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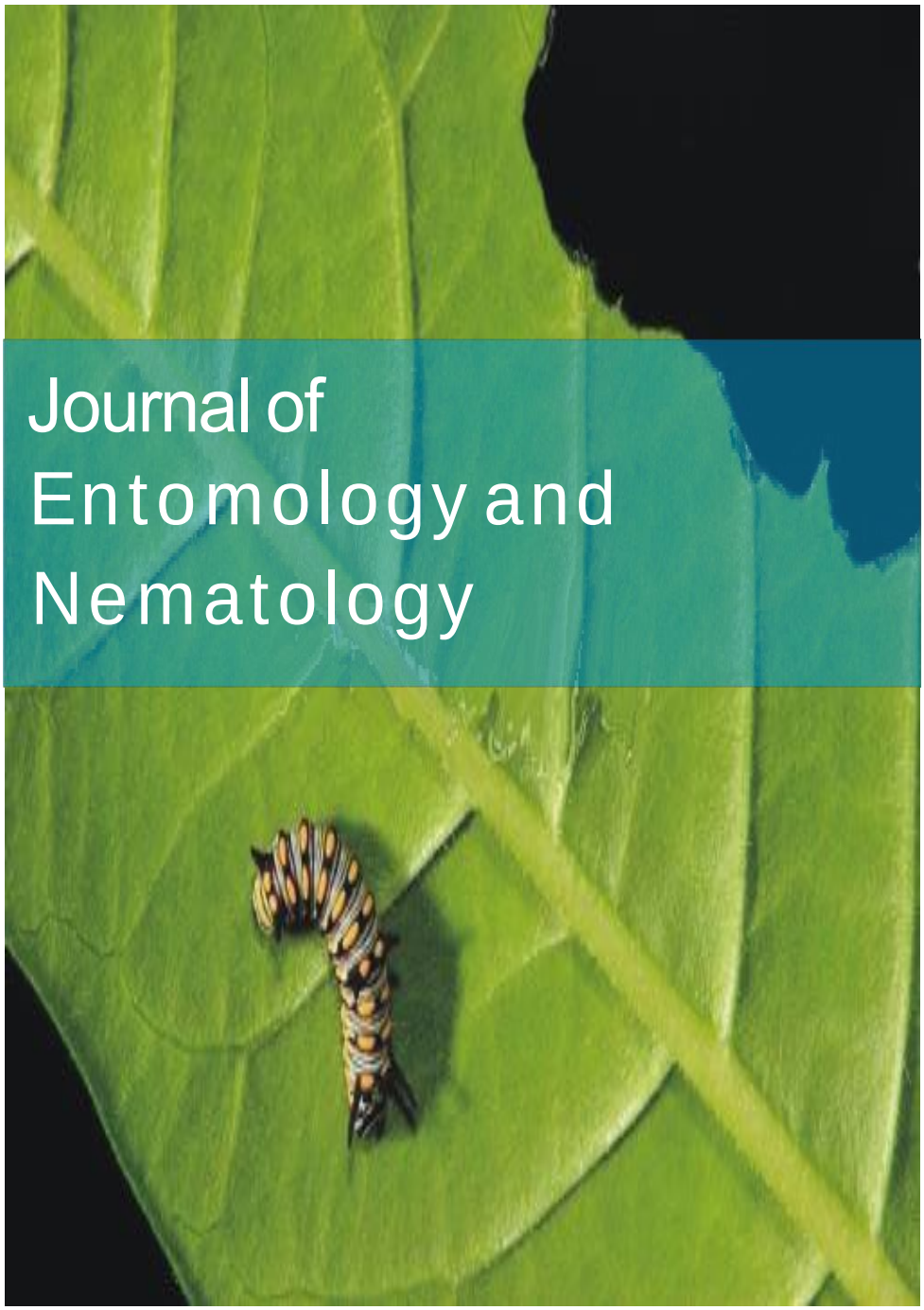

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Full Length Research Paper

Cannibalism and necrophagy in *Spodoptera frugiperda* and *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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For many years it has been known that cannibalism is a natural phenomenon in insects and has been documented in many insect orders including Lepidoptera. The present study was conducted to determine cannibalistic behavior amongst same-age 2nd, 4th and 5th instar stages of larval *Spodotera frugiperda* (FAW), *Spodotera littoralis* (CLW) or when both are combined together under laboratory conditions. The FAW larvae had a higher predatory ability than CLW larvae. However, when both same stage of FAW and CLW are combined together, the survivability and predation ability of FAW larvae were higher than for CLW. Necrophagy in FAW became more frequent as the larvae developed from young to older larvae (L2 to L4 and L5, with mortality of 24.7, 42.7 and 52.6%, respectively. CLW larvae are rarely fed entirely on their fellow larvae. Mortality due to necrophagy in CLW was highly significant among the different stages. Mortality rate due to necrophagy amongst tested stages of the 2nd, 4th and 5th instars was 1.33, 14.7, and 12.1%, respectively. Rearing FAW and CLW in laboratory shows that larval cannibalism is necessary for FAW, but not for CLW. Also, the cannibalistic behavior consequently affected the adult yield in each group of tested larvae and was stage-dependent. The percentage of emerged moths was greatest amongst CLW larvae of tested stages where no larval cannibalism was observed. The adult's yield of FAW was lower when reared alone and lowest when combined with CLW indicating that all CLW and some FAW larvae were cannibalized.

Key words: Cannibalistic behavior, fall armyworm, cotton leaf worm, larval survivorship, adult yield.

INTRODUCTION

For many years it has been known that many species of lepidoterous larvae frequently engage in cannibalism. The larvae are cannibalistic, under certain conditions killing and devouring fellow larvae, especially when competing for food (George, 1936; Joyner and Gould, 1985; Pierce, 1995; Boots, 1998; Chapman et al., 1999a). Cannibalism (that is, the killing and eating of conspecific individuals, either fully or partly), defined as intraspecific predation, is a characteristic behavioral found in a wide variety of animals, most references to this

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behavior are based on casual laboratory observations (Fox, 1975). Feeding on dead individuals of the same species is defined as necrophagy. These habits have been documented in several insect species. Some authors have suggested that cannibalism is an artifact of laboratory systems (Colinvaux, 1973) or that it occurs only in cases of severe stress, especially when alternatives, such as dispersal, are not possible (Fan et al., 1991). Richardson et al. (2010) reported that cannibalism may play different roles in the ecology of a population, such as stabilising the host-plant/insect relationship, regulating population density, and providing a selective advantage by removing conspecifics that are infected with pathogens, which reduces the rates of parasitism in subsequent generations (Richardson et al., 2010).

The Fall armyworm, *Spodoptera frugiperda* (J.E. SMITH) (FAW) and the cotton leaf worm *Spodoptera littoralis* (Boisd.) (CLW) are worldwide destructively polyphagous insects, and often cause numerous losses in almost the same agricultural crops including maize plants. The CLW is native to Africa and Israel and widely found in both Africa and the Mediterranean Europe (EPPO, 2019). The FAW is existing in the tropical and sub-tropical regions of the West and South of America (Otim et al., 2021). The FAW has been able to expand throughout Africa, much of Asia and reached Australia following its invasion into West Africa (Dahi et al., 2020). The FAW is a new invasive insect pest in the Nile Delta, Egypt (Dahi et al., 2020). Both of FAW (invasive) and CLW (local pest) insects have six larval instars and are non-diapausing insects (Hegazi et al., 1977; Du Plessis et al., 2020), so they spread searching for their plant hosts in areas far from the cold sites (Huang et al., 2013).

Cannibalism is known to occur in the cotton leafworm, *S. littoralis* when the availability of food is reduced (Fox, 1975). Cannibalistic behavior has been documented for larval of the FAW, in both field and laboratory conditions (Raffa, 1987; Chapman et al., 1999a). In order to learn more about the cannibalistic habits of these larvae, a detailed study was made under laboratory conditions. The main objective was to determine cannibalistic behavior amongst same-age 2nd, 4th and 5th instar stages of larval *S. frugiperda* (FAW) and *S. littoralis* (CLW). The incidence of larval cannibalism and necrophagy amongst same-age larvae of each of CLW or FAW was investigated using 2nd, 4th and 5th instars with not less than five trials for each instar stage/insect species. The results of present investigation would provide some useful information to avoid cannibalism during the process of mass-rearing of the present insect pests in the laboratory. This study was based mainly on lab experiments and direct estimates of rates of cannibalistic events, to fill gaps in the field evidence.

MATERIALS AND METHODS

Series of experiments were carried out in the biological control laboratory at the Department of Economic Entomology, Faculty of Agriculture, Alexandria University, Egypt using the Fall armyworm, *S. frugiperda* (FAW) and the cotton leaf worm *S. littoralis* (CLW) to investigate the rate of intra- and inter- consumption by cannibalism and necrophagy during larval development from 2nd, 4th or 5th instars to adulthood.

Insects

Each of FAW and CLW were derived from cotton and maize field-collection strains during June-August, 2023, Alexandria University farm at Abis, Alexandria, Egypt. Both insects were mass reared on simple modified semi-artificial diet according to Hegazi et al. (1977). The diet consisted of: faba beans 75 g, corn flour 75 g, Medical dried yeast 35 g, Methyl-p-hydroxybenzoate 3.5 g, ascorbic acid 3.5 g, Agar 13 g, Formaldehyde 2.5 ml, and Water (total) 700 ml. Moths were provided with 5% honey solution as nutrients and permitted for oviposition on corrugated papers.

Basic experimental methods

To investigate the relationship between different development stages of FAW or CLW larvae and cannibalism, newly molted second instars, fourth and fifth instars were prepared. The study is divided into three parts. The first and second parts were to determine intraspecific larval competition of each of the insect species. Only one larval density was used. This was, among 30 FAW larvae/trials (Exp. 1) and 30 CLW larvae/trials (Exp. 2), respectively. In part 3, we investigated the interspecific larval competition between the same instar stages of 15 FAW larvae and 15 CLW larvae/trials when they were reared together (Exp. 3). Five replicates were used/trial. For the three experiments, the host larvae were grouped into instars: newly molted 2nd. 4th or 5th (determined by their color, weight and a molted head capsule is being present (Mironidis and Savopoulou-Soultani, 2008) were used. For each replicate, *Spodoptera* larvae (30 each of the 2nd ,4th or 5th instars) that had been food-deprived - empty guts- for 3 h were released at the same time inside the rearing plastic container (115×115×35 mm). The remaining diet in each container was replaced by fresh one, each other day with 10 to 15 ml fresh standard artificial diet, taking care to form a film of adequate quantities of food availability for larvae throughout the experiments. Also, to increase the room of rearing container, 6 strips of corrugated papers (each 50×14 mm) were provided/container. On some of these papers, small pieces of the same diet were also added. So, enough room and adequate diet were available for the larvae to feed and simulating the semi-natural conditions, where the larvae feed on the diet. Each cage container covered with organdy cloth. The tested larvae were maintained at 25 ± 1°C in incubators (type Hann, Munden, Germany), under photoperiod 12:12 (light: dark), RH 75% and light intensity 8000 Lx. Light was measured with an available Weston light meter (Weston Electrical Instrument Company, Newark, NJ), located in a controlled room. Living and dead larvae and pupae were counted daily at a fixed time (between 14:30 and 15:30 p.m.), stopping only when the larvae disappeared: dead, developed into well-formed pupae or emerged as adults. To assign the event of cannibalism and necrophagy, the common use definition was used. An event of cannibalism was assigned for each missing larva, prepupa, and pupa. An event of necrophagy was considered when larva fed on deadly or no longer alive insect or fed on liquids exuded from dead insect of the same or other species or a dead larva or pupa that was observed one day, disappeared the following day. We kept tracking of individual larvae and their 'interaction' - on larva-pupal-adult stages on consecutive bioassay days. The host larvae were checked daily until adult emergence or died or killed by other larvae.

To produce large numbers of *S. frugiperda* pupae, some of the

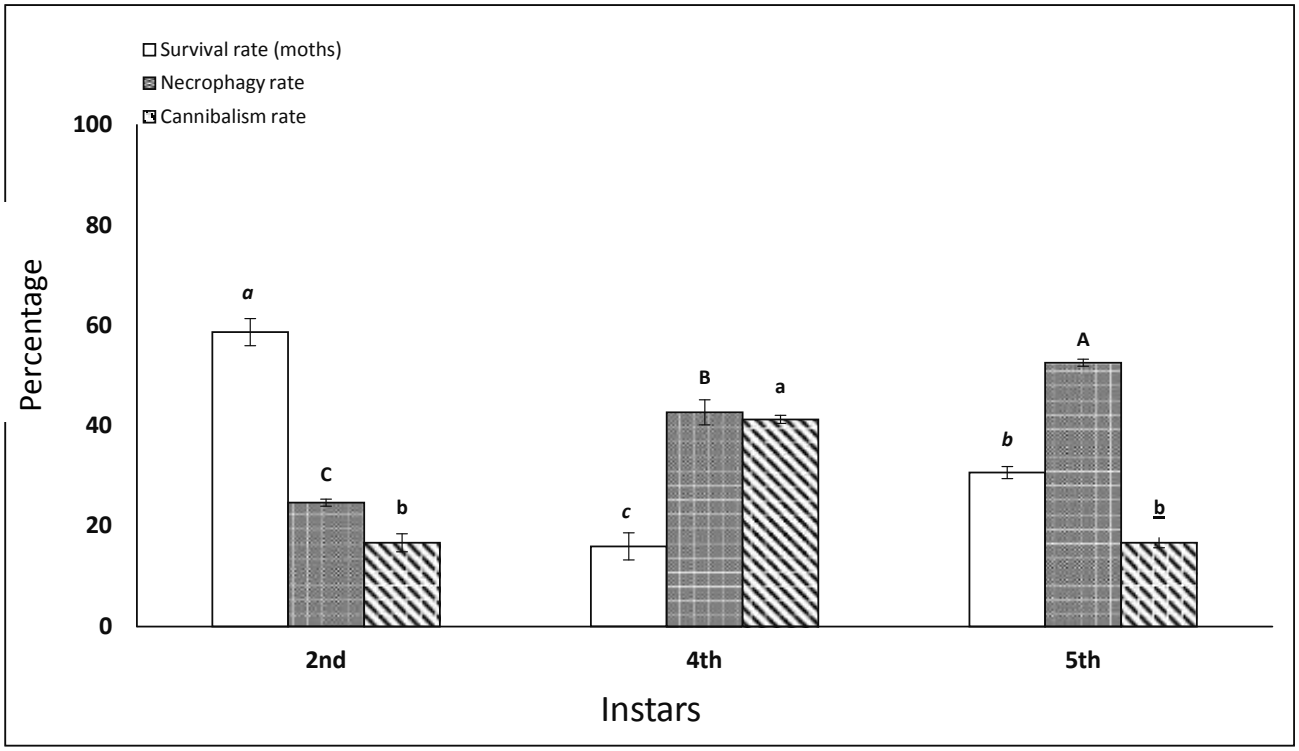

Figure 1. Rate of conspecifics cannibalism and necrophagy of rearing each of 2nd, 4th, and 5th stages of *S. frugipperda* and percentage of emerged moths in each case. For each parameter, bars with the same uppercase or lowercase letter are not significantly different (P<0.05).
Source: Authors.

Fig. 1. Rate of conspecifics cannibalism and necrophagy of rearing each of 2nd, 4th and 5th stages of *S. frugiperda* and percentage of emerged moths in each ease. For each parameter, bars with the same uppercase or lowercase letter are not significantly different (P < 0.05)

rearing plastic containers (115×115×35 mm) were provided with thin layer of artificial diet at bottom. Also, to increase the room of the rearing container, several strips of corrugated papers (each 50×14 mm) were used. Each corrugated paper was provided with small piece of diet and wrapped to make cells. Each papery cell was provided with small pieces of the same diet. The cells were placed close to each other inside the rearing cage. The last instar larvae were introduced singly at the rate of one larva/cell, so that the larvae could pupate to avoid cannibalism or necrophagy of the insect (Figure 5E and F).

Statistical analysis

Where appropriate, data were subjected to one-way analysis of variance (ANOVA) .to determine differences between means. Student's t-test was used to separate the means. Data are presented as means of number of parasitized larvae ± standard error (SE).

RESULTS

Conspecific cannibalism and necrophagy amongst same-age of 2nd, 4th and 5th instar stages of FAW larvae

Cannibalism is the practice of eating one larva on another of the same species (FAW), that is one larva fed on another and resulting in mortality, so mortality was used to record and indicate the results of cannibalism in FAW.

The consumption of conspecific individuals by cannibalism and necrophagy in different stages of FAW reared each till adult stage is shown in Figure 1. Typically, the 2nd instar larva weighed an average of 0.77 ±0.5 mg (n = 30 × 5 reps.), while weight of the 4 and 5th instars larva averaged 27.4±2.2 and 43.5±7.2 mg, respectively. When each stage of FAW was reared in test containers, 4th instar larvae were more often devouring fellow larvae. The rate of cannibalism among tested stages was significantly highest (F = 119; d.f. = 2, 12; $P <$ 0. 05). Mortality due to cannibalism of FAW (Figure 5B and C) was 16.7 ± 1.8, 41.3 ± 0.8, and 16.7 ± 1.1% for those larvae reared during their 2nd, 4th and 5th stages, respectively.

Events of necrophagy was observed when FAW larva fed on dead fellow or fed on liquids exuded from dead larva or pupa that was observed one day, disappeared next day. Necrophagy (Figure 5D) became more frequent as the larvae developed from 2nd instars to 4 and 5th instars, with mortality of 24.7± 1.7, 42.7± 1.7 and 52.6± 0.7%, respectively. Effects of conspecific cannibalism and necrophagy increased significantly (F = 64.2; d.f. = 2, 12; $P <$ 0. 05) as the larvae developed from 5th stage to adult stage. A survival rate of 58.7 ± 2.7% out of the 150 2nd instar larvae of *S. frugiperda* was able to successfully develop and reach the adult stage. This rate was highly significant (F = 87.9; d.f. = 2, 12; $P <$ 0. 05) when compared with those developed during their 4th (15.9±

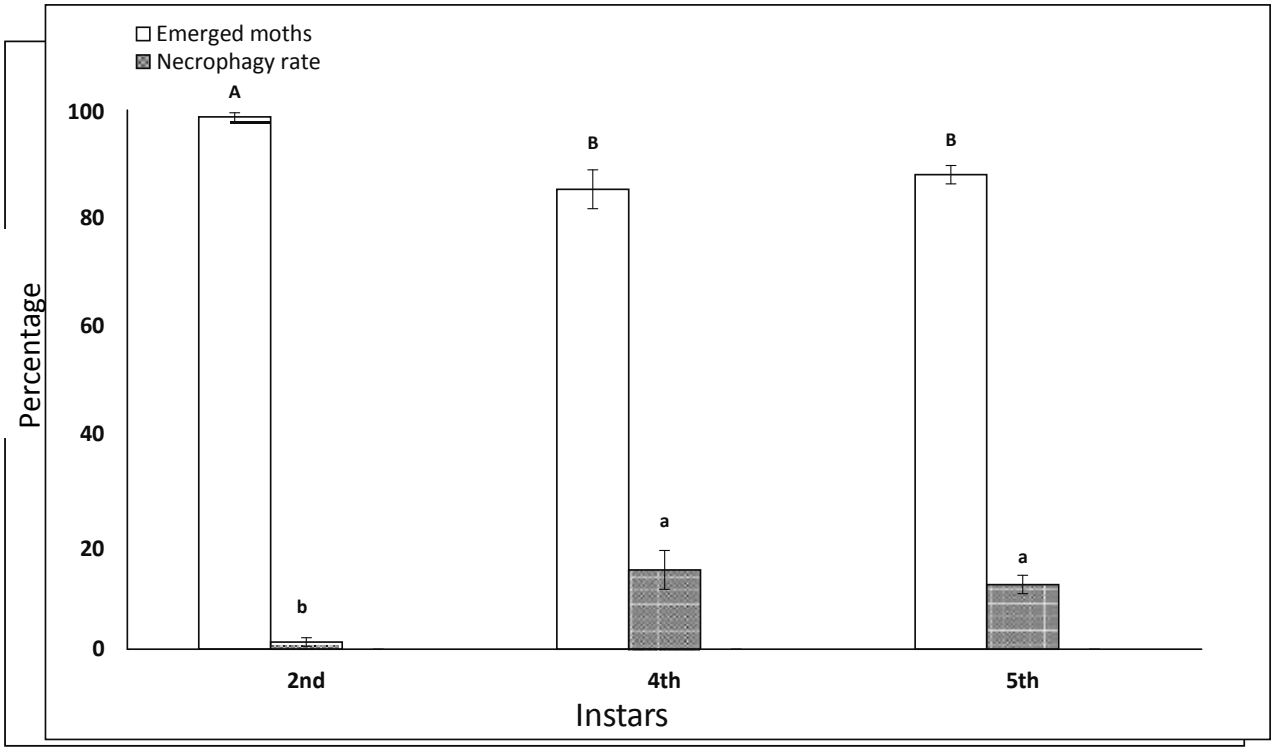

Figure 2. Rate of conspecifics cannibalism and necrophagy of rearing each of 2nd, 4th, and 5th stages of *S. littoralis* and percentage of emerged moths in each case. For each parameter, bars with the same uppercase or lowercase letters are not significantly different (P < 0.05).

Source: Authors

Fig. 2. Rate of conspecifics cannibalism and necrophagy of rearing each of 2nd, 4th and 5th stages of *S. littoralis* and percentage of emerged moths in each ease. For each parameter, bars with the same uppercase or lowercase letter are not significantly different (P < 0.05)

2.7.4%) and 5th stages (30.7 ± 1.2%).

Conspecific cannibalism and necrophagy amongst same-age of 2nd, 4th and 5th instar stages of CLW larvae

Typically, the 2nd instar LCW larva weighed an average of 0.85±0.4 mg (n = 30 × 5 reps.), while the 4 and 5th instars larva averaged 8.8 ±1.1 and 42.1 ±0.4 mg, respectively.

Larvae rarely feed entirely on their fellow larvae where abundant of food was available (Figure 5A). Cannibalistic behavior has not been observed when 150 larvae of each of 2nd, 4th or 5th (30 larvae/trial) of the CLW, were reared under laboratory conditions (Figure 2). However, events of necrophagy were observed when larva fed on dead fellow or fed on liquids exuded from dead fellow or pupa. Mortality due to necrophagy in CLW was highly significant among the tested different stages (F = 9.07; d.f. = 2, 12; *P* < 0. 05). Mortality rate amongst tested stages of the 2nd, 4th and 5th instars was 1.33 ± 0.8, 14.7 ± 3.6, and 12.1 ± 1.7%, respectively. The conspecific cannibalism due to necrophagy affected the percentage of *S. littoralis* larvae that were able to successfully develop and reach the adult stage (moths). Survival rate among the tested stages of the 2nd, 4th and 5th stages was 98.7 ± 0.8, 85.3 ± 3.6, and 88.1 ± 1.7%, respectively. The differences were highly significant (F = 9.1; d.f. = 2, 12; *P* < 0. 05).

Cannibalism and necrophagy amongst same-age of 2nd, 4th and 5th instar stages of FAW and CLW larvae when combined together

The rate of happening of cannibalism and necrophagy amongst same-age larvae of 15 FAW and 15 CLW of each of the 2nd, 4th and 5th stages became more frequent when both larvae of both stages are reared together (Figure 3). Cannibalism significantly increased (F = 20.6; d.f. = 2, 12; *P* < 0. 05) in the larvae developed during their 4th instars. Mortality rate due to cannibalism recorded 58. 7 ± 2.3, 72.7± 1.9, and 57.3 ± 1.2%, for larvae reared during their 2nd, 4th and 5th stages, respectively. However, necrophagy rates became less on the expense of higher larval cannibalism. Necrophagy rates varied significantly among larvae developed from different stages. Necrophagy rates recorded for larvae developed during their 2nd, 4th and 5th stages were 22. 7 ± 1.7, 15.3± 2.5, and 26.1 ± 1.2% (F = 8.14; d.f. = 2, 12; *P* < 0. 05), respectively. Also, rearing larvae of both species together significantly (F = 2.6; d.f. = 2, 12; *P* < 0. 05) affected the type of larval species that were able to

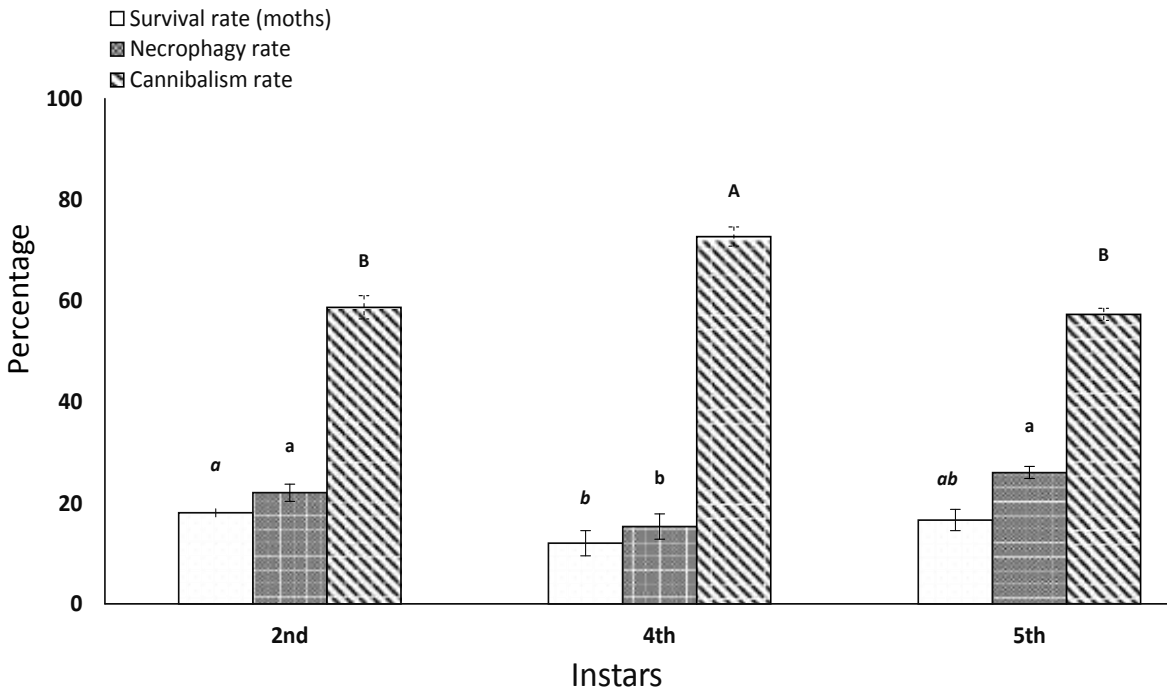

Figure 3. Effect of rearing combined larval developmental stages of each of 2nd, 4th, and 5th instars of *S. frugiperda* and *S. littoralis* on the rate of cannibalism and necrophagy and percentage of emerged moths. For each parameter, bar with the same uppercase or lowercase letter are not different (P<0.05).
Source: Authors

successfully develop and reach the adult stage (moths). The percentage of larvae that developed to adult stage were significantly different among the three groups of larval stages, with higher values observed among FAW larvae than among in the CLW larvae. Out of 150 larvae tested in each group, the emerged moths among the 2nd, 4th and 5th groups were 18. 2 ± 2.3, 12.7± 2.5, and 16.6 ± 2.1%, respectively. Out of these moths reared during 2nd and 4th stages, only 2.7 and 0.0%, respectively were CLW moths. So, most emerged moths were *S. frugiperda* indicating that all CLW and some FAW larvae were cannibalized.

Effect of cannibalistic behavior amongst same-age of FAW and CLW larvae on the adult yield (Moths)

Figure 4 shows effects of cannibalism and necrophagy of FAW and CLW combined together on adult yield. The cannibalistic behavior consequently affected the adult yield in each test group of larvae and was stage-dependent.

Always, strange cannibalistic behavior effects were observed when both same-stages of larval FAW and CLW were combined and estimated their effects on adult yield.

When the 2nd stage of larvae was tested, the percentage of survival (moths) varied significantly between the tested insect species alone or combined with other species (F = 405.4; d.f. = 2, 12; *P* < 0. 05). The percentage of emerged moths was greatest among CLW larvae reared alone (98.7± 0.8%) lower amongst FAW larvae when reared alone (58.7± 2.7%) and lowest when FAW combined with CLW (22.1± 1.7%), and only FAW moths were obtained in the latter case.

When same-ages of 4th larval stages of FAW and CLW were tested, the percentages of emerged moths varied significantly among the tested insect groups (F = 193.7; d.f. = 2, 12; *P* < 0. 05). The effect of cannibalistic behavior on moths' yield became more frequent clear amongst FAW larvae reared alone (15.98± 2.7%) and lowest when combined with CLW (12.1± 2.5%). While, the percentage of emerged moths was greatest amongst CLW larvae reared alone (85.3± 3.6%) where no larval cannibalism was observed.

When same-ages of 5th larval stages of FAW and CLW were tested, the percentage of larvae that developed to adult stage varied significantly (F = 484.23; d.f. = 2. 12; *P* < 0. 05) among insect groups. The percentage of emerged moths was greatest among CLW larvae reared alone (88.7± 1.7%) lower amongst FAW larvae when reared alone (30.7 ± 1.2%). When both species were combined, the percentages of survival moths of 5th stage group were lowest, where only 16.6± 2.1% FAW moths emerged, indicating that all CLW and some FAW larvae were cannibalized.

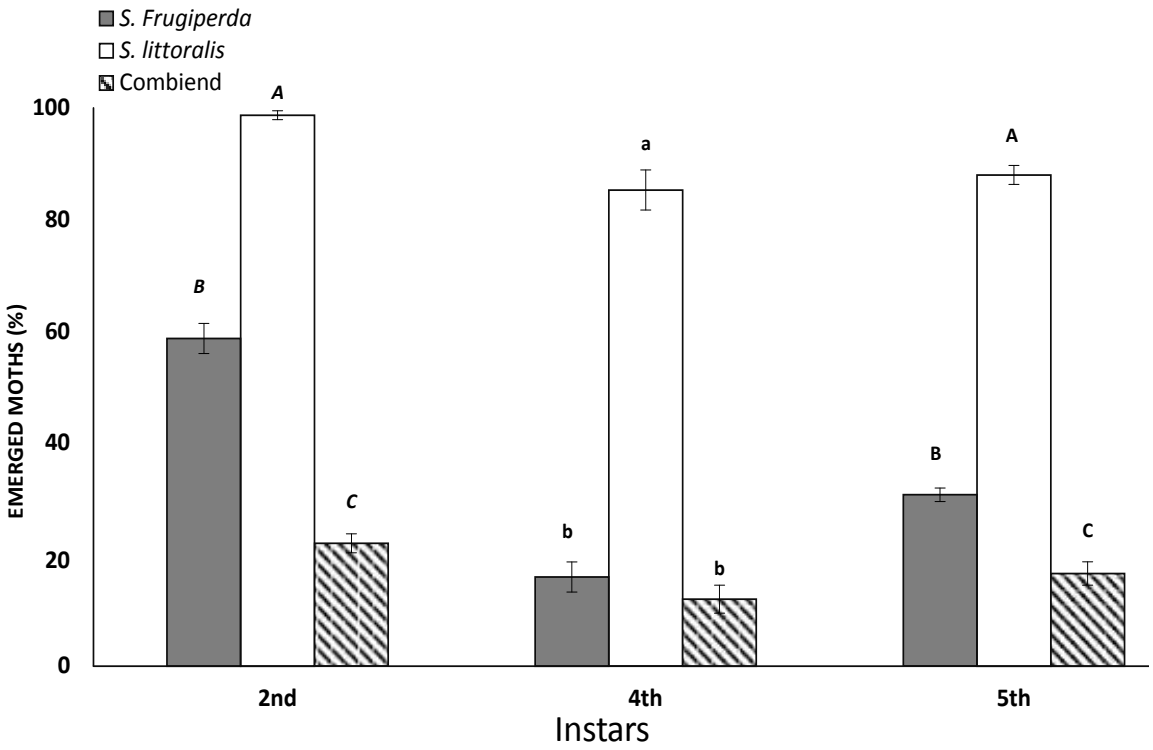

Figure 4. Effect of rearing combined larval developmental stages of each of 2nd, 4th, and 5th instars of *S. frugiperda* and *S. littoralis* on percentage of larvae that were able to successfully development and reached the adult stage (moths). For each parameter, bar with the same uppercase or lowercase letter are not different (P<0.05).
Source: Authors

DISCUSSION

Cannibalism takes place in many species of lepidopterous larvae, such as in the corn earworm, *Helicoverpa zea*, the fall armyworm, *S. frugiperda,* the cotton leafworm. The *S. littoralis* and the beet armyworm, *Spodoptera exigua* (Hübner) (Fox, 1975; Chapman et al., 2000; Williams and Hernandez, 2006; Elvira et al., 2010). In the present work, cannibalis in FAW was obligatory and the highest mortality was observed when the fifth instars reared on artificial diets was compared with those in second and fourth instars, suggesting cannibalistic behaviour occurred frequently among older larvae. The results suggested that larval development stage may be important factor affecting cannibalistic behavior in the animal kingdom.

However, Da Silva and Parra (2013) reported that cannibalism is not an important factor of mortality for larvae of FAW in laboratory rearings. Hence, individualizing the insects is not necessary to avoid larval and pupal mortality due to such negative interaction.

Cannibalism is frequently a response to high density, food shortages, conspecific behavior (Fox 1975), a size disparity between conspecific individuals (Porretta et al., 2016), environmental temperature (Start et al., 2017) or even building materials (Okano et al., 2018). Under the same larval density, sufficient larval food and space, the cannibalistic behavior was obligatory among the same developmental stages of FAW larvae and no cannibalism was observed among CLW larvae.

Cannibalism is a frequent behavior of *S. frugiperda* in laboratory culture and in the field (personal observation), even when more than adequate food is available. In June 2022, maize plants in Abis farm were heavily infested by *S. frugiperda* and destroyed the entire crop but plants were free from other lepidopterous larvae. At the same time a visit was paid to maize farm 5 km far, it was infested with *S. exigua* and other insects but free from *S. frugiperda* larvae. So, it seems that *S. frugiperda* may affect insect biodiversity in the maize plants. The present work suggests that cannibalism is evident amongst same-age larval instars of FAW and when combined with CLW, which may affect ecological and biological relationships between both species.

Kakimoto et al. (2003) reported that repeating cannibalism between same-instar larvae was lower than that between larvae of different ages. Cases in which older instar larvae were cannibalized by younger instar larvae were rare and all cases were observed in combats between larvae of adjacent instars. When the same

Figure 5. FAW and CLW larvae, A: Large numbers of CLW rearing in plastic container provided with corrugated papers with no signs of cannibalistic habits. B: Sixth-instar larva of FAW preying on CLW larva. Sixth-instar larva of FAW preying on FAW prepupa, D: complex of cannibalism and necrophagy among FAW larvae. E: papery corrugated cells to get large numbers of FAW pupae (F).
Source: Authors.

stages of FAW and CLW larvae were combined together, the predation power and survivability of FAW larvae were higher than for CLW.

Analyzing the effect of cannibalistic behavior amongst same-age of larval FAW and CLW on the adult yield suggesting that percentage of emerged moths was greatest amongst CLW larvae reared alone where no larval cannibalism was observed. The yield of adults of FAW was lower when reared alone and lowest when combined with CLW indicating that all CLW and some FAW larvae were cannibalized. The results may be useful for those culturing the larvae of these two species of *Spodoptera*. The FAW has been able to expand throughout Africa, much of Asia and reached Australia

following its invasion into West Africa.

Conclusion

The results of the present work suggested that the cannibalistic frequency in *S. frugiperda* larvae was obligatory and greatly affected by larval development stage. The frequency of cannibalism was higher in fifth-instar larvae compared with those reared during 4th or 2nd instars. Under the same rearing conditions, no cannibalism was observed among the same tested stages of CLW. When the same stages of FAW and CLW larvae were combined together, the predation ability and survivability of FAW larvae were higher than for CLW. These results suggest that the factors affecting cannibalism of FAW and CLW larvae in the laboratory rearing need to be reevaluated in biodiversity point of view.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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