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EFFECT OF SOIL TYPE, MOISTURE AND SAND COVER ON PUPATION DEPTH, SURVIVAL OF PUPAE AND ADULTS OF *ZIZYPHUS* FRUIT FLY, *Carpomyia incompleta* BECKER UNDER LABORATORY CONDITIONS

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ABSTRACT

The current study indicated that the most suitable soil type and moisture level for the emergence of Zizyphus fruit fly (ZFF), *Carpomyia incompleta* Becker (Diptera: Tephritidae) was the sandy soil containing 15% water. It was found that the highest pupation depth was about 5cm. To avoid the harmful to the friendly soil fauna, it may be suggested that if the soil fumigation or gas producing materials must be applied, it should be penetrated into the soil not more than 10cm. The effect of soil depth on the survival of pupae and adults of ZFF was studied under laboratory conditions. The results indicated that, soil depth is negatively correlated with emergence of ZFF adults. The highest rates of fly's emergence (100-80%) were observed at the lowest depths (1-15cm) with no significant differences in the percentage of adult emergence. In fact, we have recorded that highest rates of emergence (100 and 95%) were corresponded to 1and 2cm depth, respectively. The rates of emergence were gradually reduced when pupae placed on depth between 20 and 40cm. Rate of emergence at these depths was shown to be 70% at 20 cm depth and 45% at 40 cm depth. Depth of 50 cm had detrimental effect on emergence of adult fly, where the flies failed completely to emerge from the pupae (0%) at this depth. The results showed also that depth of soil between 5 and 10 cm is the most preferable for emergence of ZFF adults.

Our results are evidence that soil depth had a significant impact on emergence and survival of adults of ZFF. Therefore, we suggest that soil depth should be considered as one of the most important abiotic factors in minimizing the pest's population.

Keywords: Zizyphus fruit fly, pupa, adult, moisture, soil depth.

INTRODUCTION

The Egyptian jujube, Zizyphus – spina - christi (L.) Desf., is an ancient prehistoric fruit, that usually known as Sidr and its edible fruit as Nabq. It is a common fruit grown in the worm subtropical regions, belongs to the genus Zizyphus of family Ghamnaceae. The economic importance of Nabq in Assiut Governorate comes after citrus, dates and pomegranate (Morsy, 1971; Abdel-Galial and Darwish, 1987 and Darwish-Dalia *et al*., 2012). Zizyphus trees are grown as a windbreak or border trees and as a popular food for a lot of people. This crop is highly sensitive to the infestation with Zizyphus fruit fly, *C. incomplet*a (Farghal, *et al.*, 1981). It is well known that the females of *C. incomplet*a as the most of Tephritid flies deposit their eggs under the epidermis of Zizyphus fruits and after hatching of the eggs, the larvae feed exclusively on Zizyphus fruits. Infested fruits quickly rot and

become inedible or drop to the ground. The full feed larvae (3rd instar larvae) come out of fruits by boring an exit-hole to find a place to pupate in the soil (Kapatos, 1989; White Elsson-Harris, 1992). Pupation period lasts for 5-7 days and adult flies emerge and continue their life cycle, so this pest spends a part of its life cycle (pupal stage) inside the soil. Morsy, *et al.*, (1981) reported that a proportion of *C. incompleta* pupae enters diapause by the end of April. Although, it is known that the pest pupate in the soil, there have been no accounts of the depth at which they pupate. One of the most effective items in IPM program for fruit flies is burying the fallen fruits in the soil. Therefore, knowledge of pupation depth of ZFF is necessary as it may affect pupation success, consequently emergence of adults due to difference in temperature, moisture, mortality factors such as predation, soil characteristics (Darwish-Dalia, *et al.,* 2012) or efficiency control such as insecticidal soil drenches (Dimou, *et al.,* 2003 and Renkema, *et al.,* 2011). Thus, because of soil depth is an important abiotic factor affecting survival and developmental rates of ZFF, our simple question "Does the soil depth act as a direct mortality factor by the impact of soil pressure on pupae, or indirectly by preventing the emerged adults to make their way towards the soil surface?. The present question has still not been answered.

Therefore, the present study was carried out to estimate the soil depth at which the emergence of flies can be stopped, this may aid in developing alternative soil control methods, pupal sampling designs, assessment of natural mortality factors and management techniques in Zizyphus fruit and other fruit flies as well in Assiut Governorate.

MATERIALS AND METHODS

Source of insects:

In order to have a large number of pupae for this study, we have sampled infested Zizyphus fruits from different locations of Assiut Governorate. The fallen Zizyphus fruits under Zizyphus trees were collected in mesh pages twice a week during March and April, 2011 and transferred to the laboratory for examination.

Rearing cages:

The collected fruits were put in wire mesh cages (20x20x20 cm) with accurate holes. These cages were then suspended inside wooden crates each lined with two opposite aspects of wire mish. The upper aspect was made of glass. The frontal and rear aspects were made of wood with an opening of about 15 cm in the middle of the frontal aspect. This opening was provided with a black cloth sleeve. The base of the fund was also made of wood. Prior to experimentation, sand samples were washed, air dried, sifted through a 2 – mm mish metal sieve and dried in an oven for 6 h at 80°C to kill arthropods. Layers of sand of 2 cm for burying of the larvae during pupation were placed on the bottom of wooden cages. After that the pupae recuperated by sieving the sand.

Experimental Protocol:

To get pupae free from parasites for this trial, the stock of pupae were placed in a black jar connect with transparent plastic vial (plate 1). This unit was observed daily tell the emergence of parasite. The parasite was then attracted to the light in the transparent vial. Emerged parasites were daily released a way from the plastic vials. Adults from healthy pupae were usually emerged two days after the parasites emergence. After confirmation of complete emergence of the parasites, the remaining healthy pupae were selected and immediately used in the experiment.

Plate 1: Parasite separator at the left side and emerged parasite attracted to the light at the right side.

Effect of soil type and soil moisture on pupation depth:

Three types of soil were prepared:

1-Clay: brought in from the farm

2-Sandy: a pure sand

3-A mixture of clay and sand (1:1) by volume

Five degrees of moisture, 0, 5, 10, 15, and 20 % were prepared of soil and water volume by volume.

Plastic tubes (27 cm length and one inch in diameter) were divided longitudinally into two pieces, collected together with rubber band, closed from one end by plastic sheet and packed by the wetted soil. Two cm at the top of the unit were left without soil. Ten full grown larvae were placed on the

top of the soil, and covered with plastic sheet. Three replicates were prepared for each treatment. After 48 hours, the pupation depth was measured (Plate2).

Plate 2: A method for evaluating the pupation depth of Zizyphus fruit fly

Effect of sand cover weight on the emergence of Zizyphus fruit fly

Ten depths (3.5, 5.5, 7.5, 12.5, 17.5, 22.5, 27.5, 32.5, 42.5 and 52.5 cm) were tested to determine the effect of soil depth on emergence of Zizyphus fruit fly. Plastic tubes with a diameter of 2.4″ were cut into pieces of different lengths corresponding with the studied depths. Each length was replicated four times. Metal covers were installed on the basal ends of tube pieces. The top ends of the tube pieces were provided with plastic containers to receive the emerged adult flies (plate 3).

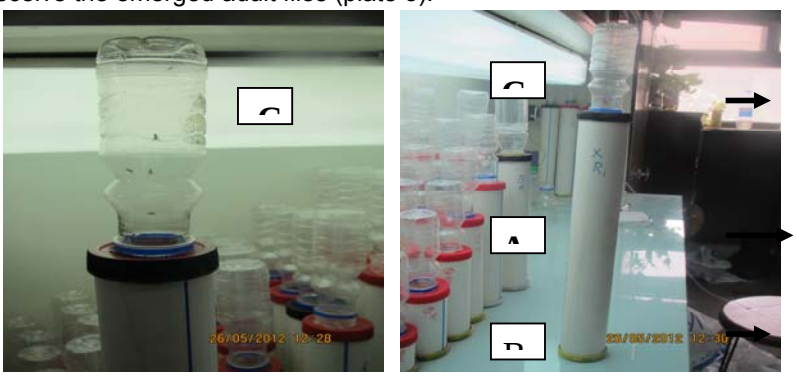

Plate 3: A tool used to determine the effect of soil depth on the emergence of Zizyphus fruit fly. A, Plastic tube 6cm in diameter and different lengths ranged from 3.5 to 52.5 cm, B lower stopper carrying the tested pupae, and C upper stopper attached to a transparent plastic vial showing the emerged flies.

Eighty healthy pupae (20 pupae/replicate) were placed on the bottom of tubes. The tube pieces were then filled with sand. The experiment was conducted in laboratory conditions at a temperature of 27±2°C and a relative humidity of 65±5%. From the first emergence, the number of adults was daily counted in order to assess the rate of emergence at each soil depth. Heights of the sand inside the plastic tube pieces were converted into so called weight of sand/cm^2 according to the following formula:

Wt. sand/cm^2 = $\pi (D/2)^2$ x H

Where:

π = a constant = 3.14

D = Tube diameter (cm)

H = Height of sand inside the tube piece

RESULTS AND DISCUSSION

Effect of soil type and soil moisture on pupation depth of ZFF:

The two-way analysis of variance of data (Table 1) indicated that the most suitable soil and moisture for the emergence of ZFF was the sandy soil containing 15% water.

Regardless of moisture content, the pupation depth was 1.68 and 1.56 cm in sandy and sand clay soils without significant difference. Beside this, the statistical analysis proved insignificant difference between moisture levels of 0,5,10 and 20%.

From the previous results it was found that the highest pupation depth was about 5cm. To avoid the harmful to the friendly soil fauna, it may be suggested that if the soil fumigation or gas producing materials should be applied, it must be penetrated into the soil not more than 10cm.

Table 1: Effect of soil type and soil moisture on pupation depth of ZFF

Soil type	Moisture percentage						
	0%	5%	10%	15%	20%	Average	
Clay	2.2	1.9	1.5	1.5	1.4	1.682	**b**
Sandy	1.9	1.5	1.2	16.2	1.7	4.492	**a**
Sand/Clay mixture 1:1	1.5	1.6	1.3	1.6	1.9	1.556	**b**
Average	1.8	1.7	1.3	6.4	1.7		
	b	**b**	**b**	**a**	**b**		
LSD For Soil Type (5%)	2.5						
LSD for Soil Moisture (5%)	0.32						
LSD for Interaction (5%)	0.55						

Effect of sand cover weight on the emergence of ZFF

As shown in Table 2 the soil depth is negatively correlated with emergence of Zizyphus fruit fly adults. The highest rates of fly's emergence (100 and 80%) were observed at the lowest depths (1-15 cm) with no significant differences in the percentage of adult emergence. Rigamonti (2004) noticed that the pupae of *Ceratitis capitata* are formed at a depth inferior to 10 cm and 90% of the obtained pupae were concentrated in the five upper centimeters. Also, Ali *et al*., (2007) reported that the higher rate of

emergence of *C. capitata* (69.3%) was observed at 2 cm depth. Similar results were reported by Al-Zaghal *et al*., (1987) who observed maximum pupae of *Dacus oleae* at depth of 2.5 - 7.5 cm in soil under olive trees in Jordan. Malem and Giliomme (1968) observed maximum pupation of *Dacus ciliatus* in dry sandy soils at depth of 1 to 2 inches. The results of the abovementioned investigators are in close agreement with our results, we have in fact recorded that the higher rates of emergence (100 and 95%) were corresponded to 1 to 2 cm depth, respectively. The data in Table (2) also show that rates of emergence gradually reduced when pupae placed at depths between 20 and 40 cm. The rate of emergence at these depths was shown to be 70% at 20 cm depth and 45% at 40 cm depth. Depth of 50 cm had detrimental effect on the emergence of adult fly, where the flies failed completely to emerge from the pupae (0%) at this depth.

Table (2): Effect of soil depth on the emergence of Zizyphus fruit fly adults.

Soil depth (cm)	Wt. soil (gm/cm^2)	No. emerged flies (mean)	Emerged flies (%)
1	1.49	20 a	100
2.5	3.73	19 a	95
5	7.45	19 a	95
10	14.90	17 abc	85
15	22.35	16 abc	80
20	29.80	14 bcd	70
25	37.25	13 cde	65
30	44.70	10 de	50
40	59.60	9 e	45
50	74.50	0 f	0

L.S.D = 2.038

Means in the column followed by the same letter are not significantly different at 0.05 level of probability.

The present results suggest that emergence of ZFF in relation to soil depth could be classified into 3 different groups on the base of percent of emergence. The first had the highest emergence rates (80-100%) with no significant differences among them; the second had moderate emergence rates (45-70%) with no considerable differences in percent of adult emergence. The third group had no emergence (0%) at all. Statistical analysis also indicated significant differences between first (1-15 cm depth) and second (20-40 cm depth) groups and between third (50 cm depth) and both first and second groups in respect to emergence percent.

Our results could be interpreted on the base that weights of soil between 1.49 and 22.35 gm/cm^2 corresponding to depths of 1-15 cm were much lighter on the pupae so that adults emerged from these pupae were able to penetrate the soil very easily, while in case of pupae placed at 50 cm depth, the weight of soil on the pupae (74.5 gm/cm^2) was too heavy so that the flies entirely failed to emerge from pupae. The present results clearly show that depth of soil between 5 and 10 cm is the most preferable for emerging of Zizyphus fruit fly adults from the pupal stage.

It may be concluded that, results of the present investigation can be used in developing no-chemical control measures and designing efficient sampling technique for the insect in the ground. Based on our results we suggest that soil depth should be considered as a one of the important abiotic factor in minimizing the pest's population.

From the previous results it may also be suggested that when burying the infested fallen fruits in the soil, the later must be covered with a layer of clay or clay sand soil not less than 50 cm.

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تأثير نوع وعمق ورطوبة التربة على حياة طور العذراء والحشرة الكاملة لذبابة ثمار النبق *Carpomyia incompleta* تحت ظروف المعمل .

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تناولت الدراسة الحالية تأثير نوع وعمق ورطوبة التربة على حياة طور العذراء والحشرة الكاملة لذبابة ثمار النبق *Carpomyia incompleta* تحت ظروف المعمل .

أشارت النتائج أن التربة الرملية التى تحتوى على ١٥% ماء هى الانسب للتعذر والتحول الى طور الحشرة الكاملة ولقد تبين ان عمق التعذر فى التربة الطينية او الطينية الرملية كان اقل منه فى حالة التربة الرملية، كما وجد ان اقصى عمق للتعذر بلغ حوالى ٥ سنتيمتراً ومن ثم ننصح بأنه عند استخدام عملية تدخين التربة او معاملتها بالمواد المنتجة للغازات السامة لا يجب ان يزيد عمق سريان الغازات اكثر من ١٠ سنتيمترات.

وقد أظهرت الدراسة أن عمق التربة يرتبط عكسياً مع خروج ذبابات ثمار النبق الكاملة وأن أعلى معدلات الخروج تراوحت بين ١٠٠، ٨٠% وقد لوحظ ذلك عند أقل أعماق التربة (١-١٥سم) دون وجود اختلافات معنوية بين هذه الأعماق فيما يتعلق بنسب خروج الحشرات الكاملة .

كذلك وجد أن أعلى معدلات الخروج والتى بلغت ١٠٠%، ٩٥% توافقت فى الواقع مع عمق ١-٢ سم على التوالى، غير أن هذه المعدلات تناقصت تدريجياً عند وضع العذارى على أعماق تتراوح بين ٢٠سم، ٤٠ سم، حيث كان معدل خروج الحشرات الكاملة ٧٠% عند عمق ٢٠سم، ٤٥% عند عمق ٤٠سم. وكان لعمق ٥٠ سم تأثير ضار على العذارى حيث فشلت الحشرات الكاملة تماماً فى الخروج (صفر%) من طور العذراء.

أظهرت النتائج أيضا أن عمق التربة بين ٥-١٠سم هو الأكثر تفضيلاً لخروج الحشرات الكاملة من العذارى، وتدل نتائج هذه الدراسة أن عمق التربة ذات تأثير معنوى على حياة وخروج حشرة ذبابة ثمار النبق الكاملة. ومن ثم نقترح انه عند دفن الثمار الساقطة فى التربة لقتل العذارى ومنع خروج الحشرات الكاملة ألا يكون غطاء الحفرة اقل من ٥٠ سنتيمتر .

قام بتحكيم البحث

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