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Mass rearing of the Fall Armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), with artificial diets and natural food sources

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ABSTRACT

This study was conducted to assess the efficacy of three agar-free artificial diets (D1, D2, and D3) for the mass rearing of fall armyworm, (FAW) *Spodoptera frugiperda* under controlled laboratory conditions (27 ± 1°C; 70 ± 5% relative humidity; 14:10 light : dark photoperiod), in comparison to natural food sources (castor leaves and lettuce leaves). The findings demonstrated that all three artificial diets supported efficient rearing of FAW. Diet D1 yielded the most favorable outcomes, resulting in the shortest total immature development period (24.25 days) and the lowest larval mortality percentage(5.00%), followed by D2 (7.00%). In contrast, larval mortality was highest on lettuce leaves (15%), followed by castor leaves (12%) and D3 (9%). Pupal mortality was highest on lettuce leaves (11.76%) and lowest on D1 (6.32%). The mean fecundity was highest for D1 (785 eggs/female), followed by D2 (730 eggs/female), D3 (665 eggs/female) and castor leaves (628 eggs/female), with the lowest fecundity observed on lettuce leaves (576 eggs/female). No statistically significant differences were detected in fertility across the diets and natural food sources. These results demonstrate how diet D1 outperforms other diets and natural food sources in fostering development and fitness indicators for *S. frugiperda*. These outcomes were necessary for the ongoing rearing of this insect in sufficient numbers to support studies on creating integrated pest management systems.

Key words: Castor leaves, Lettuce leaves, artificial diet and fall armyworm (FAW), *Spodoptera frugiperda*.

INTRODUCTION

The highly polyphagous insect known as the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), targets more than 353 distinct host plants from 76 plant families (Montezano *et al.*, 2018). It can be found all over the world, including the USA (Clark *et al.,* 2007; Bhosle, *et al.,* 2025), Africa (Goergen *et al.,* 2016), Asia (Ganiger *et al.,* 2018), and causes economic losses to several plant species such as maize, rice, sorghum, cabbage, soybean, cotton, tomato, potato, and alfalfa (Cabi, 2016). Despite its ability to feed on a wide range of host plants, FAW does less well on peanut leaves than on maize leaves. However, after 5 to 8 generations of acclimation, its performance can be greatly enhanced (Yao *et a*l., 2024). Therefore, in order to research this insect's biology, behavior, nutritional needs and pesticide susceptibility and resistance, laboratory breeding is required. According to (Silva and Parra, 2013), this knowledge is essential for creating effective integrated pest management programs.

Artificial diet is a more convenient option than natural diet for rearing FAW, as natural diet can be costly and stressful. With artificial diet, food sources were easier to obtain, preserve, and use, and a larger number of insects can be reared in less time with reduced risk of contamination from pathogens (Cohen, 2015). The artificial diet must, however, include all the nutrients required for successful rearing in the right amounts, since the pest's entire biology, including growth rate, development and reproduction, can be impacted by the type and amount of food it consumes (Huang *et al*., 2018; Bavaresco *et al*., 2004).

Agar is an expensive component used in most artificial diets used for FAW raising worldwide. This limits the development of numerous studies on FAW by making the process of rearing them costly (Kasten *et al*., 1978; Pinto *et al.*, 2019; substitute with Jin e*t al*., 2020; He *et al*., 2021; Truzi, 2021; Ge *et al*., 2022; Rindiani *et al.,* 2024). As a result, a lot of work has been done into creating artificial diets that were both inexpensive and very effective for their reproduction (Pinto *et al*., 2019).

This study aimed to assess the efficacy of three agar-free artificial diets for mass rearing *S. frugiperda*, and compare them to rearing on natural food sources.

MATERIALS AND METHODS

This study was conducted to assess the efficacy of three agar-free artificial diets (D1, D2 and D3) for the mass rearing of *S. frugiperda* in comparison to natural food sources (castor leaves and lettuce leaves). The Bollworms Research Department of the Plant Protection Research Institute, Sharkia Branch, conducted the trials in a controlled laboratory setting. A consistent temperature of 27 ± 1 °C, a relative humidity of 70 ± 5%, and a photoperiod of 14:10 hours (light : dark) were all maintained during the raising process.

Artificial diets:

The artificial diets used in this study were modified from diets used in the rearing of the spiny and American bollworms according to (Amer, 2015; Amer and El-Sayed, 2015). The compositions of these diets were described in (Tables 1 and 2).

Table 1. Components of the tested diets for *S. frugiperda* mass rearing.

Ingredients	Diet D_1	Diet D_2	Diet D_3
Wheat grated (g)	62.50	125.00	62.50
Kidney beans (g)	125.00	-	-
Yellow lentils (g)	-	62.50	-
Chick pea (g)	-	-	125.00
Ascorbic acid (g)	2.50	2.50	2.50
Sorbic acid (g)	1.25	1.25	1.25
Methyl parahydroxy benzoate (g)	1.25	1.25	1.25
Dry yeast (g)	27.50	27.50	27.50
Yeast extract (g)	2.50	2.50	2.50
Liquid milk (mL)	25.00	45.00	45.00
Vitamins mixture (mL)	5.00	5.00	5.00
Formaldehyde 34-38%(mL)	1.75	1.75	1.75

Kidney beans, *Phaseolus vulgaris*, Wheat grated *Triticum aestivum* L, Chick pea, *Cicer arietinum*, Lentils, *Lens culinaris*

Table 2. Vitamins mixture components, each 10 mL from vitamins mixture contains

Ingredients	Quantity
Panthenol	4 mg
Nicotinamide	10 mg
Vitamin A	2400 I.U
Vitamin B1	2 mg
Vitamin B2	2 mg
Vitamin B6	1.0 mg
Vitamin C	100 mg
Vitamin D3	200 I.U
Vitamin E	2 mg
Ferrous gluconate	86.4.2 mg
Calcium gluconate	50 mg
Calcium phospholactate	50 mg

Diet preparation:

Kidney beans, grated wheat, lentils and chickpeas thoroughly washed with water. For the preparation of the first diet (D1), kidney beans and grated wheat combined with water in a pot and heated for 70–80 minutes. Similarly, for the third diet (D3), chickpeas and grated wheat were processed under the same conditions. After heating, the mixtures were removed from the heat and the excess water was drained. Each mixture then minced using a meat grinder. For the second diet (D2), wheat grits were initially heated with water in a pot. After 80 minutes of heating, lentils added to the mixture and allowed to cook for an additional 10 minutes. The mixture was then removed from the heat at the 90-minute mark, drained of excess water, and minced using a meat grinder. Following this, milk added to each of the prepared mixtures and thoroughly blended. The remaining components—including vitamins, formaldehyde, ascorbic acid, sorbic acid, methyl parahydroxybenzoate, yeast extract and dry yeast were incorporated into each diet and mixed uniformly. The prepared diets were then refrigerated for 2 hours before use.

Insect:

The newly hatched larvae of the *S. frugiperda* used in this study were obtained from a culture maintained for 20 generations at the Bollworms Research Department, Plant Protection Research Institute, Sharkia branch. In this study two generation of the FAW were reared on each diet and natural food before beginning recording the experiment observation.

Natural food:

In this study, FAW larvae were fed on two natural foods.

- Lettuce leaves, *Lactuca sativa*
- Castor leaves, *Ricinus communis*

Diet evaluation:

Transferred the newly hatched larvae of the *S. frugiperda* resulted from rearing on each artificial diet (Table 1) or each natural food (lettuce leaves or castor leaves) to Petri dishes (6 cm diameter x 2 cm height) containing the same food as previous feeding for feeding for three days. In third days, four replicates each 25 larvae were transferred individually from previous Petri dishes in each diet to transparent plastic cups with cover (2.8 × 3.6 x 3.3cm) (Fig. 1) containing the same previous natural plant (lettuce leaves or castor leaves) or about 7 – 8 g from each artificial diet (Table 1). Each plastic cup wear covered by paper napkin, then covered it with her lid and placed in incubator at the same previous conditions. Larvae were examined daily until pupation with replacement of food two days' intervals in case of natural foods. Larval duration, larval mortality percentages, larva weights and pupation percentages were recorded. Pupae collected and placed in clean glass gar with tissue paper and examined daily until adult emergence to record pupal duration, pupal mortality percentage and pupal weight. Moths emerged from each diet or natural food were sexed and caged to eggs laying. Four replicates each five pairs / cage was used for each diet and natural food. Moths fed on 10 % honey solution. The cages were examined daily until moth death to determine the pre-oviposition, oviposition and post-oviposition periods and adult longevities. Egg masses were collected and counted daily and placed at previous condition until hatched. The number of deposited eggs was recorded. The growth and fitness index of different FAW stages were calculated using the equations used by (Pretorius, 1976; Itoyama *et al.,* 1999; Amer and El-Sayed, 2014).

$$\text{Growth index of larval} = \frac{\text{Pupation percentage}}{\text{Larval period (days)}}$$

$$\text{Growth index of pupal} = \frac{\text{Emergence percentage}}{\text{Pupal period}}$$

$$\text{Standardized growth index of immature stages} = \frac{\text{Pupal weight}}{\text{Larval period}}$$

$$\text{Growth index of immature stages} = \frac{\text{Emergence percentage}}{\text{Larval period + pupal period}}$$

$$\text{Fitness index of immature stages} = \frac{\text{Pupation percentage} \times \text{pupal weight}}{\text{Larval period + pupal period}}$$

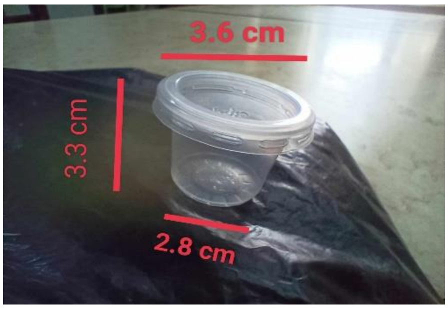

Fig. 1 Transparent plastic cups with cover

Statistical analysis:

Data were analyzed as one way ANOVA completely randomized and means were compared by LSD range test (P≤0.05 level) using Costat program, 2005. The obtained data were subjected to statistical analysis according to (Little and Hills, 1975).

RESULTS

Larval stage:

Table (3) presents the findings about how the tested meals affected the *S. frugiperda* larval stage. Depending on the diet or natural food source offered, FAW larval duration showed notable variance. Larvae raised on diet D3 had the maximum larval duration (15.75 days), whereas those fed on lettuce leaves had the shortest lifetime (14.50 days). Diet D1, diet D2 and castor leaves all had larval lengths of 15.25, 15.50, and 15.00 days, respectively. Among the diets and natural food sources that were examined, the percentages of larval mortality differed considerably. Diets D1, D2 and D3 had larval mortality percentages of 5.0, 7.0, and 9.0%, consecutively, while castor and lettuce leaves had larval mortality percentages of 12.0 and 15.0%, respectively (Table 3).

Pupal stage:

The data presented in (Table 3) demonstrate the pupal length of *S. frugiperda*, D1 and D2 showed the shortest pupal duration with values of 9 days, followed by D3 (9.25 days) and castor leaves (10.00 days). On the other hand, feeding lettuce leaves extended their pupal duration to 10.25 days. D1 had the largest pupal weight (0.235 g), while lettuce leaves had the lowest one (0.205 g). Pupal weights for castor leaves, D2 and D3 were 0.213, 0.227 and 0.219 g, respectively. When compared to natural meals, the studied diets significantly influenced the pupation percentage of *S. frugiperda*. The percentages of pupation on castor leaves, lettuce leaves, D1, D2, and D3 were 88.0, 85.0, 95.0, 93.0 and 91.0%, respectively. Pupal mortality percentages from larvae fed on natural foods and studied diets showed notable variances. Diet D1 had the lowest percentage of pupal mortality (6.32%), while lettuce leaves had the greatest percentage (11.76%). The percentage of pupal mortality was impacted in an intermediate way by castor leaves, D2, and D3.

Table 3. Immature stages of the *S. frugiperda* reared on tested diets and natural foods

Food kinds	Larval duration (days)	Larval Mortality %	Pupation %	Pupal duration (days)	Pupal weight (g)	Pupal mortality %	Total immature duration (days)
Castor leaves	15.00	12.0b	88.0cd	10.00a	0.213bc	9.09b	25.00
Lettuce leaves	14.50	15.0a	85.0d	10.25a	0.205c	11.76a	24.75
Diet D_1	15.25	5.0e	95.0a	9.00b	0.235a	6.32c	24.25
Diet D_2	15.50	7.00d	93.00b	9.00b	0.227ab	8.60b	24.50
Diet D_3	15.75	9.00c	91.00bc	9.25b	0.219abc	7.69c	25.00
P	Ns	0.000***	0.0002***	0.0008***	0.031*	0.0002***	Ns
LSD $_{0.05}$	---	1.81	3.04	0.53	0.018	1.51	---

Means in the same column followed by the same letters are not significantly (P > 0.05) different according to LSD test

Adult stage:

Adult emergence:

The emergence percentages of adults fed on natural foods and tested diets differed quite significantly, according to the data in (Table 4). Adult emergence percentages for castor leaves, lettuce leaves, D1, D2, and D3 were 90.91, 88.24, 93.68, 91.40, and 92.31%, respectively.

Adult longevity:

Table (4) provides a summary of the oviposition period of *S. frugiperda* female moths that were produced from larvae reared on natural foods and tested diets. There were notable differences in the oviposition period between the natural foods and the studied diets. While castor leaves, D2, and D3 had an intermediate impact, diet D1 produced the longest oviposition duration (5.0 days), while lettuce leaves produced the shortest (4 days). Additionally, the male longevity of the *S. frugiperda* moths fed as larvae on the tested diets and natural foods was shown to differ insignificantly. For D1, D2 and D3, castor and lettuce leaves, the female longevity was 10.00, 9.75, 10.25, 10.00, and 9.50 days, respectively.

Table 4. Effect of tested diets and natural foods on some biological aspects of adult stage of the *S. frugiperda*.

Food kinds	Adult emergence %	Pre-oviposition period (days)	Oviposition period (days)	Post-oviposition period (days)	Female longevity (days)	Male longevity (days)
Castor leaves	90.91bc	3.50	4.5	2.50a	10.00	9.75
Lettuce leaves	88.24c	3.75	4.0	1.75b	9.50	9.00
Diet D_1	93.68a	3.25	5.0	1.75b	10.00	9.50
DietD_2	91.40ab	3.25	4.5	2.00b	9.75	9.00
Diet D_3	92.31ab	3.50	4.25	2.50a	10.25	8.75
P	0,013*	Ns	**Ns**	0.006**	**Ns**	**Ns**
LSD $_{0.05}$	2.69	---	---	0.45	---	**---**

Means in the same column followed by the same letters are not significantly (P > 0.05) different according to LSD test

Fecundity and fertility:

The number of eggs lay by each female of *S. frugiperda*, D1 had the most eggs (785 eggs/female), followed by D2, D3 and castor leaves (730, 665, and 628 eggs/female) while lettuce leaves (576 eggs/female) had the fewest (Table 5). The fertility percentages of *S. frugiperda* were 94.90, 93.40, 95.16, 94.79, and 95.04 percent for castor leaves, lettuce leaves, D1, D2 and D3, consecutively. The fertility percentages varied considerably depending on whether the larvae were fed natural foods or tested diets.

Table 5. Average numbers of fecundity and fertility percentages of the *S. frugiperda* reared on tested diets and natural foods

Food kinds	Fecundity/female	Fertility %
Castor leave	628.00d	94.90
Lettuce leave	576.00e	93.40
Diet D_1	785.00a	95.16
Diet D_2	730.00b	94.79
Diet D_3	665.00c	95.04
P	<0,0001***	Ns
LSD $_{0.05}$	35.28	---

Means in the same column followed by the same letters are not significantly (P > 0.05) different according to LSD test

Growth and fitness index:

According to the findings in (Table 6), diet D1 produced the highest growth indices for *S. frugiperda* larval and pupal stages, 6.23 and 10.409, respectively. Conversely, lettuce leaves showed the lowest growth indices, with values of 5.86 for larvae and 8.609 for pupae. Diet D2 (6.00 larval, 9.881 pupal), diet D3 (5.78 larval, 9.717 pupal), and castor leaves (5.87 larval, 9.091 pupal) all showed intermediate growth indices. Diet D1 had the highest growth index for the immature stages (0.026), while Lettuce leaves had the lowest (0.020). Diet D1 had the highest fitness index (0.921), whereas lettuce leaves had the lowest (0.704). Castor leaves and diets D2 and D3 showed moderate fitness index values. D1 had the greatest standardized growth index of immature stages (3.863), followed by D2, D3, castor leaves and lettuce leaves (3.731, 3.692, 3.636, and 3.565, respectively). These results demonstrate how diet D1 outperforms other diets and natural food sources in fostering development and fitness indicators for *S. frugiperda*.

Table 6. Growth index of the *S. frugiperda* reared on tested diets and natural foods

Food kinds	Larval growth index	Pupal growth index	Immature stages growth index	Standardized growth index of immature stages	Fitness index of immature stages
Castor leaves	5.87	9.091	0.021	3.636	0.750
Lettuce leaves	5.86	8.609	0.020	3.565	0.704
Diet D_1	6.23	10.409	0.026	3.863	0.921
Diet D_2	6.00	9.881	0.025	3.731	0.862
Diet D_3	5.78	9.717	0.024	3.692	0.797

DISCUSSION

The pest's entire biology, growth rate, development and reproduction can be impacted by the type and amount of food it consumes (Bavaresco *et al*., 2004; Huang *et al.,* 2018). In this, study the *S. frugiperda* was bred with high efficiency on artificial diets, because it includes Wheat, which is a rich source of carbohydrates because it contains a high percentage of starch, which leads to an increase in the proportion of carbohydrates in this diets, which increases in its efficiency of them in breeding insects. In addition, wheat contains wheat germ and wheat germ oil, which contains B vitamins (thiamine riboflavin and niacin) which are involved in the process of metabolism and insect growth. These diets also contain in their components milk, which contains a high percentage of vitamins fats that help in the growth of insects and prevent diets from drying out and milk protein (casein), which leads to an increase in the proportion protein in these diets and an increase in its efficiency of them in breeding insects. In addition, these diets contain yeast extract and yeast that leads to an increase in vitamin B, which contributes greatly to the process of metabolism and growth of insects. It is recognized that proteins, lipids and carbohydrates affect an insect's ability to grow and develop (Soo Hoo and Fraenkel, 1966). The adult female *E. insulana's* fertility decreased because of a decrease in total lipid and protein (Kandil, 2013; Amer and El-Sayed, 2015). Low protein and carbohydrate level in diets effect on developmental period, weight and larval mortality, pupation rate adult emergence percentages (Borzoui *et al***.**, 2018). Wheat germ, wheat germ oil and casein are present among many components of artificial diets that are used in insect breeding (Pinto *et al*., 2019; Jin *et al.,* 2020; Truzi *et al*., 2021; Ge *et al.,* 2022). It was possible to rear *S. frugiperda* using soybeans based on an artificial diet (Thamrin *et al.,* 2022). When fed a diet including maize leaves as opposed to sugarcane, *S. frugiperda* fared better (Strydom *et al*., 2024). According to the study's findings, the larvae's maximum duration (15.75 days) occurred on D3, whereas the smallest duration (14.50 days) occurred on fed lettuce leaves. The larval stage mortality rates on castor leaves, lettuce leaves, D1, D2, and D3 were 12.0, 15.0, 5.0, 7.0 and 9.0%, respectively. The maximum pupal weight of any diet was 0.235 g on D1. On lettuce leaves, the lowest weight per pupae (0.205g) was noted. On lettuce leaves, the highest pupal mortality percentage of 11.76 % was noted. On D1, the pupal mortality rate was the lowest at 6.32%. On D1, there were 785 eggs per female, the most; on lettuce leaves, there were 576 eggs per female. An artificial diet made from pigweed leaves is the best food for rearing *S. frugiperda*. The development of the artificially fed FAW larval stage was comparable to that of (Pinto *et al.,* 2019), who reported a pupal mass of 253.3 mg, a larval survival rate of 92.0% and a larval stage of 15.6 days. The length of the *S. frugiperda* larval phase might vary depending on the nutritional value of each meal, according to (Cunha *et al*., 2008). The variations in the nutritional components of kidney beans, yellow lentils, castor and lettuce leaves may be the cause of the variations in the *S. frugiperda* study's developmental stages and reproductive effectiveness. Additionally, in this study, D1 had the highest fitness score (0.921), whereas lettuce leaves had the lowest (0.704). However, the effects of D2, D3 and castor leaves were in between. The best kind of food for *S. frugiperda* growth and development was corn leaves (Ginting *et al.,* 2021). When assessing the quality of food, the growth index highlights the significance of both survival rate and developing time (Sétamou *et al*., 1999; Hwang *et a*l., 2008; Amer and El-Sayed, 2014; El-Shennawy *et al*., 2022). The results showed that replacing the fall armyworm, FAW, with agar, which is frequently used in artificial diets; (Kasten *et al*., 1978; Pinto *et al.*, 2019; Tao *et al.,* 2020; He *et al.,* 2021; Truzi, 2021; Ge *et al.,* 2022). The inclusion of milk in the artificial diets used in this study, as well as the inclusion of wheat starch in the diet components, greatly decreased the expenses related to raising *S. frugiperda* on a wide scale. In order to support research activities targeted at creating integrated pest management (IPM) systems for controlling this pest, it is imperative that these results be maintained for the continuing and sustainable mass rearing of this insect.

CONCLUSION

This study assessed the effectiveness of three agar-free diets (D1, D2, and D3) for rearing *S. frugiperda* under controlled laboratory conditions, comparing their performance to natural food sources. Among the tested diets, D1 yielded the best results, demonstrating the shortest developmental period (24.25 days), the lowest larval mortality rate (5%), and the highest fecundity (785 eggs per female). In contrast, natural food sources, particularly lettuce leaves, resulted in higher mortality rates and reduced fecundity. Both D1 and D2 exhibited superior growth and fitness indices, highlighting their potential as efficient and cost-effective alternatives for mass rearing of FAW. These findings support their use in integrated pest management (IPM) strategies aimed at controlling this pest.

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التربية المكثفة لدودة الحشد الخريفية، *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) ، باستخدام بيئات صناعية ومصادر غذائية طبيعية

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أُجريت هذه الدراسة لتقييم فعالية ثلاثة بيئات صناعية خالية من الآجار(D1، D2 و D3) في التربية المكثفة لدودة الحشد الخريفية، *Spodoptera frugiperda*، تحت ظروف معملية (27 ± 1 د رجة مئوية؛ رطوبة نسبية 70 ± 5% ؛ فترة ضوئية 14:10 (الضوء والظلام)، مقارنةً بمصادر الغذاء الطبيعية (أوراق الخروع والخس). أظهرت النتائج أن جميع هذه البيئات الصناعية الثلاثة ساهمت في تربية دودة الحشد الخريفية بكفاءة. وقد حققت البيئة D1 أفضل النتائج، حيث نتج عنها أقصر فترة النمو الإجمالية للاطوار غير الكاملة (24.25 يومًا) وأقل نسبة موت لها (6.32 يومًا)، تليها البيئة D2 (24.50 يومًا) و 8.60 نسبة موت لليرقات. في المقابل، كان معدل موت اليرقات أعلى على أوراق الخس (15%)، تليها أوراق الخروع (12%) والبيئة D3 (9 %) . **كان** معدل موت العذارى أعلى على أوراق الخس (11%) وأقل في D1 بنسبة (6%) . كان متوسط الخصوبة أعلى فيD1 (785 بيضة/أنثى)، تليها (730 D2بيضة/أنثى)، وأوراق الخروع (628 بيضة/أنثى)، و D3 (665 بيضة/أنثى)، مع أدنى معدل خصوبة لوحظ على أوراق الخس (576 بيضة/أنثى). لا توجد فروق معنوية في الخصوبة بين البيئات الصناعية ومصادر الغذاء الطبيعية. توضح هذه النتائج كيف تتفوق البيئة D1 على البيانات الغذائية الأخرى ومصادر الغذاء الطبيعية في تعزيز مؤشرات النمو لـ *S. frugiperda*. كانت هذه النتائج ضرورية للتربية المستمرة لهذه الحشرة بأعداد كافية لدعم الدراسات حول المكافحة المتكاملة الآفات

الكلمات المفتاحية: الخروع ، الخس ، بيئات صناعية ، دودة الحشد الخريفية