novel semicarbazone insecticide) and Indoxicarb (oxadiazine) was proved in Brazil (Kaiser *et al*., 2021) and Australia (Bird *et al*., 2022) against this pest.

Insects have effective defense mechanisms that help them reduce harm from foreign organisms. Many enzymes contribute to this system. Enzymes of detoxification are regarded as essential in the metabolism of pesticides and are known to be involved in pesticide resistance (Xu *et al*., 2015).

The recent research aims to monitor and distinguish FAW stages in northeast Egypt for the first time, in addition to evaluating the effectiveness of some pesticides belonging to different groups and modes of action under Egyptian conditions on both field and laboratory conditions to demonstrate the efficiency of these pesticides. Additionally, observing changes in detoxification enzymes serves as a valuable indicator of resistance, potentially serving as a pivotal factor in the control of FAW.

MATERIALS AND METHODS

Distinguishing and Recognition of FAW

Since this invasive insect species did not present earlier, did not originate in this environment, and has not been identified morphologically and taxonomically in both larval and adult stages, so once after the first official announcement, (Figure 01) it was approved

Arab Republic of Egypt
Ministry Of Agriculture and land Reclamation
Central Administration of Plant Quarantine
(CAPQ)
Phytosanitary Unit

جمهورية مصر العربية
وزارة الزراعة واستصلاح الاراضى
الادارة المركزية للحجر الزراعى
وحدة الصحة النباتية

Report of first detection of Fall Armyworm (FAW) in Egypt

In accordance with ISPM № 17 (Pest reporting) Egypt would like to report the detection of the fall armyworm in Egypt

- **Name of the Pest:** *Spodoptera frugiperda* / Lepidoptera: Noctuidae) **(fall armyworm).**
- **Date of the report:** May 30th, 2019.
- **Host :** Maize (corn)
- **The status of the pest :** Present only in some areas (ISPM № 8) , very limited to specific areas as indicated below

FAW adult insects were detected in Aswan (Kom Ombo, Edfo) using pheromone traps to attract the insect, these insects were further diagnosed at Plant Protection Research Institutes.

No moth were observed for all the governorates of Upper Egypt, except in Aswan Governorate, A total of 119 moths of fall armyworms were observed in Kom Ombo and Edfu centers during the period from January 2019 to date, the insect samples were identified.

- **Geographical distribution**
  Limited to only two locations Kom Ombo and Edfu in one governorate (Aswan)

Fig 1: location of Aswan governorate in Egypt.

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Figure 1: Egyptian Central Administration of Plant Quarantine report of first detection of FAW in Egypt

for comparison conformity with what is published and documented by official sources such as FAO and CABI, 2019 and pertinent literature (Huesing *et al*., 2018) for verification. The larvae of FAW were also compared to those of other species of *Spodoptera* and other noctuids that are associated with corn.

Wild larvae collected on maize plants (cv. High Tec) were the source of the F0 S. *frugiperda* culture used in this study in Shousha village (30.521760422665587, 31.56995903905731), Zagazig district Sharqia Governorate, northern East Egypt, during 2020. The collected culture was transferred to the insectary facilities at Plant Protection Research Institute, Sharqia Branch at Zagazig, and reared continuously under controlled conditions for further detailed studies. Ultimately, Larvae from the field were assured as *S. frugiperda* based on their morphological features according to FAO and CABI, 2019 (Figure 02).

Rearing Technique

Since this insect has never been reared in a laboratory under local conditions and capabilities, we have resorted to quoting what is done in the laboratory rearing of other *Spodoptera* species with the necessary modifications to reach the best results.

Because corn is only available seasonally and in certain areas, it cannot be relied upon as a permanent source for laboratory rearing. Therefore, the culture was maintained on castor bean, *Ricinus communis*, leaves renewed daily, which is considered cheap, easily obtainable, and always available year-round. The phenomenon of cannibalism remains present when the larvae are crowded or subjected to the pressure of lack of food, but this phenomenon is less apparent when the larvae feed on castor leaves (Grobler, 2019). The insect was reared for several successive generations in the laboratory in incubators at 27±1°C and 70±5% R.H. under a 12:12 hr photoperiod, to establish a colony of laboratory strain under controlled circumstances.

Upon reaching the adult stage and laying eggs, the resulting eggs were transferred into jars covered with muslin cloth and secured with a rubber band. Once the egg hatched, the newly-formed larvae were moved into larger (2 L.) rearing jars that were provided with filter paper at the bottom of the jars to absorb any excess moisture. Fresh castor bean leaves were provided as food to the larvae; the technique of feeding on leaves varies according to the age of the caterpillars, which typically have six larval instars (Figure 03). To avoid the cannibalism phenomenon, larvae at the 3rd instar were transferred into smaller jars that the numbers did not exceed 5 individuals/jar, (0.35 L.) whereas the 4th-6th instars were reared solitary in plastic vials (3.5 X 7.00 cm) covered with perforated led. When maize plants are infested with larvae, cannibalism accounts for nearly 40% of mortality (Chapman *et al*., 2000). After completing the larval stage and transformation into prepupae, moist sawdust was placed at the base of the rearing jars to create a suitable pupation site. The formed pupae were then collected and transferred into clean jars until adult emergence (Figure 04).

The newly emerged adult moths were stored by sex based on their morphological characters (refer to Figure 05) and were then paired and placed in clean jars (0.35 L.) lined on the inside with sheets of paper. Each jar was provided with 10 % honey solution which was soaked in cotton wool, which was tied with metal wire for moth feeding. Adults are characterized by rapid movement and hyperactivity so they must be handled carefully and quickly. To prevent fermentation and the growth of microorganisms, the honey solution was renewed and replaced every day. Additionally, Fresh green corn stems were added daily into adult jars to provide oviposition sites.

FAW adult moth 20 to 25 mm long mm., resting position, b. male, the forewing generally is shaded gray, light brown, and straw-colored, with noticeable triangular white spots (patches) at the tip (apex) and near the center of the wing, arrows indicate key characters. c. female, the forewings of females are less noticeably marked, ranging from a uniform grayish brown to a fine mottling of gray and brown. Each male and females have hind wings that tend to be silver-white with a thin dark edge.

The newly laid egg masses were collected daily and transferred into the rearing jars (5 egg-masse / jar). The eggs are pale white or creamish at the time of oviposition and become dark before eclosion. Egg maturity takes 2-3 days. Eggs are usually laid in layers (masses) of approximately 150-200 eggs which are laid in layers of up to 1000-1200 eggs by each female. The female moth is usually covering the deposited egg masses with a protective, felt-like shield of grey scales from her abdomen. The female moth deposits covering scales from its abdomen on top and between the egg mass, giving it a hairy appearance (Figure 05).

Application

The efficiency of the insecticides tested listed in Table 01 was evaluated against 2nd instar larvae. Successive concentrations of each insecticide were prepared starting from the recommended concentration using distilled water.

Disks (three cm. diameter) of castor bean leaves were

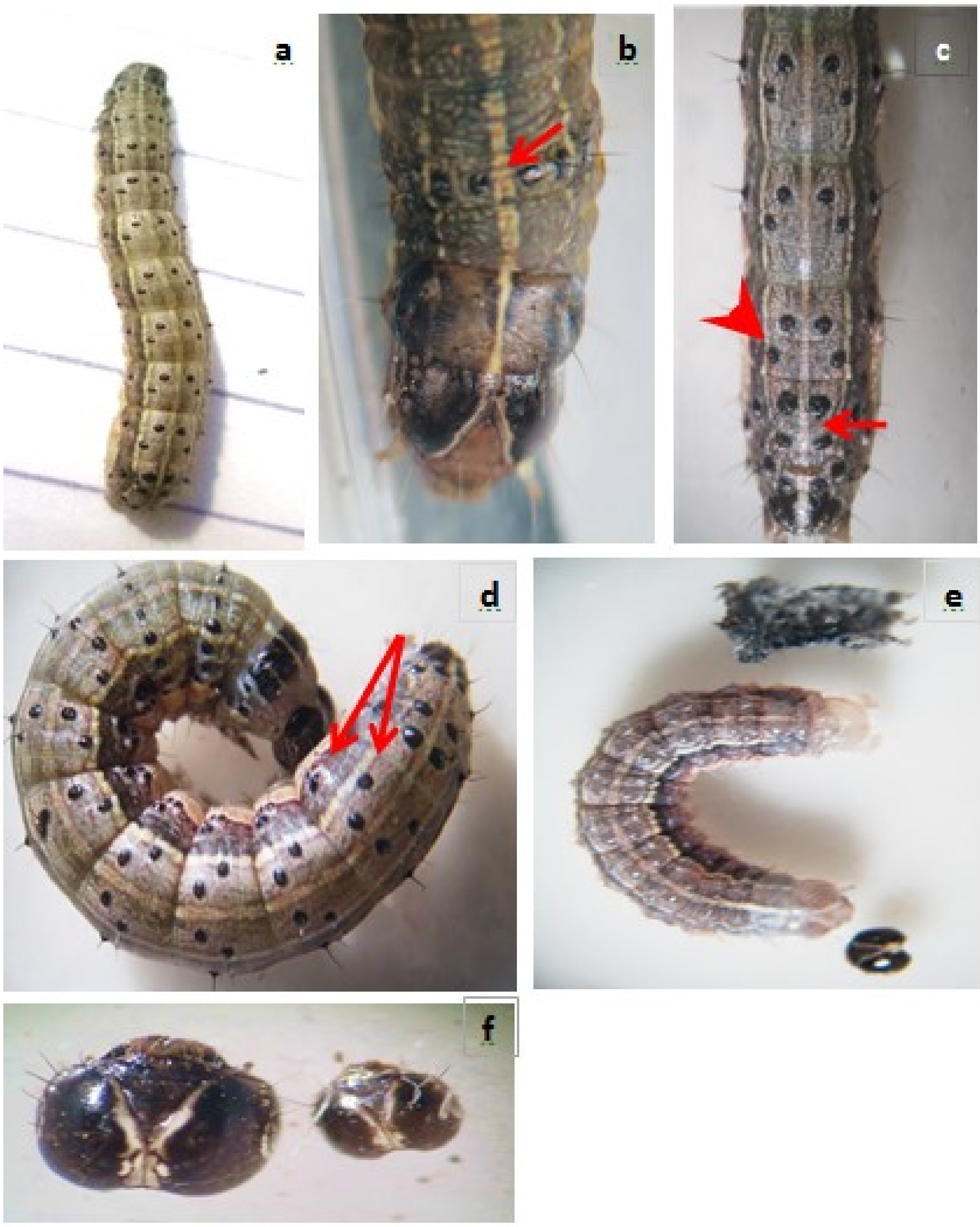

Figure 2: Distinguishing morphological features of the fall armyworm, Spodoptera frugiperda larvae

a. General view of FAW full-grown caterpillar and elevated dots distribution on the dorsum; they are usually dark in color, and the brownish body bears white subdorsal and lateral lines.

b. Inverted Y-shaped yellowish band along the front-clypeal suture, on the front of the head (face), the ecdysial line, and the pale dorsal line (arrow).

c. Close-up of the identifying feature of the FAW; spots arranged in a square on the 8th abdominal segment (arrow) (i.e., the second last segment near the end of the caterpillar), and on the upper segments, there are four smaller dots arranged in a trapeze pattern (arrowhead).

d. From the third instar upward, the dorsal surface of the body becomes brownish, with light bands on the side with a dark band in between (arrows) on a concealed larva.

e. Newly molted larva discarded behind its old cuticle and head capsule.

f. The difference in the size of early and old larval head capsules.

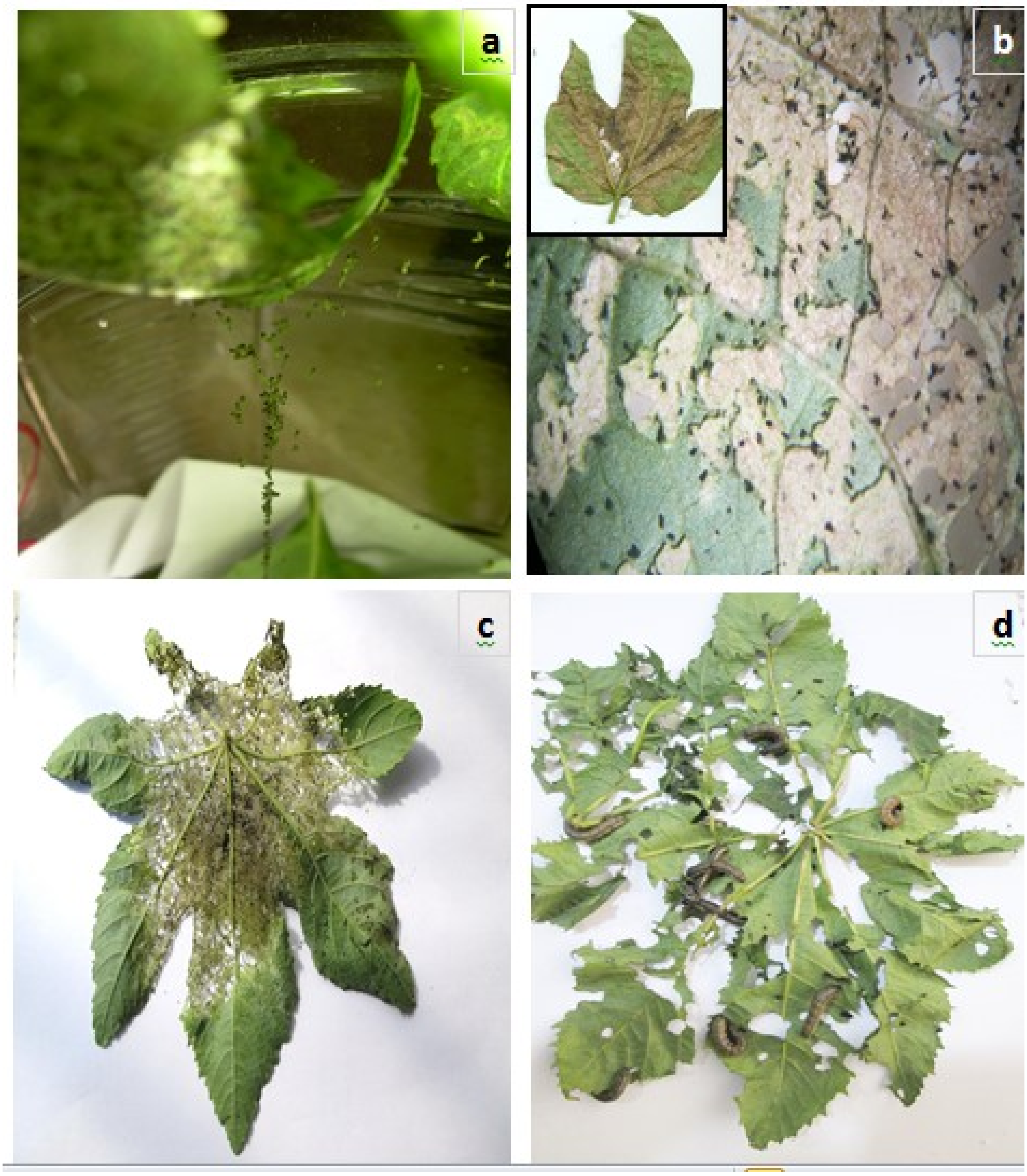

Figure 3: Damage symptoms.

a. Early instar caterpillars can spin silken threads which catch the wind and transport to new a plant.

b. Feeding by young early instar caterpillars (1^{st} and 2^{nd}) consumes leaf tissue from one side, leaving the opposite epidermal layer intact resulting in semi-transparent patches on the leaves.

c. Feeding by mid-instar larvae (3^{rd} and 4^{th}) begin to feed on both sides resulting in narrow irregular holes on the leaves.

d. Feeding by grown caterpillars (5^{th} and 6^{th}) eat from the edge of the leaves inward resulting in large irregular holes (windows) on the leaves.

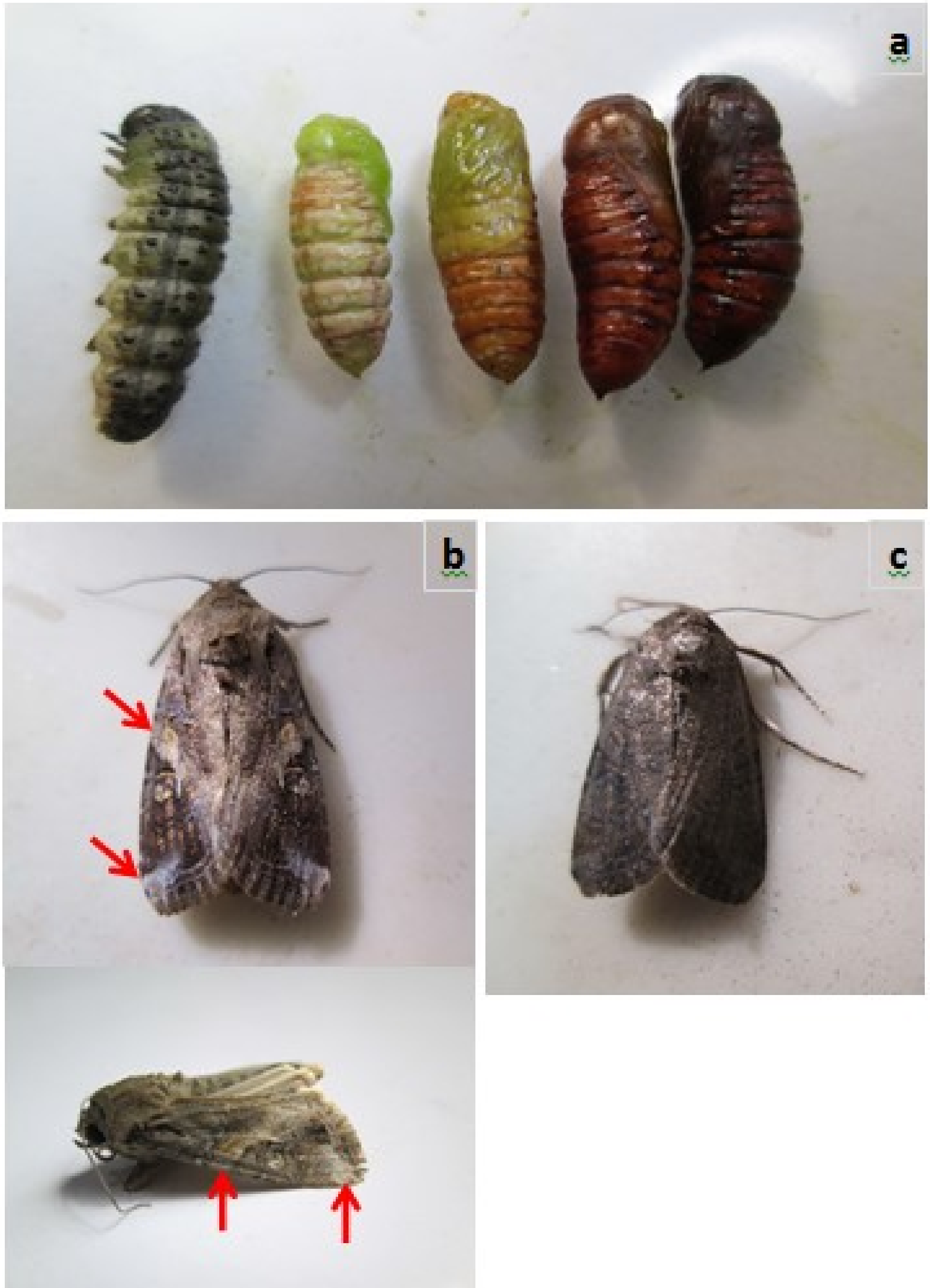

Figure 4: Pupae and adults.

a. Prepupa to left, gradual change in pupal color up to full tanning and hardening, measuring 14 to 18 mm in length. The newly formed pupa is light green with a loose texture, and within a few minutes turns to a darker color (reddish brown) and hard texture.

FAW adult moth 20 to 25 mm long mm., resting position, b. male, the forewing generally is shaded gray, light brown, and straw-colored, with noticeable triangular white spots (patches) at the tip (apex) and near the center of the wing, arrows indicate key characters. c. female, the forewings of females are less noticeably marked, ranging from a uniform grayish brown to a fine mottling of gray and brown. Each male and females have hind wings that tend to be silver-white with a thin dark edge.

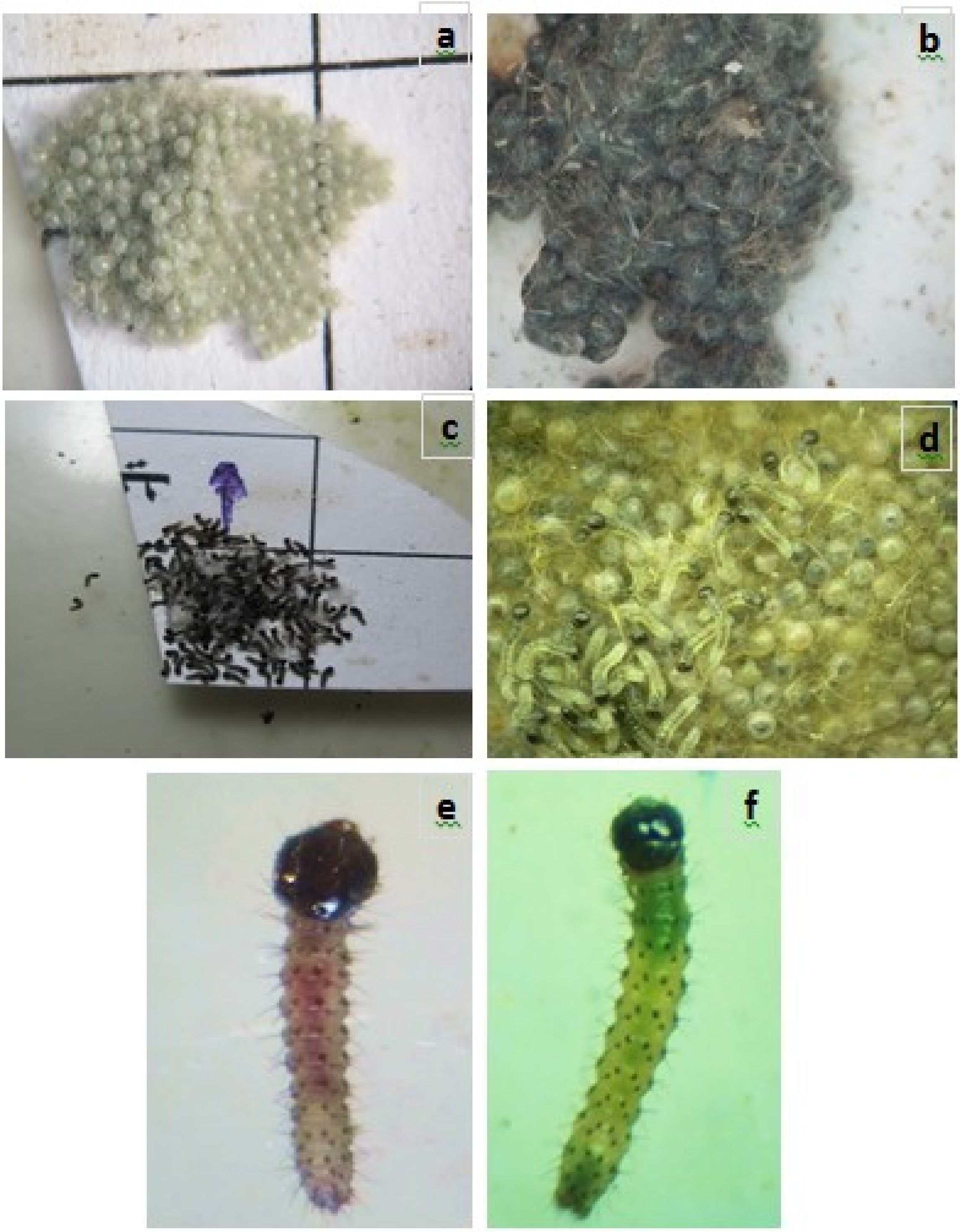

Figure 5: Eggs and newly hatched larvae.

a. The egg is dome-shaped; the base is flattened and the egg curves upward to a broadly rounded point at the apex Eggs are covered in protective scales rubbed off from the moth's abdomen though the surrounding hairs can give the impression of a yellow color.

b. eggs change to dark about to hatch collected from the oviposition cage.

c. Newly hatched neonate fall armyworm larvae on the top of eggs (egg mass), magnified (d).

e. Newly hatched neonate larvae, dark beige with a dark shining head spotted with dark dots usually bear spines.

f. Early instar fall armyworm larva turns greenish once they start feeding on leaves.

dipped in the tested concentrations for 10 seconds, allowed to dry, and offered to larvae, which starved for 4-6 hours before treatment. Larvae were placed individually into plastic vials as described above mention; each treatment was replicated five times (10 larvae per each). Control disks were dipped in only distilled water. The larvae were allowed to feed on treated disks for 24 hr.

After 24 hr, mortality percentages were recorded for all insecticides and then corrected using Abbott's formula, 1925. According to Finney, (1972) Probit analysis was used to compare the mortality percentages to corresponding concentrations. The Dose-mortality data (LC), relative potency, and toxicity index were calculated using the Sun equation, 1950.

Biochemical Assay

Samples Preparation

The preparation of samples involved the use of newly molted 2nd instar larvae of *S. littoralis* after 48 hr of treatment with LC_{25} of methomyl, indoxacarb, metaflumizone, and chlorpyrifos as well as control. Ten healthy larvae from each treatment were chosen and placed in clean jars (Greatglas Inc., Wilmington, USA), then starved for 4 hr. 5 mg of insects' tissues was homogenized in distilled water using an ST-2 Mechanic-Preczyina, Poland, glass Teflon tissue homogenizer for three minutes. The homogenates were centrifuged at 8000 rpm for 15 min at 5°C in a chilled microcentrifuge (Hetich, Germany) to eliminate hemocytes. The supernatants were transferred to clean tubes and reserved in the freezer at -20°C until needed. Three replicates were used for each.

Total Soluble Protein

Total protein concentration was assessed according to Bradford (1976) using bovine serum albumin as a standard.

AchE (EC 3.1.1.7)

To determine the activity of the acetylcholinesterase (AchE) enzyme, the method described by Simpson *et al*. (1964) was followed using acetylcholine bromide (AchBr) as the substrate.

GST (EC 2.5.1.18)

To determine the activity of Glutathione S-transferase (GST), the procedure defined by Habig *et al*. (1974) was followed using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate.

POD (EC 1.11.1.7)

The procedure given by Hammerschmidt *et al*. (1982) was followed to determine the activity of Peroxidase (POD).

CarE (EC 3.1.1.1)

Carboxylesterase (CarE) activity was determined according to the method designated by Van Asperen, with modifications (Van Asperen, 1962).

Statistical Analysis

The biochemical results were subjected to one-way analysis of variance (ANOVA) to show the differences within used insecticides. The significance of various treatments was evaluated by Duncan's multiple range test ($p < 0.05$) (Sun, 1950). One-way ANOVA was adopted to analyze the obtained data, and mean values were compared using Tukey's HSD test ($p < 0.05$). The SPSS 21.0 software (SPSS, Chicago, IL, USA) was used to analyze data. The differences between studied strains and data are presented as mean ± standard deviation, and the statistical significance was analyzed using Student's *t*-test, $p < 0.05$ was considered significant (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Toxicological Studies

Concentration values (LC_{50} and LC_{90}), toxicity index, RR, and RS were tabulated in Tables 02 and 03. The toxicant values estimated for four different insecticides on two tested strains (field and laboratory) of FAW suggested very wide variations in the values across the insect populations (Tables 02 and 03).

The lowest range in the values of LC_{50} (most toxicants) across the different strains was recorded for the new insecticide, indoxacarb (48.79 and 16.47 ppm) for field and laboratory strains, respectively. The highest range was given with metaflumizone (648.53 and 507.31 ppm), respectively. As for LC_{90}, the same precedent trend was achieved in the field strain, whereas indoxacarb was the most toxicant (208.47 ppm) and metaflumizone was the least toxicant (4263.980 ppm) (Table 2). In the laboratory strain, there is an exchange in insecticide efficiency; chlorpyrifos occupies the first

Table 1: List of the used pesticides and their related information.

Trade name	Formulation	Active ingredient and concentration	Chemical formula	Chemical Group	Commercial application rate	Mode of action	Manufacturer
Jitu®	Soluble Powder (SP)	Methomyl (90%)	$C_5H_{10}N_2O_2S$	Carbamate	200 gm/Feddan*	acetylcholinesterase inhibitor	Kafr El Zayat Pesticides & Chemicals Co., Egypt
Easo Plus®	Water-dispersible Granules (WG)	Indoxacarb (30%)	$C_{22}H_{17}ClF_3N_3O_7$	oxadiazine	60 gm/Feddan	blocking of neuronal sodium channels	Starchen Industrial Chemical, Egypt
Metazone®	Suspension Concentrate (SC)	Metaflumizone (24%)	$C_{24}H_{16}F_6N_4O_2$	Semicarbazone	100 cm^3 / 100 L	blocking sodium channels in target insects, voltage dependent sodium channel blockers	Kafr El Zayat Pesticides & Chemicals Co., Egypt
Pestban®	Emulsifiable Concentrate (SC)	Chlorpyrifos (48%)	$C_9H_{11}Cl_3NO_3PS$	organophosphorus	1 Liter/Feddan	acetylcholinesterase (AchE) inhibitor	Agrochem for Fertilizers & Chemicals, Egypt

*Feddan = 4200 m^2

position in toxicity (65.17 ppm), followed by methomyl (90.44 ppm), indoxacarb (103.78 ppm), and finally metaflumizone (1762.68 ppm) (Table 03).
Based on LC_{50} values, the resistance ratio (RR) of the field strain ranged between 1.00% and 13.291% for the most toxicant, indoxacarb, to the least toxicant, metaflumizone, respectively (Table 2). Both insecticides remained in the same order in the laboratory strain (1.00% to 30.786%), respectively (Table 03).

Results of Bioassay

The results of the bioassay of the tested insecticides showed that the field strain of *Spodoptera frugiperda* demonstrated noteworthy resistance to chlorpyrifos (9.435 fold) and methomyl (3.749 fold) but remained susceptible to indoxacarb (2.962 fold) and methoxyflumizone (1.278 fold) as compared to the susceptible strain (Table 03).

In the current research, the fall armyworm, *S. frugiperda*, was detected for the first time in corn fields at Sharqia Governorate, Egypt. Therefore, field and laboratory strains of FAW were tested for their susceptibility to four insecticides belonging to different chemical groups. Among all used insecticides, the lowest LC_{50} and LC_{90} values were recorded in both strains for indoxacarb, suggesting that it proved to be the most effective toxicant in controlling the FAW populations in our trials. The variation in LC values between both FAW strains could reflect the resistance to the tested insecticides. Generally, the FAW population collected from corn fields at Sharqia Governorate, Egypt, exhibited resistance to different tested insecticides compared to the laboratory strain; however, it is susceptible to metaflumizone and indoxacarb (1.278 and 2.962 fold, respectively) as compared to methomyl (37.49 fold) and chlorpyrifos (9.435 fold). These outcomes were supported by Yu *et al*. (2003), who recorded high resistance of the FAW field strain collected from corn in regions of Citra, Florida, against parathion (organophosphate) and carbaryl (carbamate). Recently, in corn fields, Mexican FAW populations expressed higher resistance to methomyl and chlorpyrifos (Gutiérrez-Moreno *et al*., 2019).

In Brazil, the susceptibility to indoxacarb and metaflumizone is still high against the field populations of FAW (Kaiser *et al*., 2021). This may be due to their unique mode of action by blocking sodium channels, which subsequently block the flow of sodium ions to nerve cells, preventing the transmission of nerve impulses, causing paralysis, and ultimately insect mortality (Silver *et al*., 2017). In addition to low toxicity to natural enemies and favorable eco-toxicological profiles.

These characteristics may help to delay the development of resistance and therefore, keep the field FAW population susceptible to both insecticides.

Effect of the Tested Compounds on Biochemical Assessments

Results showed that the total soluble protein level and detoxification enzymes (AchE, GST, POD, and CarE) in 2nd instar larvae were determined in the field and laboratory strains of FAW using LC_{25} of different insecticides and were compared to those of the laboratory strain. The results of the total soluble protein of tested insecticides in both field and laboratory strains of FAW are presented in Table 04. It can be seen that the laboratory strain had a significantly higher level of total soluble protein than the field strain for chlorpyrifos (0.781 fold), indoxacarb (0.584 fold), and methomyl (0.406 fold), conversely in the case of metaflumizone (1.518 fold).

Activities of the tested insecticides were significantly higher in the field strain than in the laboratory strain, ranging from 1.362 fold for metaflumizone to 2.338 fold for methomyl (Table 05). In contrast, lower activities of indoxacarb in the field strain than in the laboratory strain were recorded (0.635 fold).

GST activities toward all tested insecticides were higher in the field strain as compared to the laboratory strain (1.470 to 5.104 fold), with the exception of indoxacarb (0.664 fold) (Table 06).

Among the used insecticides, the precedent trend was also obtained in the activity of peroxidase in FAW field strain as compared to laboratory one, (Table 07). These activities ranged between 0.772 fold for indoxacarb to 1.337 fold for methomyl.

Data in Table 08 exhibited that the activity levels of carboxylesterases from field strain were significantly higher than laboratory one except for metaflumizone, methomyl recorded (14.428 fold), indoxacarb (1.511 fold), chlorpyrifos (2.985 fold) and metaflumizone (0.819 fold).

Proteins are biological molecules that play a vital role in driving the growth and metabolism processes in insects. They use proteins to construct and replace both enzymes and hormones. Moreover, proteins are needed

Table 2: Susceptibility of the second instar larvae *S. frugiperda* field strain to the tested insecticides.

Treatment	LC_{50} % (LCL-UCL)	LC_{90}% (LCL-UCL)	Slope	X^2	Resistance Ratio	Toxicity index
Indoxacarb	48.79 (40.04-58.38)	208.47 (40.04-58.38)	2.032	3.531	1.000	100.00
Methomyl	83.42 (65.43-111.29)	664.01 (375.88-1824.37)	1.422	3.537	1.710	58.49
Chlorpyrifos	181.45 (136.94-225.452)	894.32 (679.54-1334.33)	1.850	0.030	3.718	26.89
Metaflumizone	648.53 (524.73-866.49)	4263.980 (2507.19-10380.674)	1.567	2.119	13.291	7.54

-LCL, lower confidence limit; UCL, upper confidence limit; X^2, Chi square value.
-LC_{50}= lethal concentration that kills 50% of insects. LC_{90} = lethal concentration that kills 90% of insects

Table 3: Susceptibility of the second instar larvae *S. frugiperda* laboratory strain to the tested insecticides and the R/S ratio.

Treatment	LC_{50} % (LCL-UCL)	LC_{90} % (LCL-UCL)	Slope	X^2	Resistance Ratio	Toxicity index	LC_{50} R/ LC_{50} S
Indoxacarb	16.47 (7.99-31.69)	103.78 (47.28-1079.99)	1.604	1.526	1.00	100.00	2.962
Methomyl	22.25 (19.01-25.78)	90.44 (70.05-130.37)	2.104	0.665	1.53	74.056	3.749
Chlorpyrifos	19.23 (17.32-21.45)	65.17 (52.64-87.31)	2.418	2.099	1.167	85.681	9.435
Metaflumizone	507.31 (323.89-1056.54)	1762.68 (909.63-20181.06)	2.369	0.577	30.78	3.248	1.278

-LCL, lower confidence limit; UCL, upper confidence limit; X^2, Chi square value.
-LC_{50} = lethal concentration that kills 50% of insects. LC_{90} = lethal concentration that kills 90% of insects
-RR= LC_{50} of tested insecticides divided by LC_{50} of indoxicarb.
-R/S= LC_{50} of field strain (R) divided by LC_{90} of laboratory strain (S).

to build up muscles, cuticle, ribosomes, nucleic acids, egg yolk, and many other components. In this context, disruption of protein content may be considered the start of multi-malfunctioning performance in insect systems, leading to failure and mortality.

In general, the two crucial mechanisms predominantly responsible for insecticide resistance are metabolic resistance and target site insensitivity because of elevated levels of insecticide detoxification enzymes (Barres *et al*., 2016). Detoxification enzymes, including glutathione-S-transferase and acetylcholine esterase, are the main resistance mechanism in many lepidopteran species and are responsible for FAW resistance to different chemical groups (Yu *et al*., 2003). Possible mechanisms to achieve this include either eliminating the toxic components to protect cells from damage through a range of metabolic processes or playing a significant role in the detoxification of some xenobiotic compounds (Krishnan and Kodrík, 2006). Furthermore, these enzymes act as a protective mechanism against some pathogens (Shelby and Popham, 2006).

Table 4: Changes in the content of total soluble protein of the second instar larvae of *S. frugiperda* treated with tested insecticides.

Insecticides	Total soluble protein (mg/ g.b.wt) Field	Laboratory	R/S	t	*p*
Indoxicarb	9.666± 0.757[d]	16.533± 0.125[b]	0.584	15.397	0.0001
Methomyl	12.433± 0.757[c]	30.633± 1.365[a]	0.406	20.194	0.0000
Chlorpyrifos	22.466± 1.457 textsuperscripta	28.766± 1.566[a]	0.781	5.095	0.007
Metaflumizone	17.100± 0.854[b]	11.266± 0.862[c]	1.518	8.323	0.0011
$LSD_{0.05}$	1.882	2.124			
P	0.000^{***}	0.000^{***}			

- Every datum is the average of three replicates.
-Data expressed as mean±standard deviation (SD).
-R/S= mean of field strain (R) divided by mean of laboratory strain (S).
-P= Means under each variety having different letters in the same row denote a significant difference ($P \leq 0.05$) among different treated compounds.
-*p*= significant difference ($P \leq 0.05$) between tested strains.

Table 5: Changes in acetylcholinesterase (AchE) enzyme of the second instar larvae of *S. frugiperda* treated with tested insecticides.

Insecticides	AchE (ug AchBr/min/ mg protein) Field	Laboratory	R/S	t	*p*
Indoxicarb	11.30± 0.435[a]	17.800± 1.021[a]	0.634	6.942	0.002
Methomyl	12.00± 0.556[a]	5.133± 0.208b[c]	2.337	20.008	0.000
Chlorpyrifos	8.633± 0.321[b]	4.766± 0.404[a]	1.811	12.969	0.000
Metaflumizone	9.033± 0.404[b]	6.635± 0.550[b]	1.362	6.085	0.004
$LSD_{0.05}$	0.8243	1.7961			

- Every datum is the average of three replicates.
-Data expressed as mean±standard deviation (SD).
-R/S= mean of field strain (R) divided by mean of laboratory strain (S).
-P= Means under each variety having different letters in the same row denote a significant difference ($P \leq 0.05$) among different treated compounds.
-p= significant difference ($P \leq 0.05$) between tested strains.

Table 6: Changes in Glutathione S-transferase (GST) enzyme of the second instar larvae of *S. frugiperda* treated with tested insecticides.

Insecticides	GST (mmol sub. Conjugated /min/ mg protein)		R/S	t	p
	Field	Laboratory			
Indoxicarb	3.100± 0.360[c]	4.666± 0.208[a]	0.664	6.517	0.002
Methomyl	9.866± 0.416[a]	1.933± 0.208[c]	5.104	29.527	0.000
Chlorpyrifos	5.266± 0.208[b]	2.166± 0.208[c]	2.431	18.238	0.000
Metaflumizone	4.706± 0.190[b]	3.200± 0.264[b]	1.470	8.0104	0.001
$LSD_{0.05}$	0.5824	0.4210			
P	0.000^{***}	0.000^{***}			

- Every datum is the average of three replicates.
-Data expressed as mean±standard deviation (SD).
-R/S= mean of field strain (R) divided by mean of laboratory strain (S).
-P= Means under each variety having different letters in the same row denote a significant difference ($P \leq 0.05$) among different treated compounds.
-*p*= significant difference ($P \leq 0.05$) between tested strains.

Table 7: Changes in Peroxidase (POD) enzyme of the second instar larvae of *S. frugiperda* treated with tested insecticides.

Insecticides	POD (mδ O.D. /min/ mg protein)		R/S	t	*p*
	Field	Laboratory			
Indoxicarb	558.0± 12.165[b]	723.0± 19.697[a]	0.772	12.344	0.000
Methomyl	606.666± 12.433[a]	453.666± 10.263[b]	1.337	16.445	0.000
Chlorpyrifos	512.666± 6.658[c]	420.333± 9.504[c]	1.220	13.781	0.000
Metaflumizone	480.0± 10.000[d]	451.0± 8.544[b]	1.064	3.818	0.018
$LSD_{0.05}$	19.896	24.124			
P	0.000^{***}	0.000^{***}			

- Every datum is the average of three replicates.
-Data expressed as mean±standard deviation (SD).
-R/S= mean of field strain (R) divided by mean of laboratory strain (S).
-P= Means under each variety having different letters in the same row denote a significant difference ($P \leq 0.05$) among different treated compounds.
-*p*= significant difference ($P \leq 0.05$) between tested strains.

Table 8: Changes in Carboxylesterase (CarE) enzyme of the second instar larvae of *S. frugiperda* treated with tested insecticides.

Insecticides	CarE (ug Meb /min/ mg protein)		R/S	t	*p*
	Field	Laboratory			
Indoxicarb	121.0± 5.291[b]	80.06± 7.035[a]	1.511	8.053	0.001
Methomyl	151.0± 8.544[a]	10.46± 1.171[c]	14.428	28.224	0.000
Chlorpyrifos	34.03± 3.647[c]	11.40± 2.170[c]	2.985	9.236	0.000
Metaflumizone	42.66± 3.247[c]	52.06± 3.494[b]	0.819	3.413	0.027
$LSD_{0.05}$	10.518	7.751			
P	0.000^{***}	0.000^{***}			

- Every datum is the average of three replicates.
-Data expressed as mean±standard deviation (SD).
-R/S= mean of field strain (R) divided by mean of laboratory strain (S).
-P= Means under each variety having different letters in the same row denote a significant difference ($P \leq 0.05$) among different treated compounds.
-*p*= significant difference ($P \leq 0.05$) between tested strains.

Acetylcholine (Ach) is a neurotransmitter that is hydrolyzed in the nervous system of numerous organisms by the enzyme AchE. The target location of various neurotoxic pesticides, including carbamates and organophosphate insecticides, is considered the basic enzyme when detecting neurotoxicity (Ljubisavljevic *et al*., 2012).

GST has been described as playing a pivotal role as a detoxification mechanism and cellular antioxidant defense against many insecticides, protecting tissues from oxidative damage and stress (Lumjuan *et al*., 2005).

POD is a vital protective enzyme in insects that plays a role in immunity by avoiding free radical-associated destruction and defending cells against various environmental reactions (Bi *et al*., 2010).

CarE performs several physiological functions, including detoxification, metabolism of allelochemicals, tolerance metabolism of specific hormones and pheromones, degradation of neurotransmitters, and playing a crucial role in defending against xenobiotic compounds (Field and Blackman, 2003; Vogt, 2005).

Presently, there exist noticeable variations in the activities of detoxification enzymes between the two strains. The higher activities of these enzymes observed in the field strain compared to the laboratory strain serve as a key indicator for monitoring the observed resistance. This increase is one of the most common resistance mechanisms to different classes of insecticides (Oppenoorth, 1985). A similar conclusion was documented by Yu *et al*. (2003) when examining some detoxification enzymes against field and laboratory strains of *S. frugiperda*. Significant variation in the activities between the two strains as affected by different insecticides supports that indoxacarb achieves the lowest levels of the activities of the enzymes compared to the other insecticides.

Therefore, our recent study suggests that FAW demonstrates a low-risk resistance evolution to indoxicarb. Indoxicarb and metaflumizone were tested for cross-resistance in Brazil, but no evidence was discovered between them for major insecticides, and Bt proteins permit the use of both insecticides in a rotation policy (Kaiser *et al*., 2021). Conversely, due to the development of resistance, chlorpyrifos and methomyl, which are conventional insecticides, may no longer be effective in controlling FAW[30]. Furthermore, in China, the resistance to pyrethroids, organophosphorus, and spinosad were all at moderate to high levels (Zhang *et al*., 2021).

CONCLUSION

This study is the initial documentation of *S. frugiperda* occurrence in the fields of Sharqia Governorate, northern-east Egypt. Despite the crucial role of insecticides in managing FAW, their extensive use has led to the development of resistance in this pest to various chemical groups. In our study, the field strain of FAW remained susceptible to indoxacarb compared to other conventional insecticides. These findings showed that sodium channel blockers (e.g. indoxacarb) are a preferable nominee for adoption by farmers and authorities involved in IPM programs for FAW in Egypt. This could serve as a guide for developing proposed control strategies to mitigate the risk posed by this pest. The study also includes a detailed method for rearing laboratory strains. Future research should focus on continuous monitoring of resistance development to provide more data for the informed selection of pesticides.

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