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Hatching of migratory locust (*Locusta migratoria*, L.) (*Orthoptera: acrididae*) eggs at several of texture and moisture levels in semi-field laboratory

Nikolas Nik [1*], Edhi Martono [2], Nugroho S. Putra [2], Suputa [2]

[1] Department of Agrotechnology, Faculty of Agriculture, Timor University, INDONESIA
[2] Department of Crop Protection, Faculty of Agriculture, Gadjah Mada University, INDONESIA
*Corresponding author: mariaseoteme@yahoo.co.id

Abstract

Migration locust (*Locusta migratoria*, L.) or *Kembara* (Indonesian) grasshopper is one of the herbivorous insects that lay eggs on the ground. The spawning process requires the right place, humidity, and type of soil. Moist soil and suitable soil texture can accelerate the hatching of grasshoppers into nymphs. This study aims to analyze the effect of texture and soil moisture level on hatching eggs of migratory locust (*Locusta migratoria,* L.). This research was carried out in a semi-field laboratory in Kefamenanu, North Central Timor Regency. The study was conducted for three months, from October to December 2018. The research method used completely randomized designs and data analysis using the Tukey's HSD (honestly significant difference) 5% test. The results showed the texture of sandy clay loam with a humidity level of 60% (24.67 days) and 80% (22.33 days) can shorten the time of hatching eggs, and at 40% humidity on the texture of the clay lasts longer for 32.33 days.

Keywords: egg, Kembara grasshoppers, hatching, Locusta migratoria, soil moisture

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INTRODUCTION

Migratory locust (*Locusta migratoria,* L.) or *Kembara* (Indonesian) grasshopper is a vicious pest in the districts of East Sumba, East Manggarai, North Central Timor, and Belu, East Nusa Tenggara Province, Indonesia. In addition, these areas are endemic areas for migratory locust pest breeding. The dry season in the Timor region lasts long, which is about 8 months in one year. According to Zhang (2019) migratory locust is too hot in Indonesia. Some farmers work in the fields by growing corn, sorghum and rice. Therefore, migratory locust pest is a ferocious pest that is feared by farmers in this area because it can damage crops and cause crop failure. **Figures 1 and 2** show migratory grasshopper pest (*Locusta migratoria*, L.) has damaged maize in two areas of East Sumba in 2019 and East Manggarai Regency in 2020.

The nymph is almost widespread in the region, especially in the savannah. Nymphs are small grasshoppers that do not yet have wings and reproductive organs that appear after the grasshopper's eggs hatch. In general, nymphs are white but after exposure to sunlight they will change color to green or brown. In June 2020, nymphs appeared in the Palakahembi area, Pandawai District, East Sumba Regency. **Figure 3** shows the nymphs that attacked the corn leaves in Manufonu Village, Insana Utara District, North East Timor Regency in 2019. This pest has damaged farmers' crops so that the damage and losses they cause are varied as well as a high population increase (Ma *et al*., 2011).

Uvarov (1977) stated that migratory locust (*Locusta Migratoria,L*) is an agricultural pest throughout the world (Hou *et al.,*2020). Important agricultural pests in this part of the world, namely migratory epidemics, are associated with climate events such as drought or El Niño (Qi *et al*., 2007). The migratory locust is the most widely distributed orthopteran in the world and poses a potentially serious threat to agricultural crops throughout Africa, Eurasia and Australasia as a result of its propensity to outbreak in massive swarms and to migrate long distances (Tu *et al.*, 2019). *Locusta migratoria* is an economically important agricultural insect, and there is much empirical awareness about its biology (Nishide *et al.*, 2017). Therefore, migratory locust invasion has been considered a significant threat to agriculture since ancient times (Dkhili *et al*., 2019).

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Locuste outbreaks such as *Schistocerca gregaria*, *Locusta migratoria*, and *Chortoceites terminifera* regularly affect vast areas of North Africa, the Middle East, Asia, and Australia (Topaz *et al*., 2012). According to Hu (2014) the migratory locust is a major insect pest in China's range land and farm land (Li *et al*., 2020). For the desert locust, the population dynamics of *Schistocerc agregaria* (Forskål) (*Orthoptera: Acrididae*) – a major pest whose invasions begin in the desert areas of Mauritania to India (Lazar *et al*., 2019). Latchininsky (2000) stated that locusts are one of the most destructive agricultural pests in former Soviet Union countries, particularly Russia, and Kazakhstan (Löw *et al*.,2016). Locusts and grasshoppers (*Orthoptera: Acrididae*) are notorious pests responsible for the destruction of crops and pastures (Crooks and Cheke, 2014). Several locusts include *Locusta migratoria* (*Orthoptera:Acrididae:Oedipodinae*) and the locust desert *Schistocerca gregaria* (Forskål) (*Acrididae: Cyrtacanthacridinae*) are probably the most harmful agricultural insects in the world (Tokuda *et al*., 2010). Popov *et al* (1991) state that desert locust swarms typically originate in desert regions but they expansive invasion zone is among the largest of any locust and includes many agricultural areas (Le Gall *et al*., 2019). Locusts and grasshoppers (L&G) (*Orthoptera: Caelifera, Acridoidea*) disturbed grassland ecosystems. They are among the most devastating enemies of agriculturists (Alexandre *et al*., 2011). Locusts and Grasshoppers (L&G) are among the most devastating enemies of farmers when their populations grow to catastrophic dimensions (Latchininsky *et al*., 2011). Locusts and grasshoppers are abundant in natural and anthropogenic habitats such as grasslands, wetlands, agricultural fields, lawns and the like (Alexandre *et al*., 2011). Desert locust (*Schistocercagregaria*) is one of the most destructive migratory pests in the world (Wang *et al*., 2019). The Asiatic Locust (*Locusta migratoria*) has the largest distribution range for all species of locust (Malakhov*et al*.,2018). According to Ellis *et al* (1957) the desert locust, *Schistocerca gregaria* (Forskål, 1775), solitary locusts that occur in low density avoid each other and are sedentary, whereas gregarious locusts that occur in high density are attracted to each other and move long distances within a group (Maeno and Ebbe, 2018).

The distribution of migratory locusts is supported by several factors, among others, food availability, texture and soil moisture, and land use. These factors can influence the increase in population so that it is difficult to prevent. To prevent its impact on the local community, it is necessary to find the right location for the culture, namely humidity in a dry or semi-arid environment (Gomez *et al*., 2018). Desert locusts can adapt well to live in arid and semi-arid habitats where annual rainfall is around 0–400 mm. When the population is high, migratory locusts can be found in various places such as swamps, arid fields, open forests or in gutters (Schowalter, 2018). In general, grasshopper biology is often associated with abiotic conditions such as temperature, rainfall, humidity and soil texture which always interact with their environment (Le Gall *et al*., 2019).

Fig. 1. Migratory locust or called *Kembara* (Indonesian) grasshopper attacks. The ferocious pest attacked 10 hectares of grasslands and corn plantations in East Sumba in June 2019

Fig. 2. Grasshopper pest attack in the area of Watu Ngene Sub-district, Kota Komba District, East Manggarai Regency resulted in 40 hectares of maize crop failure in February 2020

Fig. 3. Grasshopper nymphs damage corn plants in Manufonu Village, Insana Utara District, North East Timor Regency in 2019.

Fig. 4. The nymph group is clustered along the roadside of Ponu Village, Biboki Anleu District, North Central Timor Regency in 2020

Fig. 5. The nymphs are grouping in the grasslands in Rindi Village, Rindi District, East Sumba Regency in 2020

Movement of grasshopper cycles vary in their habitat, both micro in certain plant species that can correlate with time, light intensity, temperature, humidity, and certain plant species (Maeno *et al*., 2016). Grasshopper monitoring must be adjusted based on time, age of grasshoppers, phase conditions and relative abundance of certain plant species. Migratory locust (*Locusta migratoria,* L.) tends to form large groups and likes to move around so that in a short time can spread over large areas. Usually individuals who migrate in groups while eating plants to improve nutrition for their development (Clark *et al*., 2013). Vegetation is one of the factors that affects the daily travel distance but vegetation density reduces the daily journey distance of migratory locusts bands (Dkhili *et al*.,2019). Groups that migrate can eat the plants they pass during the trip. Eating behavior of migratory locusts adult usually alight in the afternoon and evening until the morning before flying.

Hatching Egg

When damaging plants, adult grasshopper groups mate and lay eggs on the ground in the area. Once placed, the eggs will hatch into nymphs. Most adult grasshoppers who have laid their eggs do not maintain their eggs, unlike other insects where the hatching of eggs is facilitated by the mother (Mukai *et al*., 2012; Nishide *et al*., 2016). All locust species require moist soil for oviposition and varying degrees of moisture at different times to allow their eggs to develop and hatch (Crooks and Cheke, 2014). The incubation period can be as short as 10 to 20 days, depending on the availability and temperature of soil moisture (Crooks and Cheke, 2014). Migrating grasshopper eggs are placed on the ground making them difficult to observe. The breeding model is carried out in the breeding house so that it can know the life cycle events hatched into nymphs. This model serves as empirical information about how soil and drought conditions interact to form eggs into nymphs (Johnson *et al*., 2010). The eggs are laid in moist soil during the rainy season, which is around mid-July and the average estimated incubation period is 40 days. Nymphs from the solitary phase are mostly green and will develop in August and early September, and turn into adults from late September to October (Elballa *et al*., 2020). During the winter in North China migratory eggs are stored on the ground for 6 months, i.e from autumn to the following spring (Qi *et al*., 2007). According Padgham (1981) the eggs of the desert locust *Schistocercagregaria*, hatch during the cryophase (low temperatures) under 12-h thermocycles (Nishide and Tanaka, 2016). The eggs are typically deposited in dispersed areas (egg fields) by groups of females, and up to 1,000 egg pods were found in one square meter. The eggs are laid down 10-15 centimeters below the surface in sandy soils. Migration grasshoppers show polyphenism between grouped and solitary individuals by showing more synchronous sexual maturation as stated by He Jing *et al*., (2016) that the time of hatching gregarious grasshopper eggs is more uniform than solitary grasshoppers. Grasshopper who is ready to lay eggs must find a suitable place for oviposition (Tanaka *et al.*, 2019). The temperature range for developing eggs and nymphs is between 20-35°C (Wang *et al*., 2020). On drought-affected soils, hatching of locust migratoria eggs will be delayed and the percentage of hatching will be low (Wang *et al*., 2019). Time for a variety of soil moisture at low temperatures can cause the death of grasshopper eggs (Qi *et al*., 2007)

MATERIALS AND METHODS

This study aims to analyze the effect of texture and soil moisture on the percentage and time of hatching of migratory locust eggs. This research was conducted in a semi-field laboratory in Kefamenanu, North Central Timor Regency, for three months from October to December 2018. The materials used for the study are clay, sandy clay loam, sandy clay, water, female and male individual insects, migratory locust eggs, and cotton. The tools used for research are insect cages, digital soil moisture, tweezers, and test containers.

Experimental Sites

Sampling of soil texture was carried out using GPS in locations with migratory locust pests as information on the study area (Low *et al*., 2016). The research location consists of three places namely Manufonu, Insana Utara District, at the ordinate point S 09o12'32,9" E 124o30'14,2", Ponu, Anleu Biboki District, at S 09o08'06,7" E 124o41'12,5" and Naen, District of Kefamenanu City, at the ordinate point S09o29'48,8" E124o31'23,2". Before taking soil samples, vegetation or leaves are cleaned at the point of the sample quadrant. Then the soil sample is taken using a PVC soil ring with diameter of 5 cm and length of 7 cm. The goal is that soil conditions do not change. At the bottom of the ring is given a base with cardboard. Then, soil samples are labeled and put in plastic bags and tied tightly. Before the soil sample is analyzed in a soil laboratory, the soil needs to be dried first using wind. In addition, pieces of soil are softened by sieving using a 2 mm diameter sieving device. Furthermore, the sieve results are mixed evenly and put into small bottles that are tightly closed and labeled. Procedures for soil sampling and soil texture analysis in the laboratory as shown in **Figures 6-8**.

Fig. 6. Soil sampling

Fig. 7. Soil samples ready to be analyzed in soil laboratory

Fig. 8. The process of analyzing soil samples in soil laboratory

Migratory locust test insects are taken in farmers' fields and grasslands by using a light at night. Then the locusts were planted in the insect breeding house. During the process of breeding must be given food and modified conditions so that the migratory locusts are developing as in nature or field. The goal is that the migratory locust will mate and produce eggs for use as egg hatch test samples. The process of preparing migratory locust is as shown in **Figures 9-11**.

Fig. 9. Catching of the migratory locust with lights

Fig. 10. Making migratory locust breeding house

Fig. 11. The life of migratory locust insects in a breeding house

This experiment consists of two factors, the first is the texture of the soil with the level of treatment of clay (A1), sandy clay loam (A2), and sandy clay (A3). While the second factor is classified as soil moisture which consists of wet soils, moist soils, and dry soils. In the soil moisture test, the treatment level consisted of 40% (B1), 60% (B2), 80% (B3), and 100% (B4) soil moisture. The design used in this experiment was Completely

Randomized Design with 3 replications and 36 units of experimental units. For the process of hatching eggs at several levels of soil texture is done after analyzing soil texture samples in a soil laboratory. So you can find out the actual texture of the soil. Soil samples taken from the field are put into earth jars of 12 cm in diameter and 10 cm in height. To find out soil moisture is done by given water that has been filled with soil before then measured with Digital Soil Moisture adjusted to the treatment. In a container that has been filled with earth, a hole can be made 2 cm. Subsequently, as many as 30 migratory locust eggs were included as a result of copulation from the breed at the breeding house (Woodman, 2015). Then put into an insect cage size 30 cm x 30 cm x 30 cm and placed in a room with room temperature ± 28ºC. To regulate the measurement of soil moisture during the experiment needs to be controlled every time by adding water to maintain soil moisture in accordance with the treatment. Then count and record the time the number of eggs that hatched into nymphs in each treatment. To find out the percentage of eggs that hatch using the following formula:

$$\%\ \text{egg} = \frac{\sum \text{hatched egg}}{\sum \text{total egg}} \times 100 \quad (1)$$

The data were analyzed and tested using a 5% BNJ test (Mattjik. 2000)

RESULTS AND DISCUSSION

Migratory locust has a high reproductive rate and is able to respond to favorable climatic conditions with a rapid increase in population. Abiotic factors as a support for increasing population, including, texture and soil moisture. Both of these abiotic factors are very important for the growth and development of insect life including the migratory locust pest. In the process, individual female migratory locust needs suitable soil texture and moisture to lay their eggs. So that they can produce new individuals. However, the state of the texture and soil moisture is a factor that affects the life of grasshoppers.

The results of the texture and soil moisture test on the hatching of migratory locust eggs showed an interaction between the two factors. The highest percentage is reached at 80% humidity level but not significantly different from the 60% humidity level in the texture of sandy clay loam. In this situation the soil porisity is good enough so that the air circulation in the soil goes well and makes it easier for grasshoppers to carry out their activities. In moderately moist soils and loose soil texture will support the viability of locust eggs to hatch into nymphs. In this kind of texture can create air flow to and from the soil particles takes place smoothly, the spaces between loose particles so that they can continue the water for the growth and development of soil microorganisms. The higher the soil pores, the aeration can function well for the development of microorganisms in the soil. Then there is reciprocal interaction so it is very helpful for the process of hatching eggs (Shmuel *et al.*, 2019). Kim *et al.* (2017) states that in sandy soil conditions will provide aeration which is the process of air circulation. Furthermore, the results of Georgis *et al* (2010) showed that the distribution of nematodes varied greatly with soil type, the greatest spread and infectivity occurred in pure silica sand and coarse sand. Conversely, if the soil pores are small, the air circulation process can be inhibited. Eventually, it will slow down the growth and development of an organism in the soil. This situation is found in the texture of clay which has fine soil particle size so that the ability to store water is very high and causes egg decay or inhibition of hatching eggs of the migratory locust (diapause).

However, soil water content and temperature have an important role in the development of *Acrididae locusta* eggs. Normal egg development requires an appropriate water content. In wet or humid soil conditions the eggs will survive in the soil or decompose so they do not have the opportunity to avoid high humidity (Malakov *et al*., 2018). The texture and structure of the soil can affect the development of eggs in the field. The newly laid migratoria locust egg has a moisture content of 53 percent of the wet weight and will rise to 83 percent if it will hatch (Roonwal, 1936).

Table 1. Effect of Texture and Soil Moisture Rate on Egg Hatching Percentage

Soil Type	Moisture Level (%)			
	40%	60%	80%	100%
Clay	37.78 ± 5.09 a	95.56 ± 1.92 b	80.00 ± 8.02 b	0.00 ± 0.00 a
Sandy Clay Loam	85.56 ± 5.09 b	91.11 ± 3.85 b	97.78 ± 3.85 c	51.11 ± 3.85 c
Sandy Clay	35.56 ± 3.85 a	71.11 ± 10.18 a	43.33 ± 6.67 a	27.78 ± 5.09 b

(%± standard deviation)

Note: Numbers followed by the same letter in the same column are not significantly different based on Tukey's 0.5% further test

The highest percentage of hatching eggs is achieved in the texture of sandy clay loam at 80% humidity and 60% humidity. This is because the soil aeration is good enough so that it can simplify the process of hatching eggs in the soil. While at100% humidity the percentage of hatching eggs has decreased. This is due to the occurrence of locust egg decay and the condition of the soil texture due to the tight bonding of soil particles. So that the ability to store water is quite high and results in egg rot. The higher the humidity will inhibit air circulation resulting in a decrease in hatching activity. Low humidity can also inhibit hatching of grasshoppers. At this humidity level the soil texture is rather dry so that the activity of egg incubators can be disrupted. Makalov *et al* (2018) states that air conditions related to soil moisture levels in a complex manner can affect the process of hatching of migratory locust eggs. At the 40% humidity level tends to decrease hatching. At low humidity, the ability of eggs to absorb water tends to

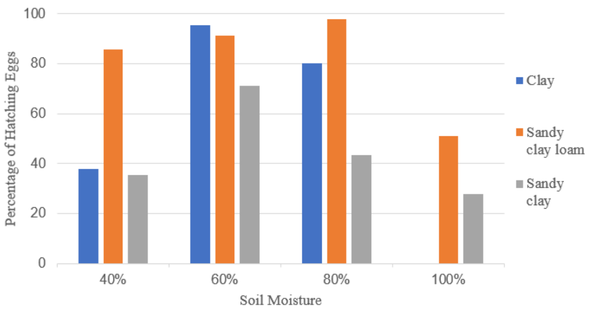

Fig. 12. Effect of soil moisture type and level on the percentage of hatching eggs

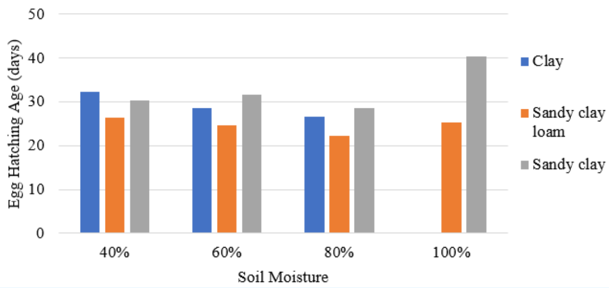

Fig. 13. Age of hatching eggs at different moisture levels and textures

Table 2. Effect of Texture and Soil Moisture on Hatch Age (days ±standard deviation)

Soil Type	Moisture Level (%)			
	40%	**60%**	**80%**	**100%**
Clay	32.33 ± 0.58 b	28.67 ± 0.58 b	26.67 ± 0.58 b	0.00 ± 0.00 a
Sandy Clay Loam	26.33 ± 1.53 a	24.67 ± 0.58 a	22.33 ± 1.53 a	25.33 ± 1.53 b
Sandy Clay	30.33 ± 0.58 b	31.67 ± 1.53 c	28.67 ± 1.53 b	40.33 ± 4.51 c

Note: Numbers followed by the same letter in the same column are not significantly different based on Tukey's 0.5% further test

slow down so that the energy possessed by egg embryos is less. This will affect the number of eggs that hatch into nymphs. This information becomes the basis for determining hereditary strength (Maeno *et al*., 2012; Van Wielendaele *et al*., 2012). This study shows that the pattern of reserves and energy utilization at each humidity level is different. It seems that differences in egg development between ovariols also cause variations in egg size. In addition, sometimes the low percentage of hatching eggs can be caused by hatching times that are not concurrent so that it is very adaptive to the humidity situation. Likewise, in the wild nature or in the field it is sometimes disturbed by rain so that eggs in one pods do not hatch at the same time but will hatch with the next day. Sometimes newly hatched eggs can be damaged by rain droplets causing death. Changes in soil moisture as an indicator to determine the success of hatching eggs (Nishide *et al*., 2017). Whereas Crooks *et al* (2014) explained that when it rains there will be lands catering so that the laid eggs will carry water currents. In unfavorable conditions the eggs can remain diapause or the eggs survive in the soil. These eggs can survive for two to five years. Thus, if climatic conditions are favorable, it can be possible for several generations to reappear (Lomer *et al*., 2001).

Egg incubation experiments in the laboratory show that eggs hatch into nymphs depending on the type of texture and soil moisture level. From these observations it can be said that the texture of sandy clay loam with humidity level of 60% and 80% can shorten the time of hatching eggs (**Table 2**).

Figure 13 shows that the texture and moisture of the soil influence the age of retention of migratory locusts at a humidity level of 60% and 80% which can accelerate the time of hatching eggs respectively at 24.67 days and 22.33 days on sandy clay loam. This might be due to the loose soil pores which will facilitate the process of air exchange in the soil making it easier for egg embryos to absorb water quickly hatch into nymphs. Eggs that absorb water well will probably be transplanted to hatch earlier, it is suspected that in larger water masses it tends to hatch earlier because of the possibility of receiving the stimulation earlier so that it hatches more quickly. The phenomenon of hatching eggs at 40% humidity on the texture of clay tends to take longer(32.33 days) to hatch, it is suspected that the laid eggs have been separated from their pods, thus slowing the hatching process. This is supported by Tanaka (2017) stated that the separation of migratory locust eggs will affect the time of hatching, eggs that are stored alone need more time to hatch than in one pods. Another thing that affects the hatching time is suspected that the soil taken may be too old so that it can hinder or delay hatching eggs. Tanaka *et al* (2017) stated that grasshopper *Schistocerca gregaria* sometimes refused to lay eggs in the sand for too long because it inhibited ovipositors from female individuals. In addition, it might also be caused by the condition of the soil dries somewhat and slows the absorption of water into the egg embryo, so that it experiences delays in the hatching of migratory locust eggs. Eggs exhibit a facultative embryo mechanism that is triggered by low soil moisture thus making water slowly available to grasshoppers eggs (Deveson *et al*., 2014).

CONCLUSION

The highest percentage of migratory locust hatching eggs is at an 80% humidity level in the texture of sandy clay loam of 22.33 days. But the results are not much different from the 60% humidity level in the same type of soil and texture, which is 24.67 days. In addition, the texture of sandy clay loam with a humidity level of 60% and 80% can shorten the time of hatching eggs, which is 22.33 days and 24.67 days. While the hatching of eggs at 40% humidity on the texture of the clay last longer which is 32.33 days. It is suspected that the eggs placed have been separated from their pods, thus slowing the hatching process. The state of the texture and moisture of the soil is an important factor in the process of hatching of migratory locust.

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