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The Effect of Soil Depth on Pupation of *Bactrocera dorsalis* Collected from Chilli

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Abstract. Fruit flies including genus *Bactrocera* undergo a process of pupation in the soil. The depth of the soil may affect the success of pupation. This study aimed to evaluate soil depth's effect on the success of *Bactrocera dorsalis* (Hendel) pupae in becoming imago, survival, normality and development time. The experiment in the laboratory consisted of eight treatments of pupation depth (0, 4, 10, 20, 30, 40, 50 and 60 cm). Pupae were put in mica tubes with a diameter of 5 cm and poured with soil granules. Replications were done four times. The survival of *B. dors*alis was significantly affected by soil depth. As the depth of the soil increased, the likelihood of becoming an imago reduced. The survival rate was lowest at a depth of 30 cm (2%±2) and the success rate for becoming an adult was best at a control depth of 0 cm (60%±5.9). Additionally, it was discovered that as pupation depth increased, the imago's survival rate decreased. The control group had a greater imago emergence survival rate (51%±4.1), whereas no pupa survived at a depth of 30 cm in the other groups. All soil depths except for 30 cm had normal imago, however, all other soil depths had anomalous imago. The range of the average development time was 8.02 to 12.57 days. The length of pupal development is influenced by the depth of pupation. At depths between 0 and 4 cm, pupal development took fewer than 10 days on average, but from 10 to 60 cm, it took more than 10 days. The regression analysis's findings point to the equation $y = -4.9524x + 35.54$. According to prediction results, a depth of 50.4 cm may be the point at which the emergence of adult *B. dorsalis* is successfully suppressed. This study recommends covering rotten fruit to inhibit the growth of fruit flies or burying it in the ground to a depth of more than 50 cm (in dry conditions) to increase the effectiveness of fruit fly management.

1 Introduction

Chilli pepper (*Capsicum annuum* L.) (Solanaceae) is one of the vegetables consumed in everyday life. The demand for chilli and its derivative products continues to increase along with population growth and technological advances [1]. This plant is an economically important vegetable in most tropical countries, including Indonesia. Indonesia is currently

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ranked fourth in chilli production and accounts for approximately 5 per cent of the total global annual chilli market share [2]. In Indonesia, chilli pepper is planted on 133,729 hectares of land, producing around 1.47 million tons in 2022 [3]. About 48 per cent of total national chilli production is concentrated in Java Island. Domestic demand for Chile is very high in Indonesia, but the production was considered low (11 tons/ha or about 60% of its potential). Pest and disease disorders are the main problems that hinder the chilli fruit harvest. Several important pests that commonly attack chilli plants, including armyworms, aphids, fruit flies, thrips and mites (*Tetranychus telarius* Linn.) [4].

Tephritidae fruit flies is one of the families belonging to the order Diptera that has a large number genera and species. There are about 4000 species divided into 500 genera. *Bactrocera* is one genus which is of great economic importance and widespread globally. Among the *Bactrocera* species, *Bactrocera dorsalis* is known to have the destructive role as a pest for numerous economic fruits and vegetables. *B. dorsalis* infests hundred host species and it is widely distributed in the equatorial zone [6-8]. *B. dorsalis* attack currently occurs in over 60 countries in Asia, Africa and America indicating its broad climatic range. This species is indigenous in the tropics including Indonesia [9]. Fruit fly infestations have a negative influence on the economy by reducing production levels and quality, raising production costs, and leading to customer refusal [7,11,12].

Female fruit flies lay their eggs in the appropriate fruit (on the surface or inside) as offspring of the hosts, often in ripening or mature fruits or vegetables. The larvae leave the fruit at the end of the third instar and bury themselves in the ground to pupate. Adult fruit flies dig higher to the soil's surface once their transformation is complete to stiffen their wings before flight [13]. The development of strategies and management for the fruit fly is mostly focused on mature adults due to their distinct life history. Examples include using methyl eugenol, customized traps, the essence lure, sterile insect approach, and botanical insecticides [14–17]. However, there is little attention on the possibility of controlling larva or pupa stages.

Efforts to control fruit flies at the pupal stage can be made by studying pupation behavior, especially soil depth. Previous research shows that hybrid *B. dorsalis* and *B. carambola*e are capable of pupation at a depth of 50 cm [18]. In the species *B. dorsalis*, several studies report that pupation generally occurs at a depth of 0 – 5.5 cm [19-21]. Pupation is also influenced by soil moisture. Most of the larvae prefer to pupate in the soil surface layer (less than 4 cm)l, while relatively few larvae move downward to the deeper layer (more than 4 cm) when the soil receives extreme water content (too much or too little) [21]. Knowledge of survival rates at different depths is important for biological, physical or mechanical control of entomopathogens by burial. One application of nematodes, change soil moisture, sanitation and burial of infected fruit [22-25]. The survival of fruit flies during pupation may be impacted by exposure to unfavorable environmental conditions. The depth of the pupation can hinder fruit fly pupae's growth and lower their survival rates. For managing soil conditions, this information can be helpful as a recommendation. There have not been any studies done on *B. dorsalis*, particularly with pupa survival rates in clay-type soil. The objective of this study is to assess the effect of soil depth on the imago's emergence of *B. dorsalis* pupae.

2 Materials and method

The *B. dorsalis* fruit flies were collected from the Sumberejo dan Pesanggrahan Villages, Batu Sub-district. A total of 1,000 larvae were collected from the orange orchards and reared in the Laboratory of Animal Diversity and Environmental Technology the Department of Biology, Universitas Brawijaya, Indonesia. Then, the fruit flies were transferred to a 50 cm

× 50 cm × 40 cm cage. The laboratory conditions were kept at room temperature (23-32°C) with a 12:12 photoperiod (light : dark). The offspring were used as experimental objects [22].

As the media for experiments, this study used soil collected from Dau Sub-regency, Malang Regency, East Java. Those samples were dug from two orange orchards (mixed with chilli) from 0 to 20 cm depth of using a soil scoop, and then both were mixed. In the laboratory, the soil was air-dried for 48 hours. Dried soils were pounded and sieved using a 2 mm mess. The soils were sterilized in an autoclave. The soil was composed of sand (10%), clay (72%), and silt (18%).

Bactrocera dorsalis pupae were taken after one day of pupating. Different soil depths were tested to determine their ability to emerge as adults. The treatment included depths of 0, 4, 10, 20, 30, 40, 50 and 60 cm. The replication was done four times, in which each replicate administered 25 pupae aged 1–2 days. Experiments were carried out by placing pupae in soil media. Soil media was put into transparent plastic and formed into a tube with a diameter of 5 cm. Approximately 10 cm of space was added to the height of the tube to ensure oxygen availability. The plastic tube is filled with soil media 1 cm thick; After that, the pupa is placed on top, and then soil is poured into the tube according to the depth of each treatment. The top of the tube was covered with a layer of cotton cloth to prevent adult fruit flies from escaping. After six days, pupa status in each treatment was checked every day until 16 days after treatment. The number of adults released and their physical condition were observed. Fruit flies are classified based on the perfection of their abdomen and wings. Imago with deformed or flawed wings are considered abnormal.

The number of adults hatching, survival, and morphological normality from each depth level was calculated, and the result was presented as a percentage, while the length of time development was presented as day. Data normality analysis was carried out. The results indicated that adult hatching, survival and morphological normality data and development times were normally distributed. Therefore, statistical analysis was suitable for parametric analysis using Multivariate Analysis of Variance. To see the comparison of each treatment level, a post hoc test was performed with the LSD essay. Data were calculated using the Excel program, while Statistical tests were processed using SPSS® version 22, and the results of the tests were considered different when $p<0.05$.

3 Results and discussion

Based on statistical analysis, the treatment of soil depth had a negative and significant effect on the survival of *B. dorsalis* pupae ($R^2 = 0.519$; $p<0.001$) (Fig. 1). Mean pupal survival rates were significantly different between soil depths ($F = 105.6$; $p<0.001$). The highest imago emergence occurred at depths of 0 cm (60% ± 5.89) followed by 4 cm (58% ± 6.22), while the lowest survival occurred at depths of 30 cm (2% ± 2) (Fig. 1). Some fruit flies were able to emerge from a depth of 60 cm showed abnormal bodies with wrinkled wings (Fig. 1). The regression formed an equation of survival of $y = -4.9524x + 35.54$. Prediction results showed that the depth that effectively suppresses the emergence of adult fruit flies is likely to occur at a depth of 50.4 cm.

Typically, *B. dorsalis* larvae pupate below the soil's surface. According to previous study, *B. dorsalis* flies might emerge with a survival rate of more than 81% at a depth of 0–2 cm, while soil moisture levels range from 30–70%. In this study, the average of imago emergence started to decline within 4 cm and 30 cm depths, while it began to increase between 30 cm and 60 cm depths. Another study demonstrated that another fruit fly species, *B. cucurbitae*, is incapable of emerging at 46 cm below ground [27]. The imago lacked adequate energy to reach the surface, which made it difficult for it to emerge from the medium at depths between 50 and 60 cm.

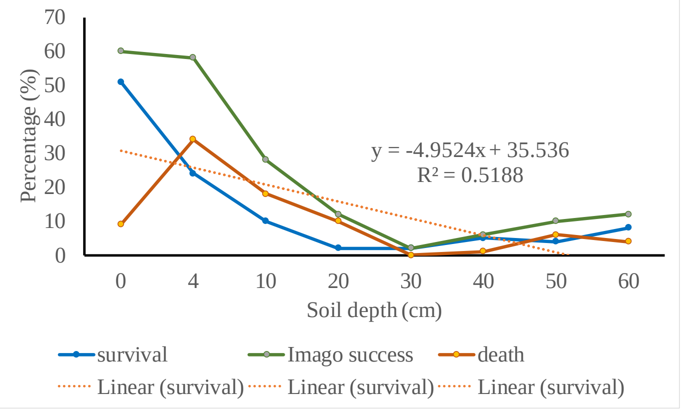

Fig. 1. The percentage of *B. dorsalis* emergence, survival and fatality in various soil depth

From the results of observations of fruit flies appearing on the surface of the soil on mica tubes, it was seen that there were fruit flies that were deformed. Compared to normal flies which have fully developed wings, bright body colour, and long stomachs (Fig. 2A). The appearance of young deformed fruit flies has the characteristics of a pale-yellow body, wrinkled wings, and a short, small abdomen. The two horizontal black lines and the longitudinal median line are barely visible (Fig. 2B). defects in the pupa generally cause imago disorders and death.

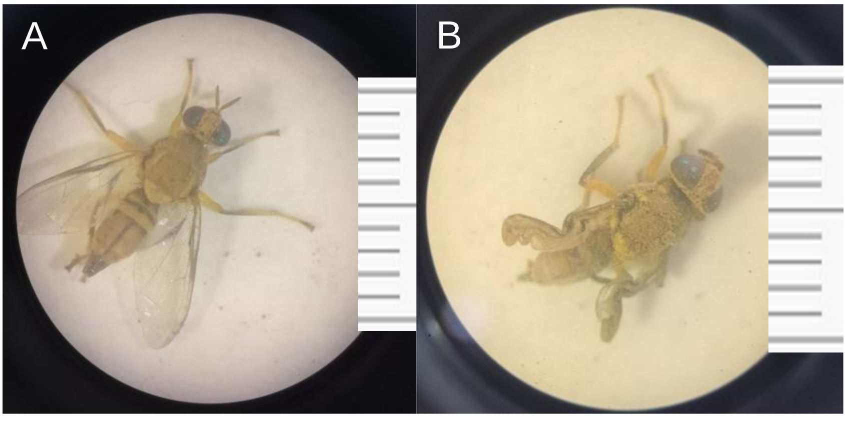

Fig. 2. Normal (A) and abnormal (B) imago

This study indicated that normal imago was hatched from all soil depths, but it was absent in 30 cm depth. In that depth (30 cm) no imago survived was found. Furthermore, abnormal/deformed imago was found in all soil depths ranging from (Table 1). The percentage of abnormal (deformed) imago ranged from 1% ± 1 to 22% ± 3.46. Analysis non-GLM analysis of variance showed that depth has a significant effect on all variables, including imago normality ($F = 16.56$; $p<0.001$), imago abnormality ($F = 6.35$; $p<0.001$) and death imago ($F = 18.8$; $p<0.001$).

Table 1. Effect of soil depth treatment on imago normality and survival of *B. dorsalis*

Normality category		Depth (cm)						
	0	4	10	20	30	40	50	60
Normal Imago (%)	46±5.29a	39±5.66a	11±3.42b	2 ± 1.15b	0b	6 ± 6b	2 ± 2b	8 ± 5.66b
Abnormal Imago (%)	14±1.15b	22±3.46b	17±3b	10±6.22ab	2 ± 2a	1± 1a	1 ± 1a	4 ± 4a
Dead Imago (%)	49±4.12a	76±4.32b	90±5.29c	98±1.15c	98±2c	95±5c	96±1.63c	92±4.9c

Note: Different letters on the same row indicate significant differences in the mean at the level of p<0.05

In general, adult abnormalities are found in the wings. Wing growth is not perfect, they appear shrunken or wrinkled (Fig. 2B), and some flies have a swollen abdomen. Soil physical and chemical characteristics, such as interstitial space, grain size, compaction, moisture and organic matter content, can affect the appearance of the adult fruit flies on the surface. The pupation success of fruit fly larvae increases in soil with large soil particles because new adults easily emerge from the pupa. Some soil properties, such as porosity and total organic matter, may decrease soil density and allow images to appear easily. Those characteristics support the emerging adult to move upward to reach the surface. In denser soils, the imago spent hard effort to reach the surface due to the low total porosity of the soil [28]. This situation can affect the movement time to reach the soil surface, so that the imago development time becomes slower than the controls. Soil porosity value was classified as good because the soil drained, and therefore, the imago could float out of the soil. However, the abnormal growth of some fruit fly adults showed that the soil depth has exceeded the normal growth capacity of imago. In other research, soil porosity, texture, density, humidity and temperature have been shown to influence the death of pupae and deformed flies [28]. Another study demonstrated that soil texture significantly affected the survival of *B. dorsalis* pupae as well as soil moisture [19, 21].

Based on the research results, this study warns of burying rotten fruit in swallow ground without control. If the rotten fruit is buried less than 50 cm deep, it needs to be covered to prevent impurities from rising to the soil surface. Furthermore, these results may also be useful in developing the effectiveness of tests for the detection of the entomopathogenic fungus that causes depth-related disease, *B. dorsalis*. Some soil fungi that cause insect diseases, such as *Metharizium anisopliae*, *Beauveria bassiana*, and *Streptomyces* sp. isolates, may lead to high fruit fly mortality. Therefore, the results of this experiment suggest that pupal depth at the time of maturation can be increased to levels that affect the performance of fungi as biocontrol agents of this *B. dorsalis* species.

Observation results show that the average pupa development time lasts between 8.01 to 12.78 days. Soil depth treatment had a significant effect on pupation time ($F=1$, $p<0.001$). The fastest development time was found at a soil depth of 10 cm, while the longest was at a depth of 50 cm. The soil depth factor influences the development time of the pupa. The results there was no difference in the average development time at a depth of 4 cm to 40 cm. While, there was a significant difference in development time of 50 cm and 60 cm (Table 3). The slow development of adult fruit flies may be due to the thick layer of soil and the length of time it takes to spread their wings. Adult fruit flies planted in the soil at a depth of 30 cm or more take longer to reach the soil surface than fruit flies planted in shallow soil. They need more energy to crawl to the surface. Worse, oxygen requirements may not be sufficient in conditions where soil aeration is not optimal. The depth of pupation affects the survival rate, normality of the imago and the length of pupal development. The average of 0 and 4 cm is less than 10 days, but at depths of 10 cm to 60 cm, the development time is more than 10 days. To control fruit flies more effectively, this research recommends burying decaying fruit in the soil to a depth of 60 cm or providing a cover so that the imago cannot escape to the soil surface.

Table 2. The development time (day after treatment) of *B. dorsalis* pupae

Treatment in soil depth (cm)	Development time (day after treatment)
4	9.14 ± 0.11
10	8.01 ± 0.06
20	12.55 ± 0.64
30	11.5 ± 0
40	12.67 ± 0
50	12.78 ± 0.54
60	11 ± 0

4 Conclusion

This study shows that the imago survival rate decreases with increasing pupation depth. Higher imago survival occurred at a control depth of 0 cm (51% ± 4.1), while lower survival occurred at a depth of 30 cm (no pupae survived). Normal imago was found at all soil depths except at a depth of 30 cm, where at this depth, all imagoes had abnormal morphology. Average development time ranged from 8.02 to 12.57 days. The depth of the soil affects the length of pupa development. The average development time for pupae at depths of 0 and 4 cm is less than 10 days, but at depths of 10 cm to 60 cm, the development time is more than 10 days. To control fruit flies more effectively, this study recommends to burry rotten fruit in the soil to a depth of more than 50 cm or burying it wrapped to prevent the development of fruit flies.

References

1. I.K. Budaraga, G. Gusriati, H. Gusvita, Z. Zulfitriyana, International Journal of Scientific & Technology Research **8**, 159-173 (2016)
2. J. Mariyono, S. Sumarno, Journal of Agribusiness in Developing and Emerging Economies **5**, 57-75 (2015)
3. Indonesia Statistic Central Agency, 2023. Data of Agricultural Product 2022 https://www.bps.go.id/subject/55/hortikultura.html#subjekViewTab3
4. J.K. Gupta, A. Bhatnagar, V.K. Agrawal, S. Mukherjee, B.K. Sharma, Journal of Progressive Agriculture **7**, 101-106 (2016)
5. A.R. Clarke. Biology and management of *Bactrocera* spp. and related fruit flies (CABI Publishing, 2009)
6. R.I. Vargas, J.C. Piñero, L. Leblanc, Insects **6**, 297–318 (2015)
7. J.F. Vayssières, M. De Meyer, I. Ouagoussounon, A. Sinzogan, A. Adandonon, S. Korie, R. Wargui, F. Anato, H. Houngbo, C. Didier, H. De Bon, G. Goergen, J. Econ. Entomol. **108**, 2213–2230 (2015)
8. M. Salmah, N.A. Adam, R. Muhamad, W.H. Lau, H.J. Ahmad, Fundam. Appl. Sci. **9**, 799–812 (2017)
9. S. Hudiwaku, T. Himawan, A. Rizali, Biodiversitas **22**, 4608–4616 (2021)
10. H. Syahfari, Ziraa'ah Majalah Ilmiah Pertanian **36**, 32–39 (2013)

11. M. Kibira, H. Affognon, B. Njehia, B. Muriithi, S. Mohamed, S. Ekesi, African J. Agric. Resour. Econ. **10**, 343–353 (2015)
12. F. Jamaluddin, A. Nurariaty, N. Amin, IOP Conference Series: Earth and Environmental Science **486**, 012146 (2020)
13. P.H. Smith, World Crop Pests. Vol. 3(B). Fruit Flies: Their Biology, Natural Enemies and Control (eds Robinson, A. S. & Hooper, G.) 325–341 (Elsevier, 1989)
14. T.E. Shelly, E. Pahio, J. Edu, Fla. Entomol. **87**, 481–486 (2004)
15. G.T. McQuate, S.L. Peck, P.G. Barr, C.D. Sylva, J. Econ. Entomol. **98**, 1170–1178 (2005)
16. A. Susanto, C. Nasahi, Y. Rumaisha, W. Murdita, T. Lestari, Agrikultura **30**, 53–62 (2019)
17. M. Sutantawong, W. Orankanok, W.R. Enkerlin, V. Wornoayporn, C. Cáceres, *The sterile insect technique for control of the oriental fruit fly, Bactrocera dorsalis (Hendel) in mango orchards of Ratchaburi Province, Thailand*, in Proceedings of the 6th International Symposium on Fruit Flies of Economic Importance. Barnes, B.N. (Ed.). Isteg Scientific Publications. Irene, South Africa, pp. 223–232 (2004)
18. A. Susanto, M.G. Faradilla, Y. Sumekar, D.H. Yudistira, W. Murdita, A.D. Permana, L. Djaya, S.N.S. Putri, Scientific Reports **12**, 4235 (2022)
19. C.G. Jackson, J.P. Long, L.M. Klungness, Journal of Economic Entomology **91**, 138-142 (1998)
20. Y. Shengyong, K. Qiong, X. Chun, C. Bin, L. Zhengyue, G. Yonghong, Journal of Mountain Agriculture and Biology **23**, 486-488 (2004)
21. B. Hou, Q. Xie, R. Zhang, Appl. Entomol. Zool. **41**, 515–520 (2006)
22. A. Godjo, L. Zadji, W. Decraemer, A. Willems, L. Afouda, Biol. Control. **117**, 68–77 (2018)
23. M. Usman, W. Wakil, D.I. Shapiro-Ilan, Biol. Control **163**, 104706 (2021)
24. L.M. Klungness, E.B. Jang, R.F.L. Mau, R.I. Vargas, J.S. Sugano, E. Fujitani, J. Appl. Sci. Environ. Manage. **9**, 5–15 (2005)
25. Z. Li, C. Chambi, T. Du, C. Huang, F. Wang, G. Zhang, C. Li, M.J. Kayeke, Insects **10**, 138 (2019)
26. Indonesian Center for Forecasting Plant Pest Organisms. Guideline for Rearing *Bactrocera* spp. in Laboratorium (Director General of Horticulture, 2014).
27. M.K. Dhillon, R. Singh, J.S. Naresh, H.C. Sharma, J. Insect Sci. **5**, 40 (2005)
28. I.R. El-Gendy, A.M. AbdAllah, Int. J. Pest Manage. **65**, 154-160 (2019)