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Life cycle *Spodoptera frugiperda* JE Smith with lettuce (*Lactuca sativa* L.) and pakcoy (*Brassica rapa* L.) in the laboratory

Siklus Hidup Spodoptera frugiperda JE Smith pada selada (Lactuca sativa L.) dan pakcoy (Brassica rapa L.) di laboratorium

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ABSTRACT

Spodoptera frugiperda JE Smith. is a new pest on maize plants in Indonesia and is polyphagous. The purpose of this study is to find out what plants are lettuce and pakcoy can be used as potential hosts for *S. frugiperda*, its life cycle, sex ratio and number of eggs produced in the laboratory. The research was conducted in October 2019 - January 2020. Treatment and observations were carried out at the Botanical Laboratory, Ahmad Dahlan University. The parameters taken were the average value of larvae length, head diameter, weight of instar larvae 3 - 6, length and weight of pupa, sex ratio, number of eggs, and life cycle length of S. frugiperda. The highest average value of all observed parameters was pakcoy leaves. The length of time needed for one life cycle is the fastest for pakcoy leaves for 37 days. This study showed that lettuce and pakcoy plants could be used as potential hosts with the best growth in the treatment of pakcoy leaves.

Keywords: *Brassica rapa*, *Lactuuca sativa*, pakcoy , lettuce, *S. frugiperda*

1. Introduction

Spodoptera frugiperda JE Smith. (FAW) is a new pest found in maize in Indonesia. This pest originates from the tropics and subtropics of America and attacks the young corn leaves. *S. frugiperda* is a polyphagous pest, because it is polyphagous, this pest has many hosts. One of the plants that is possible to host *S. frugiperda* is lettuce (*Lactuca sativa L.*) and pakcoy (*Brassica rapa L.*).

Lettuce is a seasonal vegetable that can grow well in the highlands with the best planting time at the end of the rainy season. One of the causes of decreased lettuce production is the attack of armyworm pests which in the vegetative and generative phases and only leave the epidermal layer on the leaves, while the larvae of the final instar are able to destroy all parts of the lettuce plant (Bragard et al., 2019). Lettuce and pakcoy can be used as alternative hosts for *S. frugiperda* (Caniço et al, 2021). Moreover, it had been reported that this pest also had attacked lettuce and pakcoy in several countries worldwide (Caniço et al., 2021; D. G. Montezano et al., 2018). The pest is *S. litura* F. The attack of *S. litura* on lettuce can cause farmers to lose up to 25% of production (Nurlaili et al, 2020). Apart from attacking lettuce plants, *S. litura* can also attack pakcoy which of course also results in a decrease in crop productivity and can even cause crop failure. *Spodoptera litura* attack on pakcoy plants can cause crop yield losses of up to 85% and can even cause crop failure or puso (Nurlaili et al., 2020).

The discovery of a new pest in maize, namely *S. frugiperda*, can cause concern for farmers in the future. This concern is due to whether the pests can attack lettuce and pakcoy in the future. Although there aren't any report of yield loss of lettuce and pakcoy because of

S. frugiperda attack, but we must minimie of this spread and attack in other cultivar plant. Considering this pest can make high yield lost in other cultivar plants (De Groote et al., 2020; Maharani et al., 2019; Trisyono et al, 2019). Therefore, this research needs to be done to determine whether lettuce and pakcoy can host *S. frugiperda*. This study also aims tothe life cycle and determinesex ratio of *S. frugiperda* which is given lettuce and pakcoy in the laboratory.

2. Materials and Method

Mass rearing of *S. frugiperda* from maize

This protocol was modified from (Perkins, 2009) and (Ginting *et al.*, 2021). Larvae collection was carried out in the Bantul area, to be precise in the Bangunharjo, Sewon and Sumber Agung, Jetis (7°50'48.6 "S 110°22'58.8" E). Larvae were put in a small plastic aquarium (15 cm x 10 cm x 10 cm) and fed with corn leaves and were separateed with each other. After that, larvae were then put in a 240 ml plastic cup and covered with an organdy cloth tied. Each glass contains 1 larvae. Every day, larvae were fed with young corn leaves until turn into pupae. After turn into pupa, the pupa then put in a 5 L jar and given a cotton ball that has been moistened with honey. The cotton was hung from the top of the jar and covered with an organdy cloth and waited until became a moth. The jar was placed in a room that's not exposed to sunlight and covered with a black cloth. After turning into a moth and aying eggs, the eggs were counted. Furthermore, the eggs were waited for until they hatch and turn into larvae.

Process of larva rearing *S. frugiperda*.

Alternative feed of lettuce and pakcoy leaf was prepared. Lettuce and pakcoy leaves that have been prepared were washed using running water and then cleaned with tissue. Each feed was weighed using analytical scales for 1 gram in 1 plastic cup. Feed was given to larvae in plastic cups with 3 replications. Each plastic cup contains 10 of 1st instar larvae. 3rd instar larva was separated so they don't eat each other (cannibals). The feed was changed every 2 days, starting from 3rd instar, the feed was changed every day until turns into pupa. 3rd instar larvae body length, head diameter and weight were measured to determine the weight of the larvae. After turning into pupae, the 3 days old pupae were weighed to determine the weight of the pupa. The pupa were placed in a jar and covered with an organdy cloth tied until turns into moth. After turning into moth, the jar was given a cotton ball soaked in honey and then hung it in the jar. Jar filled with pupa and moth were placed in a dark place. After that, wait for the moth to lay eggs.

Observation Parameters

Moths that hatched were counted to determine the number between male and female. Acccording to (Brambila, 2013; Deole & Paul, 2018) adults (moths) of *S. frugiperda* have forewings light brown, gray, and dark brown in males while females have gray wings with light and dark markings. In both sexes there is a white patch near the tips of the forewings. The markings in males are more prominent than females, having a gray color and bright diagonal markings on the forewings and hind wings in white. Then the eggs produced by the moth were calculated as well. The time needed for one life cycle of *S. frugiperda* was calculated.

Data analysis

The data analysis was carried out, namely starting with the Shapiro-Wilk normality test, if the data were obtained normally, then continued with the homogeneity test then the Annova continued test was carried out, but if the data obtained was not normal then a non-parametric further test was carried out with Kruskal Walis. All stages of data analysis were carried out at the 5% significance level.

3. Results and Discussion

Larvae length and head diameter

Based on the research results, the increase in larval length and the widest head diameter was found in pakcoy feed, while the shortest was found in maize feed (Table 1). The stadia development of *S. frugiperda* from egg to imago shown in Fig. 1.

Table 1. Length and diameter of the head larval *S. frugiperda* instar1 - 6.

Stadium	Control (Average ± SD)		Lettuce (Average ± SD)		Pakcoy (Average ± SD)	
	Larval length (cm)	Head diameters (cm)	Larval length (cm)	Head diameters (cm)	Larval length (cm)	Head diameters (cm)
Instar 1	0,31 ± 0,33	0,05 ± 0,00	0,15 ± 0,07	0,05 ± 0,00	0,15 ± 0,06	0,05 ± 0,00
Instar 2	0,66 ± 0,11	0,10 ± 0,01	0,79 ± 0,11	0,07 ± 0,04	0,48 ± 0,32	0,09 ± 0,03
Instar 3	1,19 ± 0,31	0,15 ± 0,05	1,36 ± 0,27	0,15 ± 0,00	1,53 ± 0,24	0,15 ± 0,00
Instar 4	1,97 ± 0,33	0,19 ± 0,02	1,84 ± 0,29	0,20 ± 0,00	2,01 ± 0,33	0,20 ± 0,18
Instar 5	2,31 ± 0,30	0,20 ± 0,00	2,55 ± 0,41	0,29 ± 0,03	2,78 ± 0,19	0,29 ± 0,07
Instar 6	2,65 ± 0,31	0,20 ± 0,00	2,75 ± 0,32	0,29 ± 0,01	3,09 ± 0,21	0,30 ± 0,00

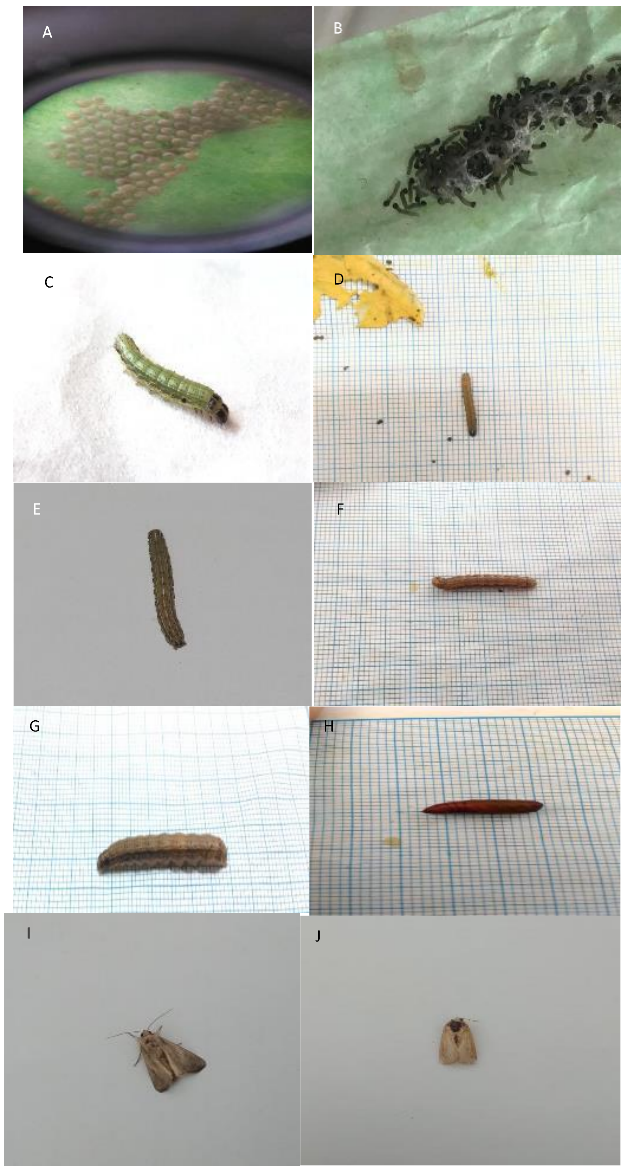

Figure 1. Stadia development of *S. frugiperda* in laboratory feed with lettuce and pakcoy; (A) egg; (B) 1st instar larva; (C) 2nd instar larva; (D) 3rd instar larva; (E) 4th instar larva; (F) 5th instar larva; (G) 6th instar larva; (H) Pupa; (I) Male *S. frugipreda*; (J) Female *S. frugiperda*

Larval weight

The average weight value of larvae shows that larvae *S. frugiperda* have the heaviest weight, namely in pakcoy leaf feed, while the lowest is in maize leaf feed (Table 2). According to Barros et al., (2010) , the development of insects depends on the preferred feed. The nutritional content of the host plant which is suitable for the growth and development of insects will cause the rapid growth and development of these insects.

Sex ratio *S. frugiperda*

According to West and Sheldon (2002), the sex ratio is the ratio of the total population between the sexes of women and men or male and female. The sex ratio produced in the maize resulted in 13 females and 16 males, while on lettuce leaves produced 13 females and 17 males, and on pakcoy leaves produced 16 females and 14 males (Table 3).

The number of eggs of *S. frugiperda*

The results of the calculation of the number of eggs produced obtained the highest yield of eggs in the pakcoy leaf treatment compared to other treatments (Table 4).

Discussion

According to (Montezano et al., 2019; Subiono, 2019), type of food will affect the weight of early development to late development in the insect cycle. Foods that contain more nutrients will support the rapid growth and development of insects as well and cause *S. Frugiperda* pakcoy fedleaves to have a longer and larger head length and diameter than other feeds. This is because the nutritional content contained in Pakcoy is higher than other treatments. This is supported by the research of (Carinhas et al., 2011) and (Oliveira et al., 2020), stated that amino acids needed in *S. frugiperda* for their growth.

The average weight value of larvae showed that larvae *S. frugiperda* had the heaviest weight, namely in pakcoy leaf feed, while the lowest was in maize leaf feed (Table 1). The content of carbohydrate in pakcoy leaf higher than other feed. Carbohydrate used in *S. frugiperda* to increase their weight (Mello da Silva et al., 2017). Not only that, the larvae prefer to eat pakcoy than maize because nutritional content in the feed. According to (Montezano et al., 2019; Subiono, 2019), the development of insects depends on the preferred feed. The nutritional content of the host plant which is suitable for the growth and development of insects will cause the rapid growth and development of these insects. In (Subiono, 2019), results showed that the tested larvae had a weight on the 6th instar of 1.84 gr. Based on the results of this study, it was found that the tested larvae had a lighter weight than the previous study, namely 0.29 gr. Although the temperature used in this study was suitable for the growth oflarvae *S. frugiperda*, the corn leaves used quickly wilted. This causes the larvae weight to be lighter than previous studies.

According to (Hardy, 2002), sex ratio is the ratio of the population between the sexes of women and men or male and female. This comparison is not only seen in the size of the population, but also in a certain period of time (Smallwood & De Broe, 2009). In this study, the highest number of sex ratios femalewas in the treatment of pakcoy leaves and maize leaves, namely 16 heads, while the number of males was as many as 14 in the treatment of pakcoy leaves and as many as 13 in the treatment of

Table 2. Weight larvae of *S. frugiperda* instar 3 - 6.

Stadium	Weight of *S. frugiperda* larvae (gram)		
	Control (Average ± SD)	Lettuce (Average ± SD)	Pakcoy (Average ± SD)
Instar 3	0,04 ± 0,02	0,06 ± 0,02	0,06 ± 0,04
Instar 4	0,09 ± 0,05	0,23 ± 0,07	0,24 ± 0,06
Instar 5	0,21 ± 0,07	0,31 ± 0,08	0,37 ± 0,07
Instar 6	0,29 ± 0,06	0,34 ± 0,07	0,39 ± 0,07

Table 3. Sex ratio *S. frugiperda*

Replication	Sex ratio of *S. frugiperda*					
	Corn		Lettuce		Pakcoy	
	Female	Male	Female	Male	Female	Male
1	6	4	3	7	6	4
2	5	5	4	6	5	5
3	5	4	6	4	5	5
Total	**16**	**13**	**13**	**17**	**16**	**14**

Table 4. Number of eggs of *S. frugiperda* in 3 treatments of

Stadium	Number of eggs from *S. frugiperda*		
	Control (Average ± SD)	Lettuce (Average ± SD)	Pakcoy (Average ± SD)
Eggs	2.195 ± 947[a]	847 ± 150[b]	3.038 ± 361[c]

Note: (a) there is a real difference, (b) there is a real difference, (c) there is a real difference

corn leaves. According to research by (Signoretti et al, 2012) stated that the sex ratio of *S. frugiperda* fed with corn leaves would produce more female sex ratios. This is because *S. frugiperda* has been conditioned to adapt to maize plants (Guera et al, 2020; Montezano et al., 2019; Wang et al., 2020). Probability of mating affected by sex ratio in the population. The higher female ratio in the population, probability to mating in the population also higher (Jenouvrier et al, 2010; Stone et al, 2007). It can be seen in male pakcoy produced 14, while in corn 13. This will cause the number of eggs produced in the next generation to be more in pakcoy leaves, considering the number of males produced is more on pakcoy leaves than in corn, even though both have the number of females that are same. However, the sex ratio in populations that are developed artificially or not naturally will of course be different from the sex ratio in populations that develop naturally in their natural habitat (Jehan et al, 2020).

The difference in the number of sex ratios produced is due to the different types of feed given (Montezano et al., 2019). Different types of feed can affect the amount of male and female sex produced, this is because each type of feed has a different nutritional content. The content in feed, especially vitamins and minerals, is needed by adult insects to increase fecundity, fertility, and balance the sex ratio (Awmack & Leather, 2002; Kavallieratos et al., 2020; Nascimento et al, 2021). Of the three treatments, the highest content of vitamins and minerals was found in the pakcoy plant. In addition, pakcoy contains vegetable fats, vitamins and minerals needed by insects for the formation of cell membranes and hormones (Oonincx et al., 2018; Roe et al, 2008; Salem et al., 2014). Fatty acids are very important in the process of egg embryo formation (oogenesis) and heavy and long pupae will produce large females and produce lots of eggs (Maurer, 2016). In addition, in themeasurement results sex ratio, the highest number of females was found in pakcoy and corn leaves, this will also affect the number of eggs produced in the next generation.

It can be seen from the three treatments given, *S. frugiperda* has a different length of time needed in one cycle. Larvae treated with corn leaf feed were able to complete one life cycle for 38 days, while in the treatment of lettuce completed one life cycle of 39 days, and in the pakcoy leaf treatment completed one life cycle of 37 days. The fastest life cycle occurred in treatment

with pakcoy leaf feed, while the slowest life cycle occurred in treatment with lettuce feed. According to (Shekhawat et al, 2018), differences in the larval period of armyworms can be related to differences in nutrition in the feed given. Protein is one of the body building nutrients that provides a lot of material for growth. This is supported by (Silva et al, 2016), who states that insects that need feed with high protein content will take advantage of the availability of these compounds for tissue formation, so that larvae reach the final instar stage more quickly. Many proteins provide the basic substance for the formation of larvae body tissue which is used to pass through the instar stages during their development, whereas carbohydrates tend to act more as a source of energy.

According to (Mello da Silva et al., 2017; Wang et al., 2020) the growth and development of insects will be faster if they get the right feed. It can be seen from the three treatments, the fastest insect development is found in the Pakcoy treatment. This is because Pakcoy has the highest protein content of the three treatments given (Mello da Silva et al., 2017). In addition to suitable feed, the growth and development of insects is also influenced by several factors. The factors that influence the duration of development of insects bred in the laboratory are (1) the abiotic laboratory environment is always constant, (2) the biotic environment is always controlled, (3) temperature, humidity, light and wind are deliberately made accordingly, (4)occurrence density dependentbehavior, and (5) the partner selection process is weakened due to limited space.

The duration required for the development of *S. Frugiperda* pakcoy by feedingleaves is 37 days. This is in accordance with the research of (Mello da Silva et al., 2017), which states that the life cycle of one generation armyworms on the pakcoy host plant is around 30-60 days, while the duration required for the development of *S. frugiperda* by feeding lettuce is 39 days. The urasi needed in the development of *S. frugiperda* by feeding corn leaves is 38 days. This is in accordance with (Igyuve et al, 2018; Sharanabasappa et al, 2018), who stated that the life cycle of one generation armyworms on corn host plants is around 38 - 55 days. The conclusions that can be drawn from this study are 1) plants Lastuca sativa L. and Brassica rapa L.can be used as alternative hosts for *S. frugiperda* JE Smith. in the laboratory, 2) the life cycle of *S. frugiperda* in the laboratory with feed lettuce namely for 39 days and pakcoy for 37 days and 3) the number of eggs produced by *S. frugiperda* fed with lettuce feed in the laboratory namely 847 eggs and produced 13 females and 17 males, while the number of eggs fed by Pakcoy in the laboratory was 3,038 and resulted in 16 females and 14 males.

4. Conflict of interest

All authors state that there is no conflict of interest regarding this study and the results of this study.

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