Heliyon 10 (2024) e32083

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

# Natural Enemies of the fall armyworm (*Spodoptera frugiperda* Smith) and comparing Neem aqueous extracts with its larvae, Gurage zone, central Ethiopia

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ARTICLE INFO

*Keywords:*
Aqueous extract
*Azadirachta indica*
Natural enemy
Parasitoids
Predators
Tachinid

ABSTRACT

The fall armyworm (*Spodoptera frugiperda* Smith) is an invasive and polyphagous insect pest. It poses a significant threat to maize crops, uncontrolled infestation can result 100 % loss. However, natural enemies play a vital role in regulating the population of this pest. Additionally, botanical sources extracts have the potential to be effective insecticides. The objectives of the study were to investigate the natural enemies of *S. frugiperda* in the Gurage zone and to compare efficacy of Neem seed and leaf aqueous extracts with *S. frugiperda* larvae, central Ethiopia. *S. frugiperda* larvae and egg masses, cocoons and larvae cadavers collected from infested maze farms. From each round collection 25 healthy and inactive larvae were sampled to rear until emerging adults. Observed predator species recorded. Neem seed and leaf aqueous extracts was tested against *S. frugiperda* in laboratory condition. The study found a diverse range of natural enemies associated with *S. frugiperda*, including parasitoids, predators, and entomopathogenic fungi. Three species of parasitoids (*Exorista xanthaspis*, *Tachina* spp., and *Charops annulipes*) were documented in Ethiopia for the first time. Predatory insects belonging to four distinct orders: Hemiptera, Dermaptera, Coleoptera, and Mantodea also identified. In particular, various Hemipterans were observed in the maize farms infested with *S. frugiperda*. In terms of Neem seed and leaf aqueous extracts, they demonstrated similar mortality rates for *S. frugiperda* larvae after 72 h, although differences were observed at 24 and 48 h. For effective management of *S. frugiperda,* more research is needed to fully exploit the potential of natural enemies and botanical source insecticides.

## 1. Introduction

Fall armyworm (*Spodoptera frugiperda* Smith) (Lepidoptera: Noctuidae) is native to tropical and subtropical regions of the Americas [1,2]. It is characterized as an invasive and polyphagous insect pest [3]. *S. frugiperda* was detected for the first time outside its native territory in central and western Africa countries in early 2016 [4,5]. Upon its arrival, *S. frugiperda* spread rapidly throughout Africa, Asia and Australia and more recently, it has invaded Europe [6].

*S. frugiperda* was first reported in Ethiopia in 2017 [7,8]. It is responsible for causing significant damage to the maize crop. Studies conducted by Kumela et al. [8] estimate that this pest leads to 32 % of the loss of maize yield. If left uncontrolled, CABI [9] warns that

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https://doi.org/10.1016/j.heliyon.2024.e32083
Received 20 March 2024; Received in revised form 24 May 2024; Accepted 28 May 2024
Available online 29 May 2024
2405-8440/

*S. frugiperda* infestation can result in a 100 % loss of the maize crop. Fortunately, several methods can be employed to mitigate the impact of *S. frugiperda*. These control techniques include the use of insecticides, biological agents, cultural practices, and integrated pest management (IPM) strategies. Chemical insecticides have been the primary means of controlling *S. frugiperda* [10]. However, the use of insecticides raises environmental concerns and causes a threat to beneficial insects, leading to unintended consequences. To effectively manage *S. frugiperda*, Dequech et al. [11] explain that it is crucial to consider the implementation of IPM strategies which prioritize natural enemies.

Natural enemies such as entomopathogens such as fungi, bacteria, viruses, and nematodes [12]; parasitoids [13] and predators [14, 15] can attack *S. frugiperda*. Parasitoids significantly reduce the eating ability and weight gain of *S. frugiperda* larvae [16]. For example, it has been shown that in individual parasitized *S. frugiperda* larvae, the parasitoid *Coccygidium luteum* causes 89 % decrease in leaf consumption [17]. There are 150 species of *S. frugiperda* parasitoids reported to exist in the Americas and the Caribbean [18]. In some African countries, including Ethiopia, Kenya, Tanzania, Uganda, Ghana, Senegal, Niger, South Africa, and Mozambique, studies have found parasitoids at various stages of the pest's development [14,17,19–25] - as well as in Egypt [26]. There are plant species that can support natural enemies and providing plant extracts as botanical insecticides [27]. Botanical source insecticides are economical, easy to-use and important alternatives to manage pests [28].

The use of botanical extracts against insect pests is efficient, cost-effective, reduces the cost of crop production, safe for humans and the environment [29]. They are also possible to incorporate them into IPM programs [28,30] which can reduce the use of synthetic chemicals [28]. IPM uses non-chemical or botanical insecticide measures to suppress pest population [31]. The Neem extract (*Azadirachta indica* Juss) has a great potential to control *S. frugiperda* compared to other synthetic pesticides [32]. Effectiveness of aqueous extract of Neem tree parts, such as powder form of leaf and seed and green leaf, against insect pests including *S. frugiperda* reported [32–34], but their effectiveness was not compared among them.

Vertebrates and invertebrates, such as birds, bats, and generalist predator insects, have been observed to directly consume eggs and larvae of *S. frugiperda* in maize fields, leading to a reduction in their population [19]. Our study focused on identifying predator insects present in maize farms infested with *S. frugiperda*. Three species of larval parasitoids of *S. frugiperd* such as *Palexoristazonata (Tachinidae), Cotesia icipe,* and *Coccygidiumluteum* (Braconidae) have been identified from Ethiopia [13]. Despite this, a thorough record of the native natural enemies of *S. frugiperda* is lacking. Therefore, the identification of native natural enemies is essential for the successful implementation of biological control programs. The Neem tree is also a common plant, which is easily available and obtained freely. Thus, the objectives of the study were to investigate local natural enemies of *S. frugiperda*, evaluate parasitism rates and relative parasitoids' abundances in the Gurage Zone, and to compare the efficacy of Neem seed and leaf aqueous extracts against *S. frugiperda* larvae, central Ethiopia.

## 2. Materials and methods

### *2.1. Description of the study areas*

This study was carried out in Gurage zone, central Ethiopia. The survey specifically focused on two districts in different agro-ecological zones within zone. As described in Table 1 geographic coordinates including altitude, latitude, and longitude were recorded for each study sites. Survey sites were selected based on the presence of *S. frugiperda* and the specific agro-ecology of the region. Two kebles or sites selected purposively to represent the lowland and midland agro-ecologies. For the first zone, known as the lowland agro-ecology, Gibe-Serite kebele from Abeshge district deliberately selected. It is located about 180 km from Addis Ababa on Addis Ababa-Jimma asphalt road. June, July and August months are rainy seasons of the year. For the second zone, the mid-land agroecology, the survey focused on the Ewan-Chuqara kebel in the Cheha district. The site is located about 166 km from Addis Ababa on Addis Ababa-Wolkite- Hossana asphalt road. This district mainly characterized by mid-land agro-ecology (Weyna Dega) climate, it receives annual rain fall of 1229 mm.

### *2.2. Assessment of natural enemies of fall armyworm (S. frugiperda)*

Natural enemies of *S. frugiperda* were investigated in randomly selected 150 small-holder maize farms between June and August 2023. Samples collected in three rounds, in each round 25 maize farms were assessed per each study site. Sampling was carried out in five plots selected in 'W' design pattern with ten maize plants from each maize farms. To avoid border effects, the first two border rows, or 1 m, on either side of the farms were excluded. Assessment was done early in the morning and late in the afternoon since *S. frugiperda* is nocturnal insect. The evaluation focused on maize farms with plants in growth stages V3 (three leaves with collar) through V10 (ten

**Table 1**
Geographical positioning reading of study sites (latitude, longitude and altitude).

| Survey sites (Agro-ecology) | Latitude (N) between | Longitude (E) between | Altitude meter above sea level (Range) | Survey Duration |
|---|---|---|---|---|
| Gibe-Serite (low-land) | 8° 14′ 22.86″ and 8° 15′ 33.85″ | 37° 34′ 16.51″ and 37° 58′ 25″ | 1100–1150 | Three rounds in June, July and August 2023 |
| Ewan-Chuqara (Mid-land) | 8° 13′ 18.12″ and 8° 13′ 33″ | 37° 46′ 43.52″ and 37° 49′ 52″ | 1850–1914 | |

leaves with collar). Farmers interviewed whether maize farms is sprayed with insecticide or not to exclude farms that had been sprayed with insecticides. In *S. frugiperda* infested maize farms, we examined the presence of natural enemies of its eggs and larvae, including entomopathogens, parasitoids, and predators. To locate areas infested by *S. frugiperda*, we searched for fresh frass and observed feeding damage on whorls and leaves. The study deliberately omitted the pupal stage of *S. frugiperda*, as it typically occurs in the soil.

### 2.3. Assessment of parasitoids of fall armyworm (S. frugiperda)

Sampling was carried out purposively by taking eggs, larvae, and parasitoid cocoons found in maize plants that had been infested by the pest. To document larval parasitism, a total of 25 healthy and inactive larvae were sampled in different stages of instar following the guidelines provided by FAO [18]. These eggs and larvae were carefully transferred to a laboratory setting to complete their development. To house *S. frugiperda* larvae, a rectangular plastic box with dimensions of 9 cm × 13 cm × 12 cm was used. The top of the box was covered with a net that had not been treated with insecticides, allowing adequate ventilation while preventing the larvae from escaping. The laboratory maintained a temperature of 25 ± 2 °C and a relative humidity of 73 ± 3 %. Within the laboratory environment, *S. frugiperda* larvae received maize leaves as their primary source of nourishment until they reached the pupal stage, following the procedure outlined by Riggin et al. [35].

Upon pupation, the pupae were carefully collected and placed in petri dishes with a diameter of 9 cm. During a span of approximately 9–12 days, the emerging parasitoids were observed, numbered, and carefully preserved for further identification. The identification process involved by observing and matching the morphological characteristics for each parasitoid species, as well as references from previously published works by Sari et al. [36], Navik et al. [37], and Prasanna et al. [38]. It is important to note that no dissections were performed to search for dead parasitoids within the dead larvae or pupae. The parasitism rate was determined using the following formula:

$$\%\ \text{Parasitism} = \frac{\text{Number of Parasitoids emerged}}{\text{Total number of FAW larvae sampled}} \times 100$$

$$\%\ \text{Total parasitism} = \frac{\text{Total number of parasitoids}}{\text{Total number of FAW larvae sampled}} \times 100$$

### 2.4. Relative abundance of parasitoids from S. frugiperda

To determine the proportionate occurrence of each species of parasitoids, a calculation was performed. This involved dividing the count of distinct parasitoid species by the total count of distinct parasitoids that emerged, and subsequently multiplying the result by 100 as represented by the following formula.

$$\%\ \text{Relative abundance} = \frac{\text{Number of individual parasitoid spp.}}{\text{Total number of parasitoids}} \times 100$$

### 2.5. Assessment of predators and entomopathogen fungi of S. frugiperda

Traps were not utilized; instead, visual observation was employed to determine the predators that targeted the larvae and eggs of *S. frugiperda*. This observation was done in five plots per each selected maze farms. The presence of species recorded not the number and life stages. These observed predators were collected as specimens. Depending on their size, the samples were carefully preserved in a 50 ml centrifuge tube, a microtube (which is a screw cup with an O-ring and a capacity of 2 ml), or a 1-L plastic jar. A graphical representation was employed to illustrate the natural enemies of *S. frugiperda*. To identify collected specimens as predators at various stages of *S. frugiperda* development published references Sari et al. [36], Prasanna et al. [38] and Ahissou et al. [39]. Some of them after confirming as they are predate on *S. frugiperda* in the field their name identified using Google based image search. Furthermore, dead *S. frugiperda* larvae resulting from infections caused by entomopathogenic fungus were observed directly in maize whorls or on the leaves. Those suspected larvae collected and keep them in laboratory to observe any fungal development. The emergence of hyphae from dead *S. frugiperda* larvae was examined with naked eye to confirm the occurrence of entomopathogenic fungal infections [39].

### 2.6. Neem (Azadirachta indica) aqueous extracts preparation

Neem (*Azadirachta indica)* insecticide made from seed powder and fresh leaves. Neem seeds and leaves collected and dried in the shade. The dried seeds and leaves were then ground into fine powder using a small an electric spice grinder machine and kept in cool dark conditions until required. Aqueous extracts prepared by mixing 100 g of each Neem seed and leaf powder separately with 1 L of water containing 0.1 % soap (10 % w/v) and allowing it to steep overnight. The extracts were filtered using cheesecloth and then used for the test. The third treatment was made from fresh leaves of Neem tree that were washed and soaked in water at a 1:2 ratio (500 g in 1 L of Water, w/v) for 24 h at room temperature to remove impurities. After being soaked, the leaves were ground into small pieces and stored in a 1-L plastic container. The plastic container was filled with water with 0.1 % soap and then placed on a shaker, and the leaves were further broken down. The solution was filtered through cheesecloth to obtain pure Neem (*A. indica)* leaf extract and then transferred to a bottle for use in the laboratory [32]. The added soap helped as a surfactant/emulsifying agent.

### 2.7. Laboratory bioassay tests

The study was carried out in a complete randomized design with five treatments: two controls (pure water and water with 0.1 % soap), 10 % Neem seed, 10 % dry leaf and green leaf (1:2 dilution of Neem extract). Each treatment was replicated three times in a rectangular plastic box that measures 9 cm × 13 cm × 12 cm. *S. frugiperda* larvae (3rd instar) collected from maize farms in the Cheha district of central Ethiopia. The feeding bioassay screening was carried out by dipping young maize leaves into each extract, waiting 1 h for the extract to dry and placed in each plastic container. Ten *S. frugiperda* larvae were released into each container with treated maize leaves. Top of a container covered with an untreated net to allow ventilation while preventing larvae from escaping. The laboratory had a temperature of 25 ± 2 °C and a relative humidity of 73 ± 3 %. Larval mortality was recorded at 24, 48, 72 and 96 h post-treatment by counting the number of dead larvae. A larva was considered dead if it could not be shifted after being placed on its dorsal surface [32].

### 2.8. Data analysis

Data analysis involved computing the percentage of parasitism and determining the relative abundance of parasitoids using Microsoft Excel 2010. Parasitism rate was calculated dividing number of parasitoids emerged by total number of *S. frugiperda* larvae sampled and multiplied by 100. While the relative abundance of parasitoids calculated by dividing the number of distinct parasitoid species by the total number of distinct parasitoids and multiplied by 100. The mean mortality of *S. frugiperda* larvae was compared among treatments using one-way ANOVA using Minitab 16. Tukey's test was used to compare the mortality of *S. frugiperda* larvae for each period tested among treatment levels, with a 95 % confidence interval.

## 3. Results

### 3.1. Natural enemies of fall armyworm (S. frugiperda)

In central Ethiopia, an investigation was conducted to identify natural enemies that inhabit maize farms infested with *S. frugiperda*. General information on the natural enemies recorded of *S. frugiperda* is summarized in Table 2. Three parasitoids and ten predator insects were recorded from eight different families (Table 2). Mortality of *S. frugiperda* larvae and parasitoid cocoons collected and parasitoids identified, including their parasitism rate and relative abundance presented in Table 3. Furthermore, this study also observed various predators of *S. frugiperda* eggs and larvae, as well as entomopathogenic fungi in *S. frugiperda* cadavers. However, it should be noted that parasitoids and entomopathogen fungi were only detected in lowland agroecology areas.

Among the recorded parasitoids, three species were identified. Two of these species, namely *Exorista xanthaspis* (Wiedemann, 1830) and *Tachina* spp., were found infesting *S. frugiperda* larvae. Both species belong to the Diptera order and the Tachinidae family (Table 2 & Fig. 1a and b). Another parasitic wasp species, *Charops annulipes* (Ashmead, 1891), emerged from the cocoons of parasitoids collected from maize farms. This particular species belongs to the Hymenoptera order and the Ichneumonidae family (Table 2 & Fig. 1c).

*Exorista xanthaspis* exhibited the highest prevalence among parasitoids, with a parasitism rate of 4 % and a relative abundance of 50 %. Most of the *S. frugiperda* parasitoids, approximately 67 %, were tachinid flies (Diptera), while the remaining 33 % belonged to the wasp insect family Ichneumonidae (Hymenoptera). Overall, the parasitism rate for *S. frugiperda* larvae was calculated at 5.3 % (Table 3).

In Fig. 2, various arrays of predator insects and entomopathogens found on maize farms infested with *S. frugiperda* are presented. In this study, we documented ten species of predatory insects belonging to four distinct orders: Hemiptera, Dermaptera, Coleoptera, and Mantodea. In particular, various Hemiptera (Bugs) insects were observed in the maize farms infested with *S. frugiperda,* actively

**Table 2**
Natural enemies recorded from *S. frugiperda* infested maize farms, central Ethiopia, June to August 2023.

| Natural Enemies | Order | Family | Natural enemy type | Site |
|---|---|---|---|---|
| *Exorista xanthaspis* [37] | Diptera | Tachinidae | Parasitoid | Gibe-Serite |
| *Tachina* spp. [38] | Diptera | Tachinidae | Parasitoid | Gibe-Serite |
| *Charops annulipes* [36] | Hymenoptera | Ichneumonidae | Parasitoid | Gibe-Serite |
| *Dalpada trimaculata*[a] | Hemiptera | Pentatomidae | Predator | Ewan-Chuqara & Gibe-Serite |
| *Veterna* spp.[a] | Hemiptera | Pentatomidae | Predator | Ewan-Chuqara & Gibe-Serite |
| *Podisus* spp.[a] | Hemiptera | Pentatomidae | Predator | Ewan-Chuqara |
| *Spilostethus pandurus*[a] | Hemiptera | Lygaeidae | Predator | Ewan-Chuqara & Gibe-Serite |
| Shield bug[a] | Hemiptera | Pentatomidae | Predator | Ewan-Chuqara & Gibe-Serite |
| Termite Assassin bugs [40] | Hemiptera | Reduviidae | Predator | Ewan-Chuqara & Gibe-Serite |
| *Doru luteipes* [38] | Dermaptera | Forficulidae | Predator | Ewan-Chuqara & Gibe-Serite |
| *Cheilomenes lunata*[a] | Coleoptera | Coccinellidae | Predator | Ewan-Chuqara & Gibe-Serite |
| *Cheilomenes sulphurea* [39] | Coleoptera | Coccinellidae | Predator | Ewan-Chuqara & Gibe-Serite |
| Mantids [39] | Mantodea | Mantidea | Predator | Ewan-Chuqara & Gibe-Serite |
| Fungi [39] | Hypocreales | Clavicipitaceae | Pathogen | Gibe-Serite |

[a] Their name identified using Google based image search.

**Table 3**
A) Parasitism rate and Mortality of collected *S. frugiperda* larvae and B) Parasitoids identified from *S. frugiperda* larvae and parasitoid cocoons, their relative abundance and parasitism rate, central Ethiopia (2023).

| A) Parasitism rat and Mortality of collected *S. frugiperda* larvae | | | | |
|---|---|---|---|---|
| **Site** | **Larvae Collected** | **Mortality by Parasitoids (%)** | **Mortality by other factors (%)** | **Emerged Adults (%)** |
| Gibe-Serite | 75 | 4 (5.3 %) | 41 (54.7 %) | 30 (40 %) |
| Ewan-Chuqara | 75 | – | 38 (50.7 %) | 37 (49.3 %) |

| B) Identified Parasitoid Species and their relative Abundance | | | | |
|---|---|---|---|---|
| Number of *S. frugiperda* Larvae or pupa | Number of Parasitoids | Species of Parasitoids | Parasitism (%) | Relative Abundance (%) |
| 75 | 3 | *Exorista xanthaspis* | 4 | 50 |
| | 1 | *Tachina* spp. | 1.3 | 16.7 |
| 5 parasitoid Cocoons | 2 | *Charops annulipes* | Emerged from collected cocoon | 33.3 |
| | | **Total parasitism** | 5.3 | |

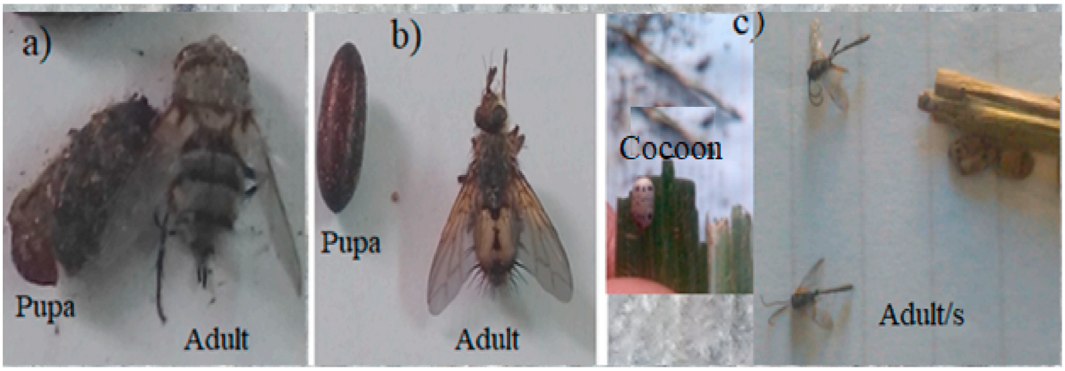


**Fig. 1.** Parasitoids recorded (a) *Exorista xanthaspis* and (b) *Tachina* spp. (Diptera: Tachinidae) from *S. frugiperda* larvae and (c) *Charops annulipes* (Hymenoptera: Ichneumonidae) (ichneumonid wasps) from parasitoid cocoons collected in *S. frugiperda* infested maize farms (Photo by: Abera H.).

preying on the larvae of *S. frugiperda* (as illustrated in Fig. 2a–f). Furthermore, Dermaptera, commonly known as earwigs, were frequently observed consuming *S. frugiperda* larvae (Fig. 2g). Ladybird beetles (Coleoptera) were consistently present in all maize farms affected by *S. frugiperda*, as is evident in Fig. 2h and i. Furthermore, mantodea, commonly referred to as mantids, were directly observed preying on *S. frugiperda* larvae in maize plants (Fig. 2j). We also observed the occurrence of *S. frugiperda* larvae being killed by an entomopathogenic fungus in maize farms infested with *S. frugiperda* (Fig. 2k).

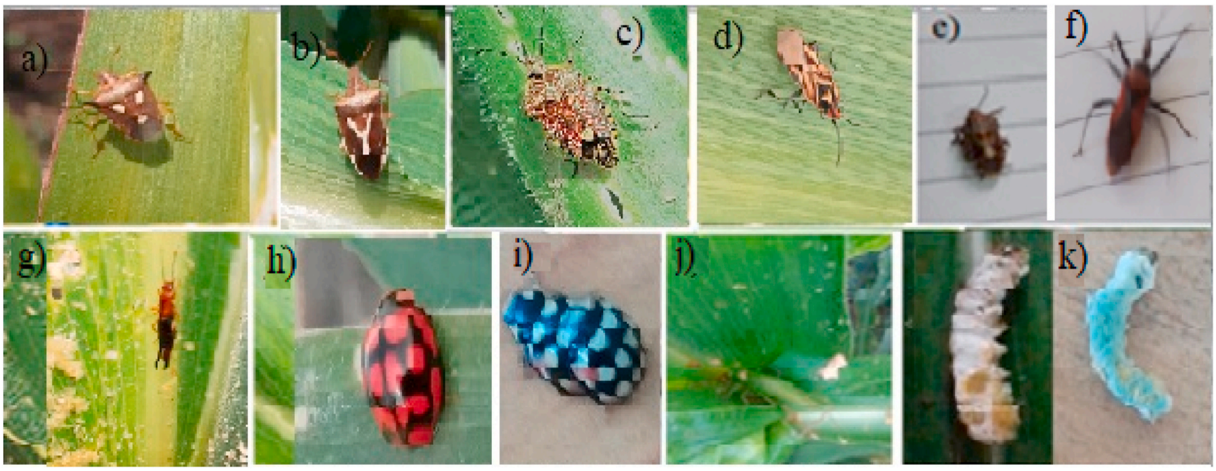


**Fig. 2.** Different predator insects and entomopathogen recorded from maze farms infested with *S. frugiperda* (a–f) Bugs/Hemiptera (a) Stink bug (*Dalpada trimaculata*), (b) Grass stink bug (Veterna spp.), (c) *Podisus* spp. (d) Seed bug (*Spilostethus pandurus),* (e) Shield bug, & (f) Termite Assassin Bugs; (g) Earwig/Dermaptera (*Doru luteipes*); (h–i) Ladybird beetles/Coleoptera, (h) *Cheilomenes lunata* & (i) *Cheilomenes sulphurea*; (j) Mantidea, and (k) *S. frugiperda* larvae killed by entomopathogen fungus on maize (Photo by: Abera H.).

### 3.2. Efficacy of Neem (Azadirachta indica) aqueous extracts with S. frugiperda larvae

Table 4 shows one-way ANOVA comparisons of mean mortality rate of *S. frugiperda* larvae at 24, 48, 72 and 96 h with different treatments. Each Neem aqueous extract form, the mean mortality of the *S. frugiperda* larva at 24 h of recorded test was below 50 %. However, at 72 h of the test, the mean mortality of the *S. frugiperda* larva recorded from the powder of Neem seed and leaf and the green leaf aqueous extracts was 82.2 %, 81.7 % and 80.0 %, respectively, with 3.33 % in negative controls. In all Neem aqueous extract forms at 96 h the mortality rate of *S. frugiperda* larvae was 100 %. There were significant differences in the percentage of mortality of *S. frugiperda* larvae between Neem seed powder and green leaf extracts at 24 and 48 h after application of the treatments at $p < 0.05$. However, there was no significant difference at 72 and 96 h in three of the aqueous forms of Neem extract.

## 4. Discussion

A study carried out in maize farms infested with *S. frugiperda* has identified various types of natural enemies, including parasitoids, predators, and entomopathogenic fungi. The presence of parasitoids was observed in lowland agroecological areas, while no parasitoids were found in collections from midland agroecology. This disparity could be attributed to ecological factors such as plant diversity, rainfall patterns, temperature, and relative humidity. Chipabika et al. [41] also mentioned that the differences between locations, rainfall, maize crop stage, pest density, and larval stage attribute the absence of parasitoid species. Quispe et al. [42] further support this assumption by demonstrating that the abundance of flowering plants and their pollen or nectar near farms influences the presence of parasitoids. The study recorded three species of parasitoids that target *S. frugiperda* larvae. These species include *Charops annulipes* (Ichneumonidae: Hymenoptera), *Exorista xanthaspis*, and *Tachina* spp. (Tachinidae: Diptera). The three species of parasitoids were recorded from Ethiopia for the first time in association with *S. frugiperda*.

Among the observed parasitoids, *E. xanthaspis* was the most prevalent and the primary cause of general parasitism. Similar observations have been made in maize farms in India, where *E. xanthaspis* was found to parasitize *S. frugiperda* larvae and pupae [37]. CABI [9] also supports this finding, stating that *E. xanthaspis* is a parasitic fly found in Africa, Asia and Europe, known to parasitize the larvae and pupae of various agricultural pests. Tachinid flies have been associated with *S. frugiperda* in multiple African countries. For example, *Drino quadrizonula* was discovered in Benin and Ghana, while *Palexorista zonata* (Curran) was identified as a parasitoid in Ethiopia, with a larval parasitism rate of 6.4 % in *S. frugiperda* [13]. Tachinid flies have also been observed to parasitize lepidopteran larvae [43]. Before the arrival of *S. frugiperda*, Tachinidae species were already known to parasitize cereal stem borer larvae in Africa [44]. These flies exhibit a wide host range and target various insect species and stages of insect development, such as obligatory parasitoids and endoparasitoids [14,38,45]. These findings support the hypothesis that tachinid flies play a key role in the biological management of insect pests. Parasites have evolved specialized adaptations to locate and attack their hosts, making them highly effective natural enemies.

A small parasitic wasp called *Charops annulipes* (Ichneumonidae: Hymenoptera) was discovered emerging from its cocoon (Fig. 1c). Our findings further confirm that *Charops* spp. has been identified as a parasitoid of *S. frugiperda* larvae, with a parasitism rate ranging from 0.2 % to 1.6 %, as reported by Sari et al. [36] in western Sumatera, Caniço et al. [21] in Mozambique and Agboyi et al. [17] in Ghana and Benin. This parasitoid was also observed in *S. litura* larvae, which is another host [46]. Similarly Estrada Virgen et al. [47] from Mexico reported that larval parasitoids of *S. frugiperda* have been detected from the orders of Hymenoptera (Ichneumonidae) and Diptera (Tachinidae). In this study, no egg parasitoids were documented, which may be due to their scarcity. Although there are some parasitoids that target the eggs and larval-pupal stage, Clarkson et al. [19] description supports the notion that the majority of *S. frugiperda* parasitoids in Africa primarily parasitize the larvae.

The discovery of dead *S. frugiperda* larvae in maize farms infested with *S. frugiperda* and affected by an entomopathogenic fungus was observed. Initially, the larvae appeared rigid and covered in white mycelium, then later turned greenish in color (Fig. 2k). Gómez-Valderrama et al. [48] similarly observed that *S. frugiperda* larvae, when killed by fungal action, exhibited a gradual stiffening process and eventually became completely coated in white mycelia. The mycelia eventually produced powdery green conidia. It is possible that the fungus responsible for this phenomenon belongs to the genus Metarhizium. Herlinda et al. [49] described Metarhizium fungi as having yellowish-white colonies that eventually transformed into dark green conidia. Similarly, *S. frugiperda* larvae infected with the

**Table 4**
Mean percentage of *S. frugiperda* larval mortality at 24, 48, 72 and 96 h after application of treatments under laboratory and pairwise comparisons by one-way ANOVA.

| Treatments | Mean percentage *S. frugiperda* larvae mortality | | | |
|---|---|---|---|---|
| | 24hrs ± SE | 48hrs ± SE | 72hrs ± SE | 96hrs ± SE |
| Neem seed (10 % powder) | 30.0 ± 5.77a | 70.0 ± 0.00a | 82.2 ± 3.33a | 100.0 ± 0.00a |
| Neem leaf (10 % powder) | 21.7 ± 3.33 ab | 56.7 ± 6.67 ab | 81.7 ± 5.77a | 100.0 ± 0.00a |
| Neem green leaf (50 % chopped) | 13.3 ± 3.33b | 50.0 ± 2.89b | 80.0 ± 6.67a | 100.0 ± 0.00a |
| 0.1 % soap | 0.00 ± 0.0c | 0.0 ± 0.0c | 3.33 ± 3.33b | 3.3 ± 3.33b |
| Water | 0.00 ± 0.0c | 3.33 ± 3.33c | 3.33 ± 3.33b | 3.3 ± 3.33b |
| F-test | 15.8 | 81.04 | 91.14 | 630.7 |
| P-Value | 0.000 | 0.000 | 0.000 | 0.000 |

Means ± SE with different letters in the same column are significantly different at $P < 0.05$ through one-way ANOVA. Tukey's test at 5 % was performed to compare means between the treatments.

entomopathogenic fungi Metarhizium were found in maize farms infested with the pest [12,50]. Firake and Behere [50] also mentioned that the *Beauveria bassiana* fungus has the ability to kill larvae of *S. frugiperda.*

We observed various predator insects, including earwigs (*Doru luteipes*), different types of bugs, ladybird beetles (*Cheilomenes lunata* and *Cheilomenes sulfurea)* and mantids. Among these, the hemiptera insects, specifically the true bugs, exhibited a higher number of species compared to other insect orders (Fig. 2). Our findings align with previous studies in America by Capinera [51] that Hemiptera groups (Pentatomidae) are important predators on *S. frugiperda*. Additionally he mentioned the predators of this pest are general predators that attack many other caterpillars. Prasanna et al. [38], also highlights the role of *Doru lineare earwigs* and *D. luteipes* in the attack of *S. frugiperda* eggs and larvae. Similarly, ladybird beetles and Mantidae, known as entomophagous insects, were also found to prey on *S. frugiperda* larvae [50,52,53]. These predator insects, including earwigs, exhibit generalist feeding behavior, targeting various stages of *S. frugiperda* [12,50], as well as aphids and stem borers in maize and rice fields [3]. Therefore, it is crucial to identify and understand the local natural enemies of *S. frugiperda* before implementing any classical biological control methods. For small-holder farmers in addition to locally available natural enemies other cheaper pest management options required. According to Isman [54] description in developing countries, plant extracts prepared from commonly available plant species that grow around the field, resulting in cheaper pest management alternatives.

It is known that the use of plant extracts against *S. frugiperda* is considered efficient, cost effective, and safe for humans and the environment [55]. Neem is one of the most effective insecticidal plant species used to control many insect pests, including *S. frugiperda* [34]. The evaluation of Neem seed and leaf aqueous extracts showed promising results, highlighting its potential as an environmentally friendly and sustainable approach to controlling this destructive pest. It acts as insecticidal activities against *S. frugiperda* compared to controls. For the first 48 h, there was a significant difference in *S. frugiperda* larvae mortality caused by seed powder and green leaf extract form. However, after 72 h, all three forms of Neem seed and leaf aqueous extract caused 80–82 % mortality in *S. frugiperda* larvae with no significance difference. In the laboratory, it was found that the percentage of larval death rose over time after the application of Neem aqueous extracts, which may imply that the bioactivity of Neem still has some residual toxicity. We observed that Neem bioactivity can effectively control *S. frugiperda.* This observation strengthens the claim of Kamunhukamwe et al. [32] that *A. indica* leaf extracts' ability to inhibit *S. frugiperda* is just as essential as synthetic insecticides. Subsequently, all extract forms were equally significant. This result supported by Kamunhukamwe et al. [32], in the laboratory *S. frugiperda* larval mortality increased over time after application of Neem insecticide, this indicates residual toxicity of the Neem bio-insecticide to the pest. It is known that Neem seed kernel and leaf extracts act as repellents, growth regulator, and larvicidal activities on insect pests [56,57]. On the contrary, Silva et al. [34] reported that Neem seed extract is more toxic than leaf extract against *S. frugiperda*.

## 5. Conclusions

The study identified various natural enemies of *S. frugiperda* in the Gurage zone, which can contribute to the management of the pest. The efficacy of Neem aqueous extracts was also demonstrated. Based on the study results, we suggest that conserving the existing parasitoids and predators in the area (conservation biological control) and utilizing locally available entomopathogenic fungi can effectively contribute to reducing *S. frugiperda* populations. Although predators can quickly devour all life stages of the target pests, parasitoids and entomopathogens can develop within or on a living host, eventually leading to its death. By promoting the presence and activity of these natural enemies through the use of biological agents and cultural practices, the negative impact of *S. frugiperda* can be minimized, thus reducing the dependence on chemical interventions. Additionally, locally available botanical source insecticides such as Neem tree are an alternative as a management strategy of the pest. Neem leaves are available than seeds, and preparation of green leaf aqueous extract is economical. Therefore, small-holder farmers are better off using the green leaf form of Neem than the seed and leaf powder forms among the three alternative aqueous extracts of Neem for the treatment of *S. frugiperda*. Further more research is needed to fully exploit the potential of natural enemies and pesticides from botanical sources.

## Data availability

Additional data will be made available on request.

## CRediT authorship contribution statement

**Abera Hailu Degaga:** Writing – review & editing, Writing – original draft, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Emana Getu Degaga:** Writing – review & editing, Visualization, Validation, Supervision, Resources.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

The authors are thankful to the maize farmers who allowed access to their farms for sample and data collection.

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