Biosaintifika 18 (1) (2026): 127-138 p-ISSN 2085-191X | e-ISSN 2338-7610
Journal of Biology & Biology Education https://journal.unnes.ac.id/nju/index.php/biosaintifika

The Effect of *Beauveria bassiana* on the Development of *Bactrocera dorsalis* Complex at Various Soil Depths

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Submitted: 2025-12-11. Revised: 2026-02-15. Accepted: 2026-04-18.

Abstract. *Bactrocera dorsalis* complex is a pest that causes losses in agriculture because it causes damage and rotting of fruits and vegetables. *Beauveria bassiana* is an entomopathogenic fungus that produces secondary metabolites that can infect pests. This study aimed to determine the effect of *B. bassiana* on the growth of B. dorsalis complex at different soil depths. This study adopted a randomized factorial design with two factors. The first factor was treatment, which consisted of control, water addition, and Streptomyces sp. application. The second factor was soil depth, consisting of eight pupation depth treatments of 0, 4, 10, 20, 30, 40, 50, and 60 cm. The pupae were placed in a mica tube with a diameter of 5 cm and covered with sieved soil. Replication was carried out four times. The observed parameters included dead pupae, successful imago, normality, and development time. *B. bassiana* treatment significantly increased pupal mortality, decreased successful survival, and the normality of the imago. *B. bassiana* treatment also significantly extended the development time of all imago parameters. Soil depth significantly affected pupal mortality and all imago parameters. Soil depth significantly affected the development time of successful imagoes, but not the development time of normal and abnormal imagoes. These findings suggest that *B. bassiana* effectively controls fruit fly pupae in soils of varying depths. This study provides a foundation for integrating entomopathogenic *B. bassiana* into fruit fly control strategies targeting the pupal stage. This research supports SDGs Zero Hunger because the result directly contributes to sustainable agriculture through biological control of the fruit fly (*Bactrocera dorsalis*), a major pest of horticultural crops, reduced crop losses, and increased agricultural productivity, as well as provides an environmentally friendly alternative to chemical pesticides.

Keywords: *Bactrocera dorsalis*; *Beauveria bassiana*; Soil Depth; Pest Control.

How to Cite: Leksono, A. S., Agustina, W. P., Salsabila, M., Rosyidah, N. F., Qurotaayunina, R. P., Mustafa, I., Kurniawan, N., Zairina, A., & Bozdoğan, H. (2026). The Effect of *Beauveria bassiana* on the Development of *Bactrocera dorsalis* Complex at Various Soil Depths. *Biosaintifika: Journal of Biology & Biology Education, 18(1)*, 127-138.

DOI: http://dx.doi.org/10.15294/biosaintifika.v18i1.35514

INTRODUCTION

Agriculture is a key sector in global economic development, providing employment opportunities and ensuring food security (Pawlak & Kołodziejczak, 2020). However, agricultural productivity is frequently threatened by pest infestations, which can alter crop production and reduce yields. Pests have substantial impacts on agriculture, extending beyond economic losses to encompass social, cultural, and political dimensions(Therville et al., 2021). Animal populations such as insects are classified as pests when their numbers are uncontrolled and result in significant losses to agricultural production. Among the most important pests affecting fruit and vegetable commodities is the *Bactrocera dorsalis* complex (Manrakhan, 2020; Jaleel et al., 2018; Vargas et al., 2015; Vayssières et al., 2015).

Bactrocera is a large genus within the family Tephritidae, consisting of approximately 750 known species, primarily distributed across Southeast Asia, Australia, the Pacific region, and the Afrotropics. Among these species, 79 share similar morphological characteristics, particularly a black scutum that is predominantly or entirely black, and are grouped within the *B. dorsalis* complex (Drew & Hancock, 2022). The

Bactrocera dorsalis complex comprises species of major economic importance due to the severe damage they inflict on fruit crops in tropical regions, including Indonesia (Aluja et al., 2024; Drew & Hancock, 2022; Hossain et al., 2020). These pests attack various host plants, including mango (*Mangifera indica* L., Anacardiaceae), banana (*Musa* spp., Musaceae), star fruit (*Averrhoa carambola* L., Oxalidaceae), guava (*Psidium guajava* L., Myrtaceae), water apple (*Syzygium aqueum*), orange (*Citrus* spp., Rutaceae), sapodilla (*Achras zapota* (L.) *P. Royen*, Sapotaceae), papaya (*Carica papaya* L., Caricaceae), peach (*Prunus persica* (L.) Batsch, Rosaceae), jackfruit (*Artocarpus heterophyllus*), grape (*Vitis* spp., Vitaceae), pomegranate (*Punica granatum* L., Lythraceae), lychee (*Litchi chinensis* Sonn., Sapindaceae), longan (*Dimocarpus longan* Lour., Sapindaceae), red chilli (*Capsicum annum* L., Solanaceae) and tomato (*Solanum lycopersicum* L. Solanaceae) (Agustina et al., 2025; Mutamiswa et al., 2021; Suputa et al., 2010; Syamsudin et al., 2022; Zhu et al., 2022). Damaged fruits become hollow and rot quickly, posing a major risk to the fresh commodity trade due to quarantine restrictions (He et al., 2023).

This pest exhibits high adaptability, allowing populations to grow rapidly and enabling its population to expand quickly across diverse climates and agricultural regions (Mutamiswa et al., 2021). *B. dorsalis* complex causes significant economic losses globally. For example, in India, infestations reduce marketable mango yields by 25–30%, resulting in estimated annual losses of USD 356 million across crops such as guava, sapodilla, citrus, and mango (Bana, et al., 2017). In Mozambique, average yield losses associated with fruit flies were estimated at 5.65 t/ha with a financial loss of USD 3428.97/ha (Canhanga et al., 2020). Similarly, guava export restrictions to the United States and Japan cause annual economic losses of approximately USD 2.5 million, while in Indonesia, the pest attacks fruits and vegetables, causing annual losses of 25–50%, and may reach 100% in mangoes (Permana, et al., 2022).

Reports of major economic losses caused by *B. dorsalis* serve as a biosecurity warning for existing fruit fly control programs. Effective management strategies require a deeper understanding of fruit fly biology to prevent infestations and mitigate potential economic damage. Controlling *B. dorsalis* larvae is challenging because their endophagous behavior makes them less susceptible to insecticide treatments (Jaffar et al., 2023). As a result, the adult stage of fruit flies is primarily targeted in control efforts using synthetic pesticides, baiting systems, and traps. Common approaches include methyl eugenol baits, standard wide-area pest management, Jackson traps, and sterile insect techniques (Vargas et al., 2015; Bajaj & Singh, 2018). However, although effective, the use of synthetic pesticides has many adverse effects with long-term use, including resistance problems, non-target effects, and harm to humans and the environment (Awan et al., 2021). Several studies reported that fruit fly species, such as *B. dorsalis* (Hendel), *Ceratitis capitata* (Wiedemann), *B. oleae* (Rossi), and *Zeugodacus cucurbitae* (Coquillett), have developed beta-cypermethrin, cyhalothrin, malathion, and spinosad resistance (Kampouraki et al., 2018; X. Li et al., 2024). Furthermore, implementing strict quarantine measures and maximum pesticide residue limits in several countries has recently increased efforts to find non-chemical control methods.

Due to such issues associated with chemical insecticides, it is essential to develop more environmentally friendly fruit fly control methods (Daane & Johnson, 2010). Biological control methods based on natural enemies, such as entomopathogenic fungi (EPF), are considered safe efforts (Dorta et al., 2020; Mantzoukas & Eliopoulos, 2020). Biological control using entomopathogenic fungi is a promising alternative to control the larvae and pupae of *Bactrocera* spp. (Hussein et al., 2018; Prince et al., 2024; Shaurub, 2022; Susanto, Permana, et al., 2022). EPF can infect fruit flies by penetrating their mycelium through the larval or pupal cuticles (Soliman et al., 2020). Several studies have evaluated the impact of EPF, such as *Metarhizium anisopliae*, *M. robertsii*, *Isaria fumosorosea*, *Glomus* spp., *Beauveria bassiana*, and *B. brongniartii*, on fly species (Diptera: Tephritidae). Some target fruit fly species include *B. zonata* (Hussein et al., 2018), *Bactrocera tryoni* (Prince et al., 2024); *Ceratitis capitata* (Ekesi et al., 2002); *B. oleae* (Sookar et al., 2008); *Bactrocera zonata* (Usman et al., 2021; Wakil et al., 2022), *B. cucurbitae* (Onsongo et al., 2022) and *Bactrocera dorsalis* (Li et al., 2024; Menzler-Hokkanen et al., 2022; Wang et al., 2021). However, research linking the potential of *B. bassiana* to *B. dorsalis*, at several soil depth levels has never been conducted. Considering the ability of fruit fly larvae to pupate in the soil, research on the effectiveness of *B. bassiana* against *B. dorsalis* at various soil depths is necessary. *Beauveria bassiana* can be cultivated in solid or liquid media. Cultivating this fungus in

liquid media is very suitable for field applications and may shorten processing time and produce no waste (Indriyanti et al., 2022). Based on this explanation, this study aims to determine the effect of *B. bassiana* on the growth of *B. dorsalis* complex at various soil depths during the pupal to imago transformation phase. The result can be applied to the control of the fruit fly (*Bactrocera dorsalis*), a major pest of horticultural crops, using an entomopathogenic bacterium. It may support agricultural productivity and provide an environmentally friendly alternative to chemical pesticides.

METHODS

Fruit Fly Collection and Maintenance

The study was conducted at the Laboratory of Animal Diversity and Environmental Technology, Department of Biology, Brawijaya University, from December 2024 to March 2025. Fruit flies were collected from chili peppers collected in Batu City, Malang. A total of 100 chili peppers were sampled, collected from the field, and maintained in the laboratory. The chili peppers were placed in 10 small plastic boxes lined with cloth on the bottom. These boxes were placed inside a larger plastic box filled with sterile sand for larval development. After two weeks, the larger plastic box was opened, and pupae were collected by sifting through the sand. Pupae were placed in transparent plastic bottles until they hatched into adults. Fruit fly identification was based on wing patterns (venation), including the number, shape, and position of brown or dark bands and spots; thoracic color and pattern; stripes or spots on the mesonotum (dorsal thorax); and scutellum coloration, following the identification key for adult fruit flies by Drew & Romig (2016) *B. dorsalis* complex samples were collected and reared for subsequent generations. Hundred pairs of newly emerged adults were reared in a 30 × 30 × 30 cm net insect-rearing cage, provided daily with a 10% sucrose solution and protein.

Culture and Propagation of Entomopathogenic Isolates

B. bassiana was obtained from a stock culture at the Faculty of Agriculture, Brawijaya University, Malang. The isolate has been tested for virulence against various pests (Afandhi et al., 2020, 2022). The isolate was subcultured on Potato Dextrose Agar (PDA) medium by streaking and then incubated for 7 to 14 days at room temperature. The purity of *B. bassiana* colonies was confirmed by observing colony morphology and microscopically at 400× magnification, stained with Lactophenol Cotton Blue (LCB). The *B. bassiana* isolate was cultured on PDA medium for 14 days at room temperature. The colonies on the agar surface were treated with 2 mL of a mixture of Tween 80 and sterile distilled water to suspend the spores. The spore suspension was stored in Falcon bottles at 4°C. Spore density was calculated using a hemocytometer in a microscope at 400× magnification. The suspension was diluted to achieve a spore density of 1 x 10^6 conidia/mL (according to the Indonesian National Standard).

B. bassiana Application to Pupae

The experiment was conducted by placing pupae in sterilized soil media. The treatments included soil without any additives (control) and *B. bassiana* application. The test was conducted using the immersion method, with fruit fly pupae dipped in 1 mL of *B. bassiana* suspension in a Petri dish for one minute for each treatment. The effect of soil depth on fruit fly adult emergence was tested using day-old pupae placed in transparent mica tubes (5 cm in diameter). Each tube was fixed onto a 20 × 20 cm piece of cardboard at the base and filled with 1 cm of soil as the bottom layer. For the 0 cm treatment, pupae were placed directly on the soil surface without additional covering, while for other treatments, soil was added to reach the assigned depth. The top of each tube was sealed with gauze to prevent adult flies from escaping. Nine soil depth treatments were tested: 0, 4, 10, 20, 30, 40, 50, 60, and 70 cm, with four replications per treatment.

Fruit Fly Abnormality and Mortality Test

Fruit fly abnormality testing was conducted by observing the growth of fruit flies from the pupal to adult stage daily for two weeks. Observation parameters included the time to adult emergence, normal adults, and abnormal adults. Fruit fly mortality testing was conducted by observing pupal mortality until the 14^{th} day. Observation parameters included the number of pupae dead and pupal morphology. Observed characteristics included changes in color, shape, and texture of the pupal cuticle. If successful adult emergence occurred, changes in the color and shape of the adult's body and wings were observed. Morphological changes in the pupae and adult fruit flies after treatment were recorded and documented for further analysis.

Data Analysis

The number of adult fruit flies hatched (imago success), survival, and morphological appearance of each treatment were calculated. The results were presented as a percentage, while the length of development time was presented in days. Data normality analysis was carried out before statistical analysis. The results indicated that the data were not normally distributed, even after transformation. Therefore, differences in the averages of successful, surviving, dead, normal, and abnormal imago across treatments and soil depths were analyzed using the non-parametric Kruskal–Wallis test. When significant differences were detected, Dunn's post hoc test with Bonferroni correction was applied for pairwise multiple comparisons. Data calculations were performed in Microsoft Excel, while statistical analyses were conducted in SPSS® version 25. Differences between means were considered statistically significant at $p < 0.05$.

RESULT and Discussion

The Effect of *B. bassiana* Treatment on the Percentage Mortality and Adult Development Type

To examine the influence of *B. bassiana* treatment on mortality and adult development type, *B. dorsalis* complex pupae were placed in soil media treated with the entomopathogen. The *B. bassiana* treatment significantly affected dead pupae ($Z = -2.675$; $p = 0.007$), successful imago ($Z = -2.675$; $p = 0.007$), and normal imago ($Z = -1.998$; $p = 0.046$), but had no significant effect on abnormal imago ($Z = -0.114$; $p = 0.910$). The percentage of dead pupae in the *B. bassiana* treatment was higher (87.78% ± 2.83) than in the control (69.58% ± 5.46), conversely, the percentage of successful pupae in this treatment was smaller (12.22% ± 2.83) than in the control (30.42% ± 5.46), as was the percentage of normal imago. The percentage of normal imago in the *B. bassiana* treatment was lower (6.81% ± 1.60) than in the control (24.03% ± 5.65). The *B. bassiana* treatment did not affect imago abnormalities. This was because the percentage of normal imago in the control was much higher, leaving a low proportion of abnormal imago (Figure 1).

A normal fruit fly has fully developed wings, bright body color, and a long abdomen. The two horizontal black lines and the longitudinal median line are barely visible (Figure 2A). Abnormal fruit flies have a pale-yellow body, a small and deformed abdomen (Figure 2B) or short, wrinkled wings (Figure 2C). Pre-treatment pupae usually have a tough body (Figure 2D). Dead pupae have a flabby body and softened cuticle. Some pupae demonstrated broken cuticle (Figure 2E).

This study demonstrated the significant effect of *B. bassiana* treatment on dead pupae, successful imago, and normal imago. Mechanistically, these effects are attributable to the infection strategy of *B. bassiana*, which penetrates the insect cuticle through a combination of mechanical pressure and extracellular enzymes such as chitinases, proteases, and lipases. Following penetration, fungal proliferation and the production of secondary metabolites (e.g., beauvericin and oosporein) disrupt host metabolism, leading to mortality or delayed development. Previous studies reporting pupal mortality between 36–60% under laboratory conditions (Li et al., 2024; Marri et al., 2016). The higher pupal mortality observed in this study compared with some previous reports suggests that soil application and interaction with soil depth can substantially enhance fungal efficacy against the pupal stage. Research with another type of entomopathogen (*Streptomyces fradiae*) showed a similar pattern in its mode of action. The entomopathogen, *Streptomyces fradiae*, also produces enzymes that can lyse the cuticle, causing damage to the pupal skin (Leksono et al., 2025).

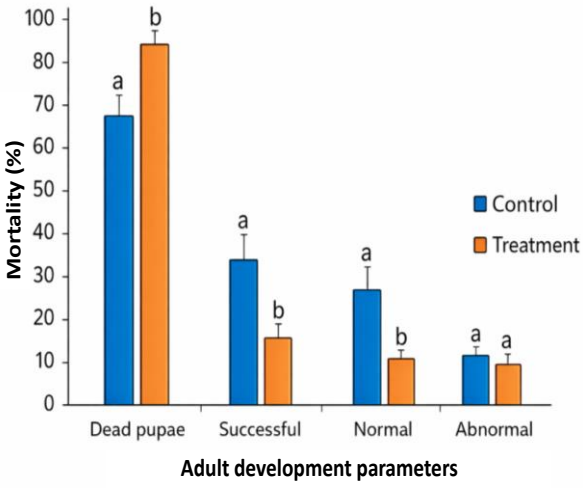

Figure 1. The effect of B. bassiana treatment on mortality and adult development of B. Dorsalis complex. The different letters (a,b,c) above the error bars indicate significant differences between the means (Dunn Post-hoc Test)

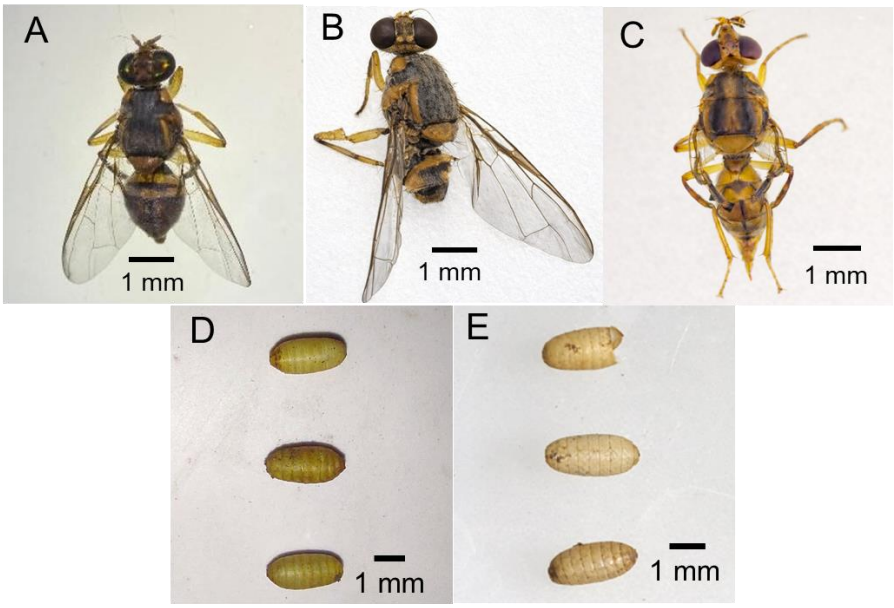

Figure 2. Imago and pupae of *B. dorsalis* complex, (A) normal imago, (B, C) abnormal imago, (D) pre-treatment pupae, and (E) dead pupae.

The Effect of Soil Level on The Percentage of Mortality and Imago Parameters

The effect of soil depth on the percentage of *B. dorsalis* complex mortality and adult development type was evaluated by putting the pupae in different soil depths (0, 4, 10, 20, 30, 40, 50, and 60 cm). The effect of soil level was significant on dead pupae (χ^2=57.043; p=0.000), successful imago (χ^2=57.043; p=0.000), normal imago (χ^2=55.168; p=0.000), and abnormal imago (χ^2=26.604; p=0.001). The mean percentage of dead pupae ranged from 27.50 to 100%. The lowest dead pupae percentage occurred at a depth of 0 cm (27.50 % ± 10.61), while the highest was at 60 and 70 cm (100% ± 0.00). That means, no imago survived at those depths. The percentage of survived imago ranged from 0.00% to 72.50%. The lowest percentage of living imago occurred at a depth of 60 and 70 cm (no survivor), while the highest was at 0 cm (72.50% ± 10.61). The normal survived imago ranged from 1.88 % to 63.75 %. The lowest percentage of normal imago occurred at a depth of 50 cm (1.88 % ± 0.91), while the highest was at 0 cm depth (63.75 % ± 13.78). The percentage of abnormal imago ranged from 1.25 to 11.88 %. The lowest percentage of abnormal imago occurred at a depth of 50 cm (1.25 % ± 1.25), while the highest was at 30 cm (11.88 % ± 4.30) (Table 1).

Table 1. The mean percentage (± SE) of dead pupa, successful imago, normal, and abnormal imago across various soil depths (0, 4, 10, 20, 30, 40, 50, and 60 cm)

Level (cm)	Dead pupae (%)	Successful imago (%)	Normal imago (%)	Abnormal imago (%)
0	27.50 ± 10.61a	72.50 ± 10.61a	63.75 ± 13.78a	8.75 ±4.41ab
4	55.63 ± 6.64ab	44.38 ± 6.64a	33.75 ± 8.80a	10.63 ±4.67ab
10	70.00 ± 7.50ab	30.00 ± 7.50ab	25.00 ± 7.07ab	5.00 ± 0.94ab
20	80.00 ± 6.55abc	20.00 ± 6.55abc	8.13 ± 2.98abc	11.88 ±6.19ab
30	83.75 ± 4.09abc	16.25 ± 4.09abc	2.50 ± 0.94bc	13.75 ±4.30b
40	94.38 ± 2.58bc	5.63 ± 2.58bc	3.75 ± 1.57bc	1.88 ±1.32ab
50	96.88 ± 1.32bc	3.13 ± 1.32bc	1.88 ± 0.91bc	1.25 ±1.25ab
60	100.00 ± 0.00c	0.00 ± 0.00bc	NA	NA
70	100.00 ± 0.00c	0.00 ± 0.00bc	NA	NA

Note: Different letters in the same column indicate significant differences at the level of $P < 0.05$ Dunn Post hoc Test

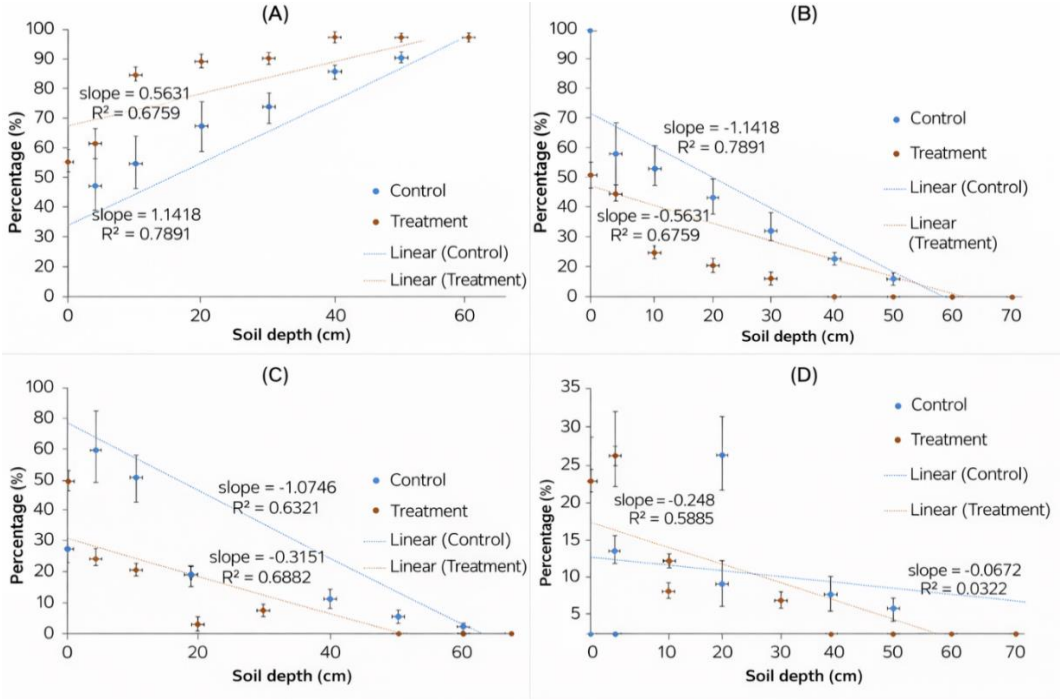

Figure 3. Correlation between the means of dead pupa and adult parameters: dead pupae (A), successful imago (B), normal imago (C), and abnormal imago (D) with soil treatments

The percentage of dead pupae in both treatment and control formed a positive relationship with soil level (Figure 3A), while that of successful adults formed a negative relationship with soil level (Figure 3B). The *B. bassiana* treatment effectively reduced survival rates at low depths and decreased further with increasing depth. This resulted in a gentler slope. Of the surviving adults, both normal and abnormal adults were negatively correlated with soil depth (Figures 3C and 3D). The *B. bassiana* treatment was quite effective in reducing the normality rate of adults at low depths and decreased further with increasing depth, resulting in a gentler slope (Figure 3C). The *B. bassiana* treatment increased the percentage of abnormal adults at low depths, but at depths greater than 20 cm, the effect was reversed (Figure 3D).

The second finding of this study is the significant effect of soil level on all parameters (dead pupae, successful imago, and normal imago (χ^2=55.168; p=0.000) and abnormal imago (χ^2=26.604; p=0.001). These patterns can be explained by soil physicochemical properties. Increasing soil depth is typically associated with higher compaction, reduced porosity, and limited oxygen diffusion, all of which hinder pupal respiration and adult emergence. At the same time, soil texture and pore size distribution influence the persistence and infectivity of fungal conidia by facilitating physical contact between spores and the host cuticle. Fine or compacted soils can trap emerging adults, leading to wing deformities, abdominal malformations, or failure to reach the surface, whereas coarser soils with higher porosity facilitate emergence but may still allow effective fungal infection.

The percentage of dead pupae from all samples after the *B. bassiana* treatment was higher (87.78%) than in the control (69.58%). Interestingly, pupal mortality was higher at shallow depths than at high depths (Figure 3A). At shallow depths, pupal success rates were generally high and decreased with depth. This indicates that *B. bassiana* application effectively increased pupal mortality. Mortality rates highly depend on the fruit fly stage, treatment method, and EPF concentration (X. L. Li et al., 2024; Marri et al., 2016). Previous studies reported that adult mortality in *B. bassiana* treatments using concentrations of 10.6–26.5 x10^7 ranged from 1–9.25%, while pupal mortality ranged from 36.25–46% (Marri et al., 2016). Another study observed the development of pupal mortality rates over time at different temperatures and conidia concentrations, ranging from 13–34°C and 1x10^3–0.56x10^{11} conidia/mL. The study results showed that *B. bassiana* strain B4 at a concentration of 1.0×10^7 caused pupal mortality ranging from 14.67% to 60% (Li et al., 2024).

Table 2. Correlation and significance of the effect of different soil levels on imago parameters (dead pupa, successful imago, normal imago, and abnormal imago)

Statistical value and significance	control	treatment
Dead pupa		
Correlation (*r-value*)	0.888	0.822
t-value	5.118	3.821
p-value	0.001	0.007
Successful imago		
Correlation (*r-value*)	-0.888	-0.822
t-value	-5.118	-3.821
p-value	0.001	0.007
Normal imago		
Correlation (*r-value*)	-0.795	-0.827
t-value	-3.468	-3.885
p-value	0.010	0.006
Abnormal imago		
Correlation (*r-value*)	-0.179	-0.767
t-value	-0.483	-3.164
p-value	0.644	0.016

Soil depth was positively and significantly correlated with pupae mortality in both control (r = 0.888; p = 0.001) and treatment (r = 0.822; p = 0.007). Soil depth was negatively and significantly correlated with successful imago in both control (r = -0.888; p = 0.001) and treatment (r = -0.822; p = 0.007). Soil depth was also negatively and significantly correlated with normal imago in both control (r = -0.795; p = 0.010) and treatment (r = -0.827; p = 0.006). Soil depth was also negatively and significantly correlated with normal imago in treatment (r = -0.767; p = 0.016), but it was not significant for normal imago in control (Table 2).

Soil depth significantly affected pupal mortality and all imago parameters. Pupal mortality increased with depth, while imago success and normality decreased with depth. Abnormalities fluctuated with soil depth. High mortality at depths greater than 30 cm may be due to soil physicochemical factors. Soil texture, particularly the size distribution of pore spaces, affects the infectivity of spores, such as those of *Beauveria bassiana*, by mediating physical contact (Sukalkar et al., 2018). In this process, the physical properties of the soil change as the pore size increases and the bulk density decreases. Physical and chemical characteristics of the soil, such as interstitial space, grain size, compaction, water content, and organic matter content, can affect the emergence of adult fruit flies on the soil surface (Agustina et al., 2025; Jaronski, 2010). The success of fruit fly larval pupation increases in soil with large soil particles because adult fruit flies can quickly emerge from the pupa. Several soil properties, such as porosity and total organic matter, can reduce soil density and make it easier for fruit flies to emerge (Jaronski, 2010). Soil texture can also cause abnormalities in adult fruit flies, such as in the wings and abdomen. Wing growth is imperfect and appears wrinkled or shriveled, and some flies have swollen abdomens. By contrast, fine soil has low porosity and may clump when exposed to water. As a result, the imago has difficulty exiting the pupa and emerging to the soil surface. These properties prevent emerging adult fruit flies from moving upward to reach the surface. In denser soils, adult fruit flies struggle to reach the surface due to the low total soil porosity (Jaronski, 2010).

The Effect of *B. bassiana* on the Adult Development

This study indicated that the effect of *B. bassiana* was significant on fruit fly imago parameters. The effect was significant on the mean development time of successful imago (Z=-3.764; p=0.000), normal imago (Z=-2.365; p=0.018), and abnormal imago (Z=-2.314; p=0.023) (Table 5). In general, the application of *B. bassiana* extended the development time of all imago parameters. The mean development time of successful imago in the *B. bassiana* treatment was significantly longer (13.00 days ± 0.17) than in the control (12.07 days ± 0.28). The mean of development time of normal imago in *B. bassiana* treatment was significantly longer (12.82 days ± 0.23) than in the control (11.91 days ± 0.28). The mean development time of abnormal imago in the treatment was also higher (13.24 days ± 0.14) than in the control (12.23 days ± 0.29) (Figure 4).

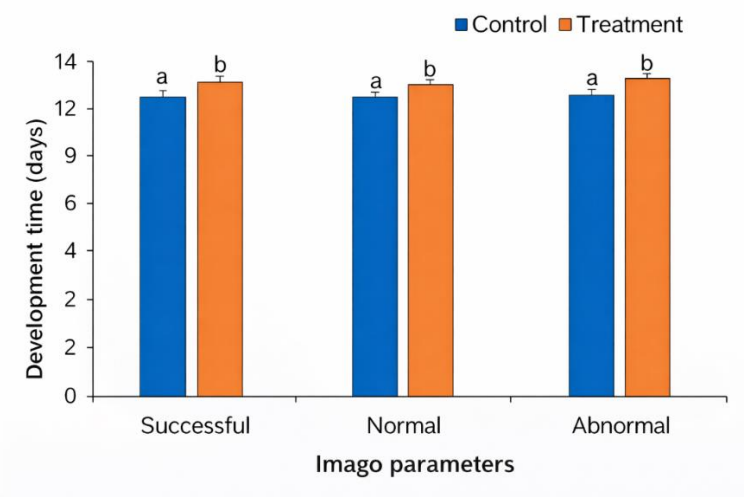

Figure 4. The effect of different treatments on the development time of imago parameters. The different letters (a,b,c) above the error bars indicate significant differences between the means (Dunn Post-hoc Test)

Table 3. The mean development time (± SE) of successful, normal, and abnormal imago across various soil depths (0, 4, 10, 20, 30, 40, 50, and 60 cm)

Level (cm)	Successful imago (%)	Normal imago (%)	Abnormal imago (%)
0	12.46 ± 0.22abc	12.36 ± 0.19ns	13.32 ± 0.24ns
4	12.21 ± 0.18b	12.05 ± 0.15ns	12.88 ± 0.07ns
10	12.19 ± 0.45b	12.09 ± 0.44ns	12.43 ± 0.48ns
20	12.11 ± 0.34b	11.82 ± 0.46ns	12.16 ± 0.35ns
30	13.12 ± 0.20bc	12.75 ± 0.63ns	12.88 ± 0.23ns
40	12.13 ± 0.13a	12.00 ± 0.00ns	12.50 ± 0.50ns
50	13.25 ± 0.25c	13.33 ± 0.33ns	13.00 ± 0.00ns
60	NA	NA	NA
70	NA	NA	NA

Note: NA = not available; ns = not significant

The Effect of Soil Depth on The Development Time of Imago Parameters

The effect of soil level on the development time of *B. dorsalis* complex was evaluated by putting the pupae in different soil depths (0, 4, 10, 20, 30, 40, 50, and 60 cm). The result showed that the effect of soil depth was significant on the development time of successful imago (χ^2=13.429; p=0.036). There was no significant different on the development time normal imago (p>0.05) and abnormal imago (p>0.05) at various soil levels (Table 6). The mean development time of successful imago ranged from 12.11 to 13.25 days. The longest development time occurred at a depth of 50 cm, while the fastest was at a depth of 20 cm. The mean development time of normal imago ranged from 11.82 to 13.33 days. The longest development time occurred at a depth of 50 cm, while the fastest was at a depth of 20 cm. The mean development time of abnormal imago ranged from 12.16 to 13.32 days. The longest development time occurred at a depth of 0 cm, while the fastest was at 20 cm depth (Table 3).

B. bassiana treatment significantly increased pupal mortality and decreased adult success and normality. *B. bassiana* treatment also significantly prolonged the development time of all adult parameters. Soil depth significantly affected pupal mortality and all adult parameters. Soil depth significantly affected the development time of successful adults, but not the development time of normal and abnormal adults.

B. bassiana treatment also significantly extended the development time of all imago parameters. *B. bassiana* attacks on *B. dorsalis* resulted in a longer development time for successful imagoes (13.00 days) compared to the control (12.07 days). This is likely due to enzymatic effects that can disrupt metabolism, resulting in delayed eclosion of surviving fruit fly pupae.

Soil depth significantly affects the development time of successful adults, but not the development time of normal and abnormal adults.

Treatment significantly affects the average development time of successful and normal adults, but not of abnormal adults. In this study, the fastest pupal development into an adult occurred at a depth of 20 cm, while the slowest occurred at a depth of 50 cm. This result aligns with other previous studies. In other studies, pupal development time differed significantly at different soil levels (Leksono et al., 2024; Susanto et al., 2022). Previous studies reported that soil depth affects the development duration of *B. dorsalis* and *B. carambolae* hybrid pupae. The average development time ranged from 8.88 to 10.63 days. It was relatively stable at depths of 4–40 cm and dramatically longer at 50 and 60 cm (Susanto et al., 2022). Previous research also showed that the average pupal stage duration ranged from 8.01 to 12.78 days. The fastest development occurred at a depth of 10 cm, and the slowest at a depth of 50 cm (Leksono et al., 2024). In this study, pupal development time fluctuated from 12.46 days at a depth of 0 cm, then decreased to 12.11 days at a depth of 20 cm, then increased at a depth of 30 cm (13.12 days), decreased at a depth of 40 cm (12.13 days), and increased at a depth of 50 cm (13.25 days).

The novelty of this study lies in its integrated assessment of entomopathogenic fungal efficacy across a vertical soil gradient, simultaneously evaluating mortality, adult emergence, morphological quality, and developmental timing of *B. dorsalis* complex. Unlike previous studies that examined either fungal pathogenicity or soil depth independently, this research demonstrates how soil depth modulates both lethal and sublethal effects of *B. bassiana* at the pupal stage. From a scientific perspective, this study advances understanding of soil-mediated host–pathogen interactions in dipteran pests and provides empirical evidence that pupal-stage targeting in soil is highly effective for biological control. From a practical and societal standpoint, the findings support the use of *B. bassiana* as an environmentally friendly alternative to chemical insecticides in fruit fly management. By identifying soil depths where pupae are most vulnerable, this research offers actionable guidance for optimizing biological control strategies within integrated pest management programs, contributing to sustainable agriculture and reduced pesticide reliance. This research supports SDGs Zero Hunger, because the result directly contributes to sustainable agriculture through biological control of the fruit fly (*Bactrocera dorsalis*), a major pest of horticultural crops, reduced crop losses, and increased agricultural productivity, as well as providing an environmentally friendly alternative to chemical pesticides.

CONCLUSION

B. bassiana treatment significantly increased pupal mortality, decreased the success rate, and affected the normality of the imago. *B. bassiana* treatment also significantly extended the development time of all imago parameters. Soil depth significantly affected pupal mortality and all imago parameters. Soil depth significantly affected the development time of successful imago, but not the development time of normal and abnormal imagoes. These findings suggest that *B. bassiana* is effective in controlling fruit fly pupae in soils of varying depths. This study provides a foundation for integrating entomopathogenic *B. bassiana* into fruit fly control strategies targeting the pupal stage. Future research is needed to examine lethal concentration levels and application in the larval and imago phases.

ACKNOWLEDGMENT

The authors thank the Rector and Director of the Directorate of Research and Community Service, Universitas Brawijaya, for supporting this study. This study received the financial support from Universitas Brawijaya through Visiting Lecturer Program.

AUTHOR CONTRIBUTION STATEMENT

All authors contributed significantly to the work reported in this manuscript. ASL, IF, and NK conceived and designed the study, conducted the data analysis, and prepared the manuscript. NFE, RPQ was responsible for data collection. AZ supported data visualization. HZ assisted in the literature review and provided substantial feedback during the manuscript drafting process. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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