Innovations

Preferences of the Fall Armyworm *Spodoptera Frugiperda* (J.E. Smith) (Lep., Noctuidae) to Different Maize Cultivars Grown In Jordan

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Abstract: *This study aimed at determining the pest's preferences for various maize cultivars grown in Jordan. The experiments were conducted at the Southern Ghor Agriculture Directorate, Ghor Al-Safi, Karak, Jordan in 2022. Multi-choice preference and non-choice susceptibility experiments were set up to determine the least preferred cultivar by the early and late larvae of the pest. Egyptian White Maize, Aqeeq F1, Egyptian Red Maize, Merkur F1, Thailand A528, and Asgrow, were used in the experiment. The results of the preference experiment revealed that Egyptian White Maize is the least preferred maize cultivar for both early and late larvae, followed by the other four cultivars: Aqeeq F1, Egyptian Red Maize, Thailand A528 and Merkur F1, while Asgrow represented significantly the most preferred plant cultivar. In the susceptibility experiment, for the early larvae, the findings indicated that the maize cultivar, Egyptian White Maize and Aqeeq F1 were significantly the least preferred cultivars, followed significantly by Merkur F1, Egyptian Red Maize and Asgrow, Thailand A528 represented significantly the most preferred cultivar. Forlate larvae, Egyptian White Maize and Aqeeq F1 were significantly the least preferred cultivars, followed by Egyptian Red Maize, while Thailand A528, Merkur F1 and Asgrow represented significantly the most preferred cultivars. In the multi-choice preference and non-choice susceptibility experiments, there was a positive and significant correlation between the cultivars and the leaf area consumed by the early and late larvae. In addition, there was a significant interaction between feeding type (separately or together) and each of larvae type and cultivar.*

***Keywords**: Spodoptera frugiperda, fall armyworm, invasive pest, ecofriendly management, maize.*

1. Introduction

Maize, also called corn (*Zea mays* L.)comes in the 2nd position of the most cultivated cereal crops after wheat globally. It is one of the most important cereals used as grain and forage by humans and animals. The total maize production in the world is about 1.16 billion tons, occupying an area of about 202 million hectares in 2020 (FAO, 2020). In Jordan, the maize cultivated areas are rapidly increased in the last 3 years with a total area of 629 hectares in 2020 for sweet maize "yellow maize" and 106 hectares for white maize. This increasein maize cultivation could be attributed to the climate in Jordan Valley which provide ssuited environment for producing high quality and quantity of sweet maize and fodder maize (Jordan Statistical Yearbook, 2020).

Recently, the fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lep., Noctuidae) is becoming a key invasive insect pest causing huge yield losses to many crops, especially maize nationwide (Deshmukh et al., 2021). *S. frugiperda* has over 200 years of history in the USA (Edosa and Dinka, 2021). In 2016, the pest was detected for the first time in some countries of Africa, and it distributed to almost the whole of Africa (Allen et al., 2021), and hereafter in different parts of Asia in 2018 (Hussain et al., 2021). Recently, *S. frugiperda* invaded Europe and Australia (Parra et al., 2022). It has now reached 109 countries globally (Zhao et al., 2022). The pest could damage approximately 353 host plant species (Chen et al., 2021). It well known that the pest has the ability to survive on many host plants, but it has a high preference for maize (Ngangambe and Mwatawala, 2020). The larva is the most dangerous stage and feeds on the leaf by scraping green tissues (Badhai et al., 2020). The pest is considered an economical insect pest due to its polyphagous habit, voracity (Chen et al., 2021), high reproductive capacity (Zhang et al., 2021), long adult dispersal (Deshmukh et al., 2021), and multiple generations/year (Edosa and Dinka, 2021). These characteristics make *S. frugiperda* a risky pest to maize.

The principal approach of pest management adopted by farmers in much of the world is the use of synthetic pesticides (Al-Zyoud, 2012; Al-Zyoud et al., 2021). Due to the rapid global invasion of *S. frugiperda*, there is a pressing need to understand the management options for this serious pest (Overton et al., 2021). The suppression of *S. frugiperda* appears challenging and difficult due to its short life cycle, high fertility, wide host plant spectrum, voracious feeding habit, fast reproduction and multiplication, and ability to distribute across wide geographical regions (Niassy et al., 2021). The management of *S. frugiperda* includes various approaches like monitoring and trapping (Koffi et al., 2021), cultural control (Niassy et al., 2021), chemical control (Nboyine et al., 2022), plant resistant cultivars (Correa et al., 2021), botanical control (Paredes-Sanchez et al., 2021), genetically modified crops (Eghrari et al., 2022), and biological control(Santos et al., 2021).

Utilizingplant cultivars that possess resistant against pests is a good control tactic due to their efficiency, safe for human health,less environmental risk, and a main component of integrated pest management (IPM) (Al-Zyoud et al., 2009, 2015). In this sense, maize germplasm with native genetic resistance to *S. frugiperda* was developed (Prasanna et al., 2018). Among 10 sweet maize genotypes, the genotypes of sweet corn, namely Tropical Plus, Teea Dulce, Doce Cubano MG 161, and Doce Flor da Serrawere found to have antibiosis resistance to *S. frugiperda* due to slower insect development (Crubelati-Mulati et al., 2020). Sanches et al. (2019),found less *S. frugiperda* preference for the cultivar, Zapalote Chico than for the other tropical popcorn genotypes.

It is hypothesized that farmers facemany challenges to eradicate *S. frugiperda*including the non-existence of any structured IPM program. The use of combination of differentcontrol approaches through IPM program is an effective method to suppress pests in a sustainable manner. Thus, addressing *S. frugiperda* problem in Jordan is needed in order to efficiently control this destructive pest on a sustainable process.Thefindings show that respondents are very highly concerned about human health, food safety, and the riskof environmental pollution, and they have a real desire to reduce the use of pesticides. Respondents had a

moderate level of knowledge about food safety, pesticides' side effects, pesticides' residues in food orfeed, and usage of pesticides in homes and gardens. According to the respondents, the most commonway that people are exposed to pesticide residues is by consuming pesticide residues in food (Al-Dawood, etal., 2023). The main goal of the current study was to investigate the preferences of the pest to different maize cultivars grown in Jordan, and the susceptibility of maize cultivars to the pest, and to establish an IPM program taking into account the least preferred maize cultivar. The most important outcome of this study is to contribute to the global pool of knowledge regarding *S. frugiperda*, and to fill in a gap in the literature regarding this destructive pest.

2. Materials and methods

2.1. Location and maintenance of the insects

The experiments were conducted in a rearing room at the Southern Ghor Agriculture Directorate, Ghor Al-Safi, Karak, Jordan in 2022. The environmental conditions during the experiments in the rearing room were 27±3°C temperature, 60±10% RH, and 16: 8 h (L: D) photoperiod. Thousands of live *S. frugiperda* larvae were gathered by the researchers from highly infested maize fields in Ghor Al-Safi, and taken directly to the rearing room for further determination of the needed larval instars using a Binocular microscope, our experience in the size of the larval instars, and using the ruler once necessary to measure the length of the larvae.

2.2. Maintenance of plants and experimental procedures

For the preference and susceptibility experiments, six maize cultivars were used. They were Egyptian White Maize, Aqeeq F1, Egyptian Red Maize, Merkur

F1, Thailand A528, and Asgrow. All maize cultivars were obtained from private companies in Jordan. Seeds of six different cultivars of maize were each sown in three pots of 12×12 cm (diameter, height) under field conditionsduring April, 2022, and no fertilizers or insecticides were applied to the plants. Meanwhile, irrigation of the potted plants was taken place regularly. This step was taken to ensure adequate host plant materials for *S. frugiperda* larvae during the experiments. The L_1–L_2 (early larvae) and L_4–L_5 (late larvae) instars of *S. frugiperda* were used in the current experiments. The preference experiment was conducted in the abovementioned rearing room. All the experiments were conducted in Petri-dishes of 3×11 cm (height×diameter), filled partially with a 0.5 cm layer of wetted cotton pad. EachPetri-dish' lid had a hole closed with fabric to ensure ventilation.

For the preference of *S. frugiperda* for maize cultivars, six maize cultivars were investigated in a multi-choice experiment. The experiment was a Complete Randomized Design (CRD). Freshly picked leaf discs of 5 cm^2 area of each cultivar (5 cm^2 area of each cultivar × 6 cultivars = 30 cm^2) were cut from each uninfested maize cultivars via a blade, and 6 leaf discs each all the tested cultivars were kepttogether above the pads of cotton in a labeled Petri-dish with two larvae of either L_1–L_2 (early larvae) or L_4–L_5 (late larvae) instars/Petri-dish to measure the leaf area consumed by the pest larvae. Hereafter, the Petri-dishes containing the leaf discs of the different cultivars were maintained under the previously mentioned rearing room conditions for a period of two days for the early larvae and for one day for the late larvae for larval feeding. Thereafter, the larvae were removed from the Petri-dishes, and the leaf area consumed was measured. Thirty replicates were used for each cultivar in the preference experiment.

For the susceptibility (non-choice experiment) of the different maize cultivars for the pest larvae, two leaf discs (each 5 cm^2) (2 × 5 = 10 cm^2 area from each cultivar) were provided for the larvae. The leaf discs of each cultivar were separately placed in a labeled Petri-dish on the cotton pads. Hereafter a larva of either L_1–L_2 (early larvae) or L_4–L_5 (late larvae) instars/Petri-dish was kept per each Petri-dish with each cultivar under the previous mentioned rearing room' conditions for a period of two days for the early larvae and for 1 day for late larvae for feeding.Thereafter, all the larval individuals were removed, and the mean consumption area of each leaf was measured for all maize cultivars. Twenty replicates were used for each cultivar in the susceptibility experiment.

2.3. Statistical analysis

The Proc GLM of the Statistical Package Sigma Stat version 16.0 (SPSS, 1997) was used for the statistical analysis of the experimental data. The obtained data were statistically analyzed via 1-way ANOVA to detect if there are any differences in the consumption of the larvae from the different cultivars (Zar, 1999). Once significant differences were detected, differences among several means were compared by using the Least Significant Difference (LSD) Test at P≤0.05 (Abacus Concepts, 1991). In addition, the correlations between the cultivar type and the consumption by the early and late larval instars in the multi- and non-choice experiments were calculated by using Spearman's correlation method (Zar, 1999).

3. Results

3.1. Preference experiment

Average leaf area of maize cultivars consumed by *S. frugiperda* larvae (early larvae: L_1–L_2 or late larvae: L_4–L_5) by feeding together upon six different maize cultivarsis summarized in (Fig. 1) The results showed that for the early larvae, the maize cultivar, Egyptian White Maize was significantly the least preferred cultivar recording a total consumed leaf area of 0.19±0.05 cm^2, followed by the four cultivars; Aqeeq F1 (0.62±0.11 cm^2), Egyptian Red Maize (0.74±0.14 cm^2), Thailand A528 (0.84±0.16 cm^2), and Merkur F1 (0.99±0.16 cm^2) which were significantly at a bar with each other. However, the cultivar, Asgrow with a consumed leaf area of 1.54±0.19 cm^2showed significantly the most preferred maize cultivar (F=9.429; 5, 180 df; P=0.000) (Fig. 1A). For the late larvae, Egyptian White Maize was significantly the least preferred cultivar recording to the total consumed leaf area of 1.99±0.37 cm^2, followed by the four cultivars; Aqeeq F1 (2.08±0.31 cm^2), Egyptian Red Maize (2.10±0.33 cm^2), Thailand A528 (2.14±0.30 cm^2), and Merkur F1 (2.43±0.32 cm^2) which were significantly at a bar with each other. However, the cultivar, Asgrow with a consumed leaf area of 2.66±0.29 cm^2indicated significantly the most preferred maize cultivar (F=9.445; 5, 180 df; P=0.000) (Fig. 1B).

3.2. Susceptibility experiment

Average leaf area of maize cultivars consumed by *S. frugiperda* larvae (early larvae: L_1–L_2 or late larvae: L_4–L_5) by feeding separately upon six different maize cultivarsis summarized in (Fig. 2). For the early larvae, the results of susceptibility of maize cultivars to *S. frugiperda* showed that the maize cultivar, Egyptian White Maize (1.57±0.13 cm^2) and Aqeeq F1 (1.71±0.31 cm^2) were significantly the least preferred cultivars, followed by Merkur F1 (2.63±0.56 cm^2), Egyptian Red Maize (3.47±0.64 cm^2), and Asgrow (3.49±0.66 cm^2) which were significantly at a bar with each other, while Thailand A528 with a consumed leaf area of

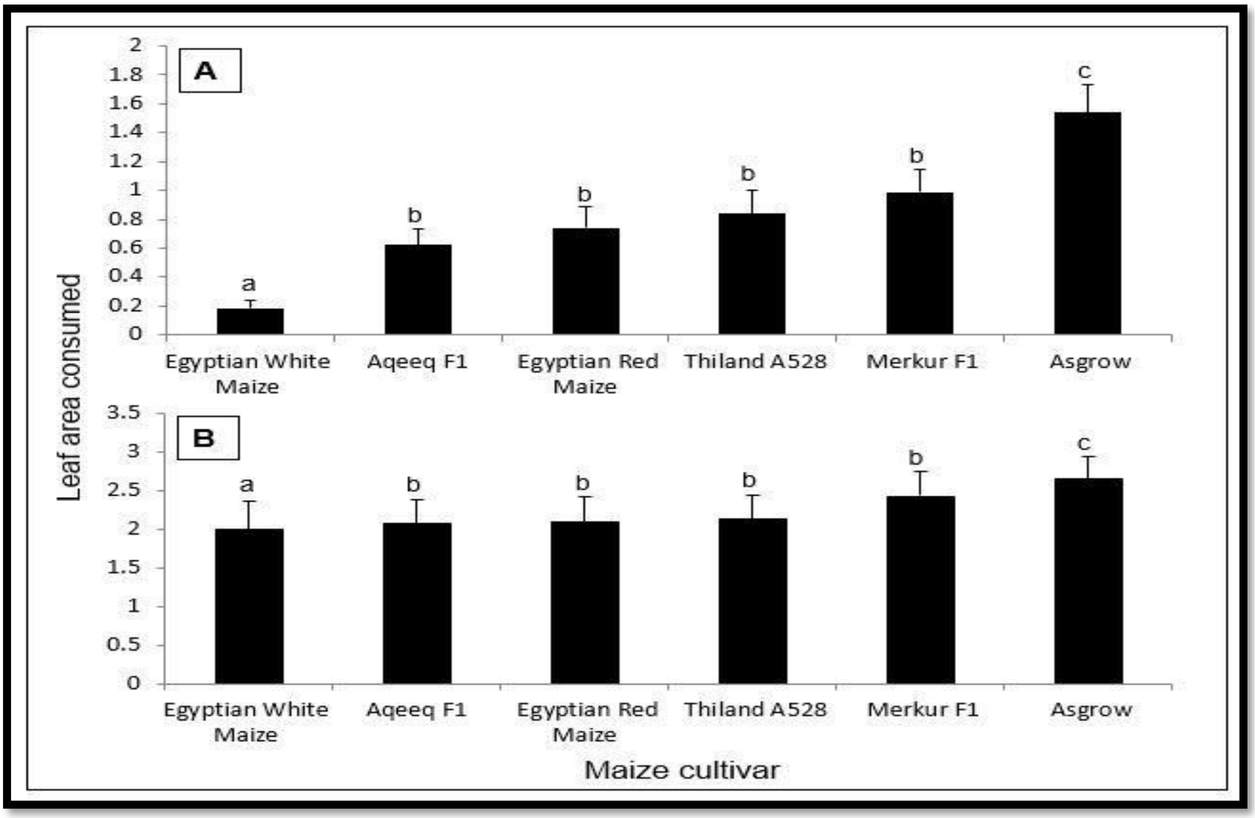

Fig 1. Average (±SEM) leaf area (cm^2) consumed by the early larval instars (L_1–L_2) (A) and the late larval instars (L_4–L_5) (B) of *Spodoptera frugiperda*by feeding together (multi-choice preference experiment) upon six different cultivars of maize. [Different small letters above bars indicate significant differences among the different maize cultivars within the same larval instar at P≤0.05 (1-factor ANOVA)].

4.55±0.71 cm^2 indicated significantly the most preferred maize cultivar (F=4.508; 5, 120 df; P=0.001) (Fig. 2A). For the late larvae, Egyptian White Maize (1.20±0.56 cm^2) and Aqeeq F1 (1.35±0.57 cm^2) were significantly the least preferred cultivars, followed by Egyptian Red Maize (2.82±0.93 cm^2). However, the cultivars; Thailand A528 (3.98±0.99 cm^2), Merkur F1 (4.30±0.91 cm^2), and Asgrow (4.43±0.86 cm^2) showed significantly the most preferred maize cultivars (F=3.156; 5, 120 df; P=0.010) (Fig. 2B).

3.3. Correlation analysis

In the multi-choice preference experiment, a positive significant correlation was found between the cultivar and the consumption by the early larval instars (r=0.421, P=0.000) at 0.01 probability level, and a positive non-significant correlation of the late larval instars (r=0.141, P=0.060), as well as a positive significant correlation for both larval instars together (r=0.253, P=0.000) at 0.01 probability level. In the non-choice susceptibility experiment, the results indicated a positive significant correlation between the cultivar and the consumption by the early larval instars (r=0.192, P=0.036) at 0.05 probability level, and late larval instars (r=0.346, P=0.000) at a probability level of 5%, as well as a positive significant correlation for both larval instars together (r=0.263, P=0.000) at a probability level of 5%. Furthermore, there was a significant interaction between larvae type and feeding type (separately or together) (F=12.278; 1, 600 df; P=0.000), as well as between feeding type and maize cultivar (F=4.864; 5, 600 df; P=0.000).

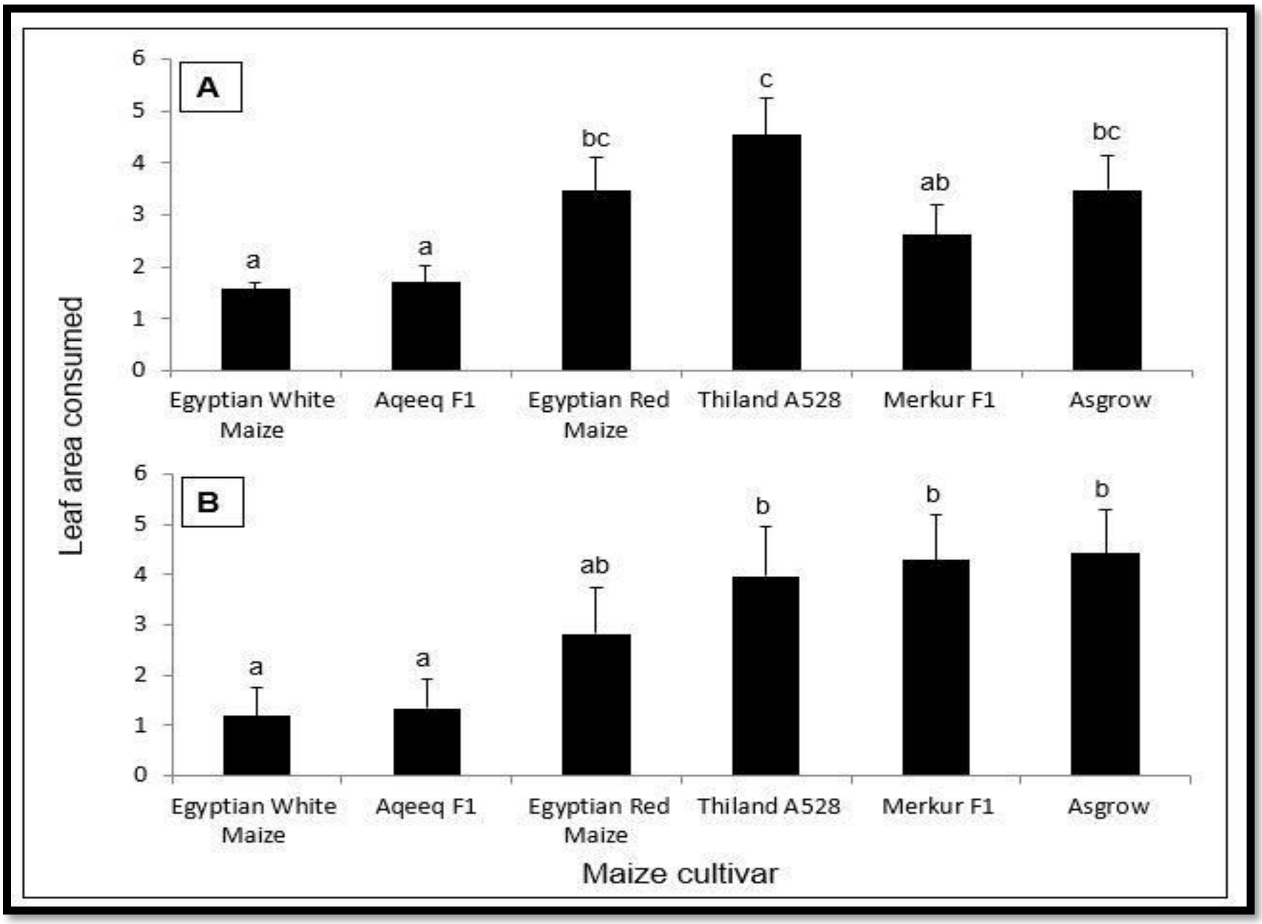

Fig.2. Average (±SEM) leaf area (cm^2) consumed by the early larval instars (L_1–L_2) (A) and the late larval instars (L_4–L_5) (B) of *Spodoptera frugiperda*by feeding separately (non-choice susceptibility experiment) upon six different cultivars of maize. [Different small letters above bars indicate significant differences among the different maize cultivars within the same larval instar at P≤0.05(1-factor ANOVA)].

4. Discussion

This study evaluated several maize cultivars in the local market due to the importance of the low-cost and environmentally friendly role of resistant plant varieties in overcoming pest damage (Bhusal and Chapagain, 2020; Paredes-Sanchez et al., 2021). The studied maize cultivars were selected based on the ones most widely used by farmers in Jordan. However, in the preference experiment, the results showed that for both early and late larvae the maize cultivar, Egyptian White Maize is significantly the least preferred, followed by four cultivars; Aqeeq F1, Egyptian Red Maize, Thiland A528, and Merkur F1, while Asgrow was significantly the most preferred cultivar. In the susceptibility experiment, for the early and late larvae results showed that the maize cultivar, Egyptian White Maize and Aqeeq F1 are significantly the least preferred cultivars, while Thiland A528, Merkur F1 and Asgrow were significantly the most preferred cultivars. The findings of this study indicated that no cultivar has a complete resistance to *S. frugiperda*, and the Egyptian White Maize cultivar was the most resistant to larval feeding among the 6 studied cultivars in both preference and susceptibility experiments. It is believed that resistance can be due to many reasons, i.e. the leaf fiber content could influence the consumption of many Lepidoptera species (i.e., moths) (Hong et al., 2012). Hedin et al. (1996), stated that the cellulose, crude fiber, and hemicellulose contents were higher in the maize whorls of resistant cultivars to *S. frugiperda* than the non-resistance ones. Moreover, it was found that lipids contents in the maize leaves play a key role in *S. frugiperda* larvae'sdevelopment and reproduction. When the larvae of *S. frugiperda* fed on leaves free of cuticular lipids (removed), the larvae had more weight and fast development than when they fed on leaves with cuticular lipids (Yang et al., 1993a). In another study, *S. frugiperda* early larvae had the ability to travel for longer distances and they were crawled faster on the upper leaves of the plant that characterized by a smooth appearance leafthan on lower leaves, which characterized by a dense array of wax crystals (Yang et al., 1993b). Andama et al. (2020), suggested an effect of antibiosis of maize hybrid against *S. frugiperda* larvae. Morales et al. (2021), suggested that other physical characters, i.e.,toughness and/or thickness of the plant leaves, and the structure of cuticular compounds may be involved in the plant preference reduction of certain cultivars. Another possible reason for less preference of the pest larvae is the presence of the anti-feedants or repellent chemicals in some maize cultivars. However, the lipophilic constituents of plant leaf surfaces (i.e., esters, fatty acids, and alkenes), and secondary plant metabolites are well known to promote test-biting of the pest and subsequent the feeding in several insect pests (Schoonhoven et al., 2007).

The multiplicity of selection, preference and resistance factors in different cultivars requires a thorough chemical analysis of the compounds found in the leaf tissues of the maize cultivars to detect their role in host-plant preferences. Nevertheless, very limited progresses have been done in developing resistance maize lines against the *S. frugiperda*. Transgenic maize hybrids (Bt-maize) expressing *B. thuringiensis* toxins can lead to a damage reduction by *S. frugiperda* (Burtet et al., 2017). Maize hybrids express the following proteins; Cry1A, Cry2A, Cry1F, and/or Vip3Aa20 could be used to reduce the pest damage and infestation. The major problem with the transgenic approach to suppress *S. frugiperda* is the durability of the insecticidal toxins, especially for single-toxin Bt, as resistance to Cry1F has been recorded in some countries (Farias et al., 2014; Huang et al., 2014). Conventional breeding has identified many effective ways of resistance to *S. frugiperda*;such as the rapid accumulation of proteins or phytochemicals (maysin, chlorogenic and aspartic acids, and cell wall/cellulose buildup) that enable plants to be toxic to the pests or cause pest starvation or other herbivores that feed on them (Constabel and Kurz, 1999). In addition to this induced direct defense tactic, the indirect defense mechanism possibility is through attracting the natural enemies of the pest (Chuang et al., 2014). Selection of plant host by *S. frugiperda* larvae and adults has been found to be affected by plant volatile materials, which can be used in establishing or developing the push-pull approach to control *S. frugiperda* (Rojas et al., 2018). Plant characters such as densities of both leaf hairs and/or cuticular wax layer were also stated to lessen plant foliar damage (Williams et al., 2000).

Crubelati-Mulati et al. (2020), reported that among 10 maize genotypes, the Teea Dulce, MG 161, Doce Flor da Serra, Doce Cubano and Tropical Plus have antibiosis resistance mechanism against *S. frugiperda* due to slower insect development. In addition, Sanches et al. (2019) found a lower *S. frugiperda* preference for Zapalote Chico rather than for other tropical popcorn genotypes. Ojumoola et al. (2022) tested the seasonal difference in the abundance of the fall armyworm larvae using 25 maize cultivars in Nigeria. They found that the cultivars,BR-9928DMR-SR and BR-9943DMR-SR are moderately attacked by the pest. Nelly et al. (2021) studied the population of the pest and level of the fall armyworm attack on five maize cultivars in Indonesia (NK7328, NK212, Bisi 18, Pioneer 32, and Pertiwi), and found that the pest attack rate was significantly different among the cultivars. The lowest attack percentage was on Bisi18, while NK212 was found to be the most preferred cultivar indicating by a high pest population and attack percentage as compared to other cultivars. Furthermore, Baudron et al. (2019), found that *S. frugiperda* damage was higher for the maize varieties, PAN413 and SC600 as compared to SC500 variety. Rosa-Cancino et al. (2016) investigated the attraction, performance and feeding preference of the fall armyworm larvae reared on hybrid (Pioneer P4063W) and landrace (Tuxpeno) of maize in Mexico. They reported that the fifth larval instar were more attracted to Pioneer P4063W maize plants than to Tuxpeno plants.

In conclusions, the fall armyworm is a key invasive insect pest causing huge yield losses to maize. This is a very alarming situation for Jordanian farmers. Thus, Jordan has begun to address the *S. frugiperda* problem. We have the basic information on maize resistant cultivars as a main IPM tactic to manage the pest in an ecofriendly manner. The Egyptian White Maize cultivar was the most resistant to larval feeding in both preference and susceptibility experiments, thus, maize farmers should start growing this cultivarof maize to reduce pest infestation. The outcomes of this study should be transferred to the Natural Agricultural Research Centre and Extension Department at the Ministry of Agriculture in Jordan to be distributed to the maize farmers in the country. Furthermore, the dissemination of the study results should be done through conducting farmer field schools (FFS) and workshops for the maize farmers. Nevertheless, it appears that future studies should focus on surveying the whole country to detect the pest whether on maize or other crops. Filed studies on the same cultivars tested in this study or more other cultivars should be done.The resistance factors in different cultivars require further analyzing the compounds in the leaves of these maize cultivars to know their role in host-plant resistance.

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