# Doleschallia bisaltide (Lepidoptera: Nymphalidae) profile and oviposition preference on Graptophyllum pictum (L.) Griff.

**Peni Lestari**
National Research and Innovation Agency
**Tri Lestari Mardiningsih**
tril003@brin.go.id
National Research and Innovation Agency
**Dewi Sartiami**
IPB University
**Wage Ratna Rohaeni**
Indonesian Agency for Agricultural Instrument Standardization
**Rohimatun Rohimatun**
National Research and Innovation Agency
**Bruce Ochieng Obura**
International Potato Center
**Ifa Manzila**
National Research and Innovation Agency

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# Abstract

*Doleschallia bisaltide* Cramer (Lepidoptera: Nymphalidae) is a main pest on *Graptophyllum pictum* (L.) Griff. in the Lepidopteran order, the first-instar larval performance is determined by adult oviposition. An adult oviposition preference can be used to determine the resistance level of some *G. pictum* accessions in early-stage breeding programs. The study aimed to identify the profile of *D. bisaltide* and its oviposition preferences on *G. pictum* accessions. The herbivore-host interaction was utilized as a guide to select cultivars resistant to *D. bisaltide* and identify repellent characteristics for the insect. The research was conducted at the Indonesian Spice and Medicinal Crops Research Institute (ISMCRI), using nested Randomized Complete Block Design (RCBD). *G. pictum* accessions nested on flight periods. The study revealed that insect mating occurred from dusk to night, while oviposition occurred the following morning. The adult mating was dominantly in the humid area, suggesting a potential population outbreak during the rainy season. The highest oviposition preference was observed for accessions with higher anthocyanin-to-chlorophyll content. In contrast, the lowest oviposition was recorded for accession 12 content of higher chlorophyll-to-anthocyanins. *D. bisaltide* oviposition preferences were influenced by leaf pigment, saponins, and the C/N ratio. Phytochemicals such as flavonoids and glycosides in *G.pictum* leaves acted as oviposition stimulants for specialists such as *D. bisaltide.*

# Introduction

The caricature plant, *Graptophyllum pictum* (L.) Griff. (Syn. *Justicia picta* Linn.), belonging to the family Acanthaceae is known in Indonesia as *handeuleum* (Sundanese), *wungu* leaf (Javanese) (Sartika & Indradi 2021), and purple pudding (Umami et al. 2020). This species is integral to Indonesian ethnobotany and is renowned for its medicinal properties, such as anti-hemorrhoid, antimicrobial, anti-inflammatory, analgesic, and wound-healing effects (Rahmawati et al. 2020). Additionally, *G. pictum* leaves are reported to alleviate coughs (Umami et al. 2020) and plaque reduction in teeth (Makkiyah et al. 2021). The health benefits of the *G. pictum* are attributed to various active compounds, including alkaloids, flavonoids, steroids, saponins, glycosides, and emollients, present in the leaves (Lestari et al. 2015), along with information on its pharmacological effects (Hutagalung 2019; Makkiyah et al. 2021).

*Doleschallia bisaltide* Cramer (Lepidoptera: Nymphalidae) is a specialist (monophagous) insect known to feed leaves of the Acanthaceae family, particularly *G. pictum* and *Asystasia gangetica* (L.) Anders (Handayani *et al.* 2019). Yield losses of *G. pictum* leaves due to *D. bisaltide* larvae have been reported to range from 300.28 to 745.86 cm² per plant, equivalent to 70% of the leaves, based on (Lestari et al. 2015). *G. pictum* leaves are essential for harvesting for medicinal purposes; therefore, larvae infestation poses a risk of reducing the quantity and quality of its harvest.

Understanding herbivore-plant interactions is crucial in developing plants resistant to herbivores (Wouters et al. 2016; Chabaane et al. 2022; Kamaliah et al. 2022), including the oviposition preferences of adult insects on host plants. Early instar larvae of Lepidopterans can only access food resources near their eggs (Wouters et al. 2016; Griese et al. 2020). Thus, oviposition preferences of adults on specific plants and the plant's antixenosis and antibiosis mechanisms are crucial in determining plant resistance levels against pests. Insect host preferences are influenced by plant morphology, which can serve as a selection factor in breeding initiatives for resistant varieties, this may serve as a selection factor (Lestari et al. 2015). Feeding preferences of *D. bisaltide* across Acanthaceae species and within *G. pictum* have been studied, along with population fluctuations in nature (Adi et al. 2021). However, information on oviposition preferences within *Graptophyllum* species still needs to be available. Based on literature studies, this is the first study to discuss the interaction of *D. bisaltide* and *G. pictum* regarding oviposition preference and plant phytochemical characteristics. Identifying the behavior of *D. bisaltide* adults and larvae is the first step in designing strategies for its management in *G. pictum* plantations, including developing resistant plant varieties.

Insects naturally exhibit varied responses to plants with diverse characteristics. Plant chemistry, especially for female butterflies, plays a role in determining niches for oviposition and feeding activities (Griese et al. 2020). (Sokolinskaya et al. 2020) explained that Insects have created an impossible-to-miss and one of the foremost advanced chemoreception frameworks, which abuses at the slightest three receptor superfamilies giving discernment of scent and taste and chemical communication in these creatures. Badenes-Pérez (2023) said that there is an effect of host plant indolic glucosinolate content on oviposition preferences and the complexity index of glucosinolates and aliphatic glucosinolates without sulfur-containing side chains on total oviposition is smaller in *P. rapae* than in *Plutella xylostella* L. (Lepidoptera: *Plutellidae*), another lepidopteran that specializes in containing plant glucosinolates. Variations in genetics, nutritional status, and environmental conditions cause significant differences in the phytochemical content among varieties under the same species. This diversity explains the oviposition preference and feeding preferences of some insects to some varieties over others.

This study aims to explore the oviposition preferences and larvae development of *D. bisaltide* reared on 13 *G. pictum* accessions. The herbivore-host interaction in this study is used as a guide to develop *G. pictum* cultivars resistant to *D. bisaltide.*

# Material and Methods

The research was conducted during three flight periods of *D. bisaltide* adults: the end of the rainy season (May-June), the beginning of the dry season (June-July), and the peak of the rainy season (November-December). The length of each period was adjusted until the last adult died. The research took place in the greenhouse of the Indonesian Spice and Medicinal Crops Research Institute (ISMCRI) (nowadays the Indonesian Agency for Agricultural Instrument Standardization/IAAIS), Bogor, Indonesia (240 m above sea level). The number of rainy days and total rainfall during the three flight periods are shown in Table 1.

Table 1
Number of rainy days and total precipitation during the study

| Flight period | Number of rainy days (day) | Total precipitation during a month (mm) |
|---|---|---|
| May-June | 33 | 771.0 |
| June-July | 23 | 501.4 |
| November-December | 37 | 693.4 |

Data was obtained from the weather station at the ISMCRI Research Station

Preparation of Test Plants

Thirteen *G. pictum* accessions were obtained from the germplasm collection of the Department of Agronomy and Horticulture, IPB University. Their tolerance level to *D.bisaltide* was described in Table 2. Cuttings that were 15 cm long were planted in sandy soil. Healthy and uniform seedlings, 30 days old, were transplanted into a planting medium mixed with soil, manure, and rice husks charcoal (1:1:1). Seedlings were maintained under 55% light intensity using artificial shade. Plants were watered twice a day. The 250 ml of balanced NPK (16-16-16) liquid fertilizer (concentration 2 g/L water) was applied to the plants every two weeks. Plants were in the vegetative phase during the testing period.

Table 2
Tolerance level of *G. pictum* accessions to *D. bisaltide* larvae

| Accessions | Resistance level |
|---|---|
| 1 | Medium resistant |
| 2 | Susceptible |
| 3 | Medium resistant |
| 4 | Medium resistant |
| 5 | Susceptible |
| 6 | Medium resistant |
| 7 | Resistant |
| 8 | Resistant |
| 9 | Susceptible |
| 10 | Medium resistant |
| 11 | Medium resistant |
| 12 | Medium resistant |
| 13 | Resistant |

Tolerance level on every *G. pictum* accession to *D. bisaltide* based on feeding preference (non choice test) (Lestari et al. 2015)

Preparation of Test Insects

The adult insect population in this experiment from a field sample first breed generations, originated from eggs collected from the *G. pictum* population in the ISMCRI collection garden. Eggs were kept in a rearing box. After hatching, the first instar larvae were moved to another box until they reached the third instar stage, then transplanted into a 0.25 $m^3$ cylindrical mesh cage until they became pupae (Schäpers et al. 2015). During rearing, larvae were fed a mixture of leaves from the 13 tested *G. pictum* accessions every morning and evening. Pupae were transferred to the rearing box. The hatched butterflies were reared in another mesh cage and maintained until testing. Food sources for *D. bisaltide* butterflies were nectars from flowers of *Ixora* sp. and *Hydrangea* sp., as well as cotton moistened with a 10% concentration of multiflora honey solution.

Experimental Design

The experiment was carried out in three flight periods: May-June (end of the rainy season), June-July (transition from rainy to dry season), and November-December (peak of the rainy season). In each experimental set, 13 *G. pictum* accessions were arranged based on a Randomized Complete Block Design with three replications and five plants per group.

The morphological characteristics and mating activity of *D. bisaltide* were observed in the adult population during the experiment. Oviposition preference testing was carried out based on the choice method. Fifty pairs of butterflies that came out from pupae on the same day were flown in a greenhouse containing the *G. pictum* accessions tested. Dead butterflies were replaced until the seventh day of flight, then started to count the adults. The profile of *D. bisaltide* was observed daily until the last adult died. The lifespan of the butterflies was calculated based on the reduction in the number of male or female butterflies compared to the initial number flown.

The frequency of visits of *D. bisaltide* adults was calculated based on the number of egg batches on a particular accession. The oviposition preference of *D. bisaltide* was estimated based on the total number of eggs laid on each plant accession compared to the total number of eggs laid on all plants during one flight period (%). The larger the egg batches and the more number eggs laid on accession, the higher the *D. bisaltide* adult preference for that accession (Lestari et al., 2015; Griese et al., 2020). The eggs counted were those laid on that day. First instar larvae were selected and maintained on each accession (non-choice) to observe their development. The frequency of adult visits, oviposition preference, number of eggs, and larval growth and development were counted daily during the experiment. Environmental variables, such as temperature and humidity, were recorded daily using a thermohygrometer (data not shown).

Observation of *G. pictum* Leaf Pigments

The leaves chlorophyll, anthocyanins, and carotenoids were measured using spectrophotometric methods based on the Sims & Gamon (2002) method. The other phytochemical substances were examined in the ISMCRI laboratory utilizing these techniques: nitrogen was measured using the Kjeldahl method; calcium was identified via the Atomic Absorption Spectroscopy (AAS) method; organic carbon was determined by spectrophotometry; and fiber was assessed based on the gravimetric method (SNI 01-2891-1992).

# Data Analysis

Data were tabulated and prepared using Microsoft Excel 2019 (www.microsoft.com, USA). The normality of data distribution was analyzed using the Shapiro-Wilk test. Data were analyzed using analysis of variance followed by the Tukey post hoc test ($\alpha$ = 5%). The relationship between observed variables was estimated using Pearson correlation in Minitab v.18 software (www.minitab.com). Mattjik & Sumertajaya (2013) suggest that correlation analysis describes the nature of the relationship between one character and another. The closer it is to 1 or -1, the stronger the correlation between the two characters. Conversely, the closer it is to 0, the more unrelated the two characters are.

## Results

Profile of *D. bisaltide* on *G. pictum*

The adult of *D. bisaltide* has brown wings, ranging from yellowish-brown to grayish-brown. Male *D. bisaltide* butterflies have characteristically darker wings with white patches on the lower-part wings, while female butterflies do not have these spots (Fig. 1). The average lifespan of butterflies in this study was 9.00 ± 7.00 days across the three flight periods. Characteristics of *D. bisaltide* are shown in Fig. 2.

Insect of *D. bisaltide* has four life stages: egg, larva, pupa, and adult (butterfly). Generally, *D. bisaltide* butterflies take flight around 8 o'clock when the weather is clear and end at sunset. In contrast, *D. bisaltide* mating activity starts in the afternoon, before sunset, and ends at night. The mating process begins with flying together as a calling stage, continuing with chasing (mating stage) (Ma et al. 2019). The butterflies who have found their mate then separate themselves and perch in pairs on the ceiling of the screen house. The male and female abdomens unite until the mating process is complete (Fig. 1). The mating process lasts six to eight hours, as (Ma et al. 2019) report on other Lepidopterans (*Scopula subpunctaria* Herrich-Schaeffer). As many as 40 of 50 pairs of butterflies copulate near water sources. The following day, the female will lay eggs on the underside of the leaves. During the test period, mating occurred within the first 3 weeks.

The eggs of *D. bisaltide* were pearly white when laid (Fig. 2a), turned cream, and finally became clear with black spots at the poles (Fig. 2b) when ready to hatch. If they turned black (Fig. 2f), they failed to hatch. The black spot was the head of the larva. The eggs were spherical (round) with a diameter of ± 1 mm. They bent inward at one pole (Fig. 2c). *D. bisaltide* adults laid all eggs, or divided into several egg batches, under the leaf surface (Fig. 2d). Sometimes, eggs were laid on the stem (Fig. 2e). The egg numbers reach 50 eggs. The eggs would hatch within 4 days (Fig. 1). The number of eggs laid per female differed between flight periods ($n = 3$, $p < 0.001$) as well as between replicates per period ($n = 6$, $p = 0.002$). Females laid more eggs during the flight period in November-December (125.78 eggs), followed by June-July (96.57 eggs) and May-June (51.20 eggs) (Table 3). However, the hatching percentage was highest in May-June and differed from June-July (Table 4). More eggs were laid in plant populations near water sources.

Table 3
Egg numbers in 13 *G. pictum* accessions during the three flight periods

| Accession | Number of eggs (eggs)* | | | Average |
|---|---|---|---|---|
| | May-June | June-July | November - December | |
| 1 | 67.70d | 143.18a | 114.57a | 108.48a |
| 2 | 54.33d | 130.14c | 135.44c | 106.64a |
| 3 | 67.16b | 82.64h | 143.73h | 97.84a |
| 4 | 54.45c | 102.19e | 164.61e | 107.08a |
| 5 | 56.21e | 117.11b | 149.56b | 107.63a |
| 6 | 40.84i | 61.08j | 129.25j | 77.06a |
| 7 | 66.69g | 100.10d | 100.21d | 89.00a |
| 8 | 21.92k | 119.21d | 132.35d | 91.16a |
| 9 | 76.88a | 102.85f | 125.73f | 101.82a |
| 10 | 52.78h | 86.94i | 152.35i | 97.36a |
| 11 | 43.27j | 97.43g | 148.90g | 96.53a |
| **12** | **7.64l** | **21.48k** | **19.05k** | **16.06b** |
| 13 | 55.76f | 91.03f | 119.43f | 88.74a |
| Average | 51.20c | 96.57b | 125.78a | |

Columns with the same letter are not significantly different based on the Tukey post hoc at α = 5%. Each period is tested using Kruskal-Wallis

The more significant number of egg batches found on one plant indicates that the more frequently adults visit that plant, the higher the oviposition preference of *D. bisaltide* females on that accession. The lowest visiting frequency was obtained for accession 12, followed by accessions 6 and 13, whereas accessions 1, 5, and 9 were most regularly visited (Fig. 3). The number of egg batches laid in other accessions varies in each period. The number of eggs laid in accession 12 was lowest in each flight period (May-June ($H = 2.94$, $p = 0.003$), June-July ($H = 3.37$, $p = 0.001$), November-December ($H = 2.85$, $p = 0.005$)). This value was only 1.33% of the eggs in 13 plant accessions, followed by accessions 6 and 13. In contrast, most of the *D. bisaltide* adult individuals laid eggs in accessions 1, 5, and 9 with a percentage of eggs ≥ 10% of the total eggs. The larvae development stage lasts around 14–16 days, followed by a pupation period of 14 days (Fig. 4).

Table 4
Hatched egg presentation in 13 *G. pictum* accessions during the three flight periods

| Accession | Hatched Egg (%) | | | Average |
|---|---|---|---|---|
| | May-June | June-July | November - December | |
| 1 | 76.38ab | 63.14ab | 63.29a | 67.60ab |
| 2 | 67.50ab | 31.34b | 44.00a | 47.61b |
| 3 | 57.59ab | 55.63ab | 70.28a | 61.17ab |
| 4 | 38.15b | 67.72ab | 76.50a | 60.79ab |
| 5 | 62.84ab | 69.68ab | 70.24a | 67.58ab |
| 6 | 73.20ab | 57.34ab | 63.13a | 64.56ab |
| 7 | 73.05ab | 61.82ab | 57.60a | 64.16ab |
| 8 | 50.11b | 47.09ab | 47.22a | 48.14b |
| 9 | 59.68ab | 34.72b | 45.23a | 46.55b |
| 10 | 60.55ab | 71.15ab | 73.34a | 68.34ab |
| 11 | 77.12ab | 39.00ab | 37.58a | 51.24b |
| 12 | 97.62a | 55.51ab | 58.40a | 70.51ab |
| 13 | 80.50ab | 80.42a | 76.99a | 79.30a |
| The average season | 67.25a | 56.50b | 60.29ab | |

They live on the lower surface of leaves. No cannibalism was found, except for fifth instar larvae, which ate young pupae. When hatched, *D. bisaltide* larvae had pale yellow bodies with a shiny black head region and soft hair on each body segment (Fig. 4a). After they ate the leaves, the bodies of the first instar larvae were blackish, like jelly (Fig. 4b). In the second instar larvae, bristly hairs were developed on all the body segments (Fig. 4c). The third instar larvae began to display white lines on their dorsal and lateral sides (Fig. 4d). These lines become more prominent as they develop to the fourth and 5th instars. From the fourth instar larvae, orange sockets appeared on the lateral sides and metallic blue sockets on the dorsal sides (Fig. 4e and 4f). The fifth instar larvae had reached maximum body size, the most extended lifespan, and the highest eating ability (Fig. 4f). The larvae need 2.04 ± 0.09 days for the first instar, 2.14 ± 0.22 days for the second instar, 2.42 ± 0.28 days for the third instar, 2.70 ± 0.30 days for the fourth instar, and 5.85 ± 0.64 days for fifth instar. At the end of the fifth instar, the larvae ceased feeding and underwent a morphological change in which their bodies shortened and hung down, forming the shape of a letter J with their heads at the bottom.

The pupae of *D. bisaltide* had an obtecta shape, characterized by prospective antennae, wings, and legs that appear faint and are tightly attached to the body. The pupa was wrapped in cremaster thread. The color of the pupa was initially pink (Fig. 4h), gradually becoming light brown to dark brown, with a yellow tinge in some parts (Fig. 4i). Butterflies would emerge in the next 7–9 days (Fig. 4j). There were neatly arranged black dots on the dorsal side of the pupa. The highest mortality occurred during the initial phase of pupa formation, likely due to the consumption of soft pupal tissues by fifth instar larvae. The extent of damage to the pupa can significantly impact the success of metamorphosis into a butterfly as an adult form of *D. bisaltide*. The emerge-adult can take one of three forms: perfect, deformed, or deceased.

### Characteristics of *G. pictum* and Its Relation to *D. bisaltide* Oviposition

The results revealed significant variations in the percentage of anthocyanin, chlorophyll, and carotenoid pigments, suggesting differences in the composition of these pigments among the accessions. The highest concentration of anthocyanin pigments was in accession 1, and the lowest was in accession 12 (Table 5). The proportion of anthocyanin to chlorophyll to carotenoid pigments varied among accessions, with accession 1 having a ratio of 5:3:2 and accession 12 of 1:6:3. In addition, eleven other accessions exhibited a similar proportion of anthocyanin to chlorophyll to carotenoid pigments, with a ratio of 4:4:2. Visually, the 12 *G. pictum* accessions have similar leaf colors, viz. reddish purple to blackish purple. Only accession 12 has a green-white variegation color (Fig. 5).

Table 5
Proportion of pigment content on the leaves of *G. pictum*

| Accession | Leaf pigment proportion (%)* | | |
|---|---|---|---|
| | Anthocyanin | Total chlorophyll | Carotenoid |
| 1 | 52.23 | 28.81 | 18.96 |
| 2 | - | - | - |
| 3 | 43.67 | 35.87 | 20.46 |
| 4 | 38.87 | 38.87 | 22.27 |
| 5 | 39.54 | 36.95 | 23.51 |
| 6 | 36.86 | 40.91 | 22.23 |
| 7 | 36.11 | 41.09 | 22.80 |
| 8 | 38.36 | 37.62 | 24.02 |
| 9 | 42.77 | 35.78 | 21.45 |
| 10 | 36.44 | 41.19 | 22.37 |
| 11 | 35.51 | 41.38 | 23.11 |
| 12 | 13.80 | 60.08 | 26.11 |
| 13 | 36.21 | 41.20 | 22.58 |

*) Measured on the third leaf of a fully opened shoot using the (Sims dan Gamon 2002) method. Pigment measurements were not carried out on accession two due to insufficient availability

The relationship between the number of eggs and the pigment content and secondary metabolites of 13 *G. pictum* accessions showed that the number of eggs was significantly positively correlated with the anthocyanin content, C/N ratio, and saponin content of *G. pictum* leaves and negatively correlated with chlorophyll content (Table 6). In contrast, the frequency of *D. bisltide* visits was uncorrelated with any plant traits. However, the correlation value is relatively high in leaves with high saponin content or low calcium content.

Table 6
Correlation values between plant characteristics and egg number and frequency of adult arrival

| *G. pictum* characters | Correlation value with | |
|---|---|---|
| | Egg number | Visiting frequency |
| Anthocyanin | 0.71** | 0.03 |
| Chlorophyll | -0.74** | -0.11 |
| Carotenoid | -0.51 | -0.14 |
| C organic | 0.03 | 0.10 |
| Nitrogen | -0.52 | -0.15 |
| C/N ratio | 0.63* | 0.13 |
| Calcium | 0.37 | -0.59 |
| Fiber | 0.16 | 0.21 |
| Alkaloids | -0.38 | -0.10 |
| Saponin | 0.62* | 0.51 |
| Flavonoids | -0.02 | 0.32 |
| Triterpenoids | 0.31 | 0.22 |
| Steroids | -0.26 | -0.44 |

Note: * means significant (P value < 0.05) and ** means very significant (P value < 0.01) based on Pearson correlation analysis

## Discussions

Like autumn leaves, the brown wings are characteristic of *D. bisaltide*, making it known as the autumn-leaf butterfly (Fig. 1). Several publications link the variation in wing color of *D. bisaltide* to its functions, such as mimicry, mating preferences, defense from predators, and response to pollutant concentrations in the environment (Sekimura & Nijhout 2017; Penz & Williams 2020). Sekimura dan Nijhout (2017) concluded that the evolution and diversity of Nymphalidae wings are influenced by genetics and changes in environmental temperature. Both larvae and adults of *D. bisaltide* are active from morning to evening, but mating activity generally occurs in the afternoon to evening, with ovipositioning the following day. The long duration of mating causes damage to the hind wings of *D. bisaltide* adults (Fig. 1). Most Nymphalidae butterflies (depending on the species) are reported to actively mate and lay eggs at dawn, dusk, or both (Penz & Williams 2020).

Forty of the fifty pairs of *D. bisaltide* chose water sources for copulation, indicating that the insect population increases in the rainy season or in areas with high humidity. Observations showed that most eggs were laid during the rainy season (November-December) (Table 3), as well as the hatching percentage (Table 4) when the number of rainy days and precipitation was higher than in the dry season (Table 1). Air humidity in November-December reaches 90%, decreasing to 48–53% from June to July and around 60% from May to June. This study supports Adi et al. (2021), who stated that humidity affects the fecundity of adult *D. bisaltide*. Based on the average total number of eggs (Table 3) and the frequency of adult visits in three flight periods (Fig. 3), it was concluded that adult *D. bisaltide* females had the lowest oviposition preference in accession 12 and the highest in accessions 1, 5, and 9.

The number of eggs laid is unrelated to the number of eggs that hatch but is in line with feeding activity. The lowest percentage of hatched eggs was in accession 9 (46.55%), and the highest was in accession 13 (79.30%). Meanwhile, our previous study on feeding activity showed that accessions 5 and 9 were susceptible to *D. bisaltide* larvae, while accessions 7 and 12 were resistant (Table 2) (Lestari et al., 2015). Both accessions 7 and 12 were rich in fiber content. Apart from insect factors, such as food consumed and the genetics of adults, plant factors also influence egg hatching. The presence of herbivore eggs acts as an inducer of plant defense mechanisms, and then they respond physiologically (Hilker & Fatouros 2016) by changes in secondary metabolic contents or structure. The presence of insect eggs is an essential form of early-stage plant defense evolution. The ability to detect and create unsuitable conditions for egg development potentially inhibits eggs from hatching, resulting in fewer larvae consuming the plant (Griese et al. 2020).

Plant characteristics influence the interaction of herbivore insects with their hosts through antixenosis and antibiosis mechanisms. There is a role for plant chemicals in differential preferences between host races related to distantly related plant taxa (Katte et al. 2022). Each adult was not labeled in this study, so whether individual butterflies return to the same plant for subsequent oviposition cannot be tracked. However, Nataraj et al. (2024) explained that insects return to similar flowers or plants based on previous experience and limit their visits to other potential oviposition niches. It has been proven that larvae-infested and uninfested maize plants affect the oviposition preference of three different Lepidoptera species (Ntiri et al. 2018). The decision of adult female insects to oviposit on a plant is made after landing on the leaves influenced by the physical and chemical characteristics of the plant. Females were found to prefer plants with high stomatal density, a large stomatal area, and fewer trichomes as oviposition hosts, and the depth of egg placement was determined by leaf thickness (Zhang et al. 2021).

During the host search phase, flying insects like Lepidoptera rely on sight and smell to identify their host plants. This research showed that plant color influences insect landing and ovipositioning activities. Accession 12 is the least choice, while accessions 1, 5, and 9 are the most preference. The proportion of anthocyanins to chlorophyll and carotenoids is thought to help *D. bisaltide* find its host (Table 5). Apart from leaf pigment, the egg-laying preference of *D. bisaltide* is also related to high saponin content and C/N ratio (Table 6). Anthocyanins (flavonoids) and saponins, glycosides, are reported as deterrents and antifeedants in many generalist insects, such as *Spodoptera littoralis* Boisduval. and *Helicoverpa armigera* Hubner (Boate & Abalis 2020). In constrast, we suspect that plants with high anthocyanin concentrations, namely accessions 1, 5, and 9, tend to be more frequently selected by *D. bisaltide* adults for oviposition. In contrast, accession 12, with the lowest anthocyanin content, is the least preferred by *D. bisaltide* to oviposit, as Cepero et al. (2015) concluded that Monarch butterflies prefer purple color to green ones.

The behavior of monophagous insects (specialist insects), such as *D. bisaltide* revealed that toxic secondary metabolites in plants are stimulants for specialist insects, which generalist insects avoid (Wouters et al. 2016). Generalist insects have a range of multiple hosts from different families, while specialist insects are monophagous or oligophagous, eating plants in the same family that release defense compounds.

Adult females generally lay eggs on the lower surface of young leaves for several pairs of leaves later. Consistent with the theory that the phytochemical content of plants is distributed with varying concentrations between plant parts Idris et al. (2023) explained that secondary metabolites are not synthesized in young leaves but are translocated from fully developed leaves to young leaves. Thus, the first instar larvae can easily feed on and digest the soft tissues of the plant. The choice of young leaves as a place to lay eggs is thought to be an insect's response to its host's defense.

The diversity of characters between *G. pictum* genotypes influences egg-laying activity but only slightly influences larval development. This pattern is also observed in other specialist insects, such as *Spodoptera frugiperda* J.E. Smith on corn (Wouters et al. 2016) and *Euphydryas editha* Bayansis on three different host plants (Haan et al. 2018). All larvae that feed on different *G. pictum* accessions can complete the larval cycle within 14–16 days, two days faster than larvae fed on *A. gangetica*, based on research by (Handayani *et al.* 2019). However, the duration of each *D. bisaltide* larval instar obtained in this study was shorter than the observations of Sartiami et al. (2009) in the field. This suggests that an unlimited food source enables the larvae to reach their maximum size earlier.

## Conclusion

Flying activities of *D. bisaltide* and its larvae-feeding activities occur during the day. Their mating activities take place in the dusk until evening. Meanwhile, oviposition activities were held the following morning. The insects prefer the leaf with higher anthocyanin-to-chlorophyll content, high saponin content, and a higher carbon-to-nitrogen ratio. They tend to avoid leaves with high calcium content. The variation in leaf characteristics among *G. pictum* genotypes affects oviposition preference, though it has only a minor impact on larval development. Accession 12 was suspected to be resistant to *D. bisaltide* based on oviposition preference. Therefore, it is necessary to conduct genetic studies on accessions 12 to use it as donor parents in a breeding program of *G. pictum* for resistance trait to *D. bisaltide* attack.

## Declarations

# Consent for publication

Not applicable.

**Conflict of Interest:**

The authors have no competing interests to declare relevant to this article's content.

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# Author Contribution

PL, TLM, DS – conceptualization, ideas, and research design formulation. PL, WRR, RR, BOO - observation and analysis data, writing the manuscript. PL, TLM, WRR, BOO, IM – insect collecting and mass rearing. PL, DS, RR, IM – morphology identification. PL, TLM, DS, WRR, RR, BOO, IM – writing, editing, managing all communication, and approving the manuscript. All authors listed have significantly contributed to the article’s development and writing.

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# Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

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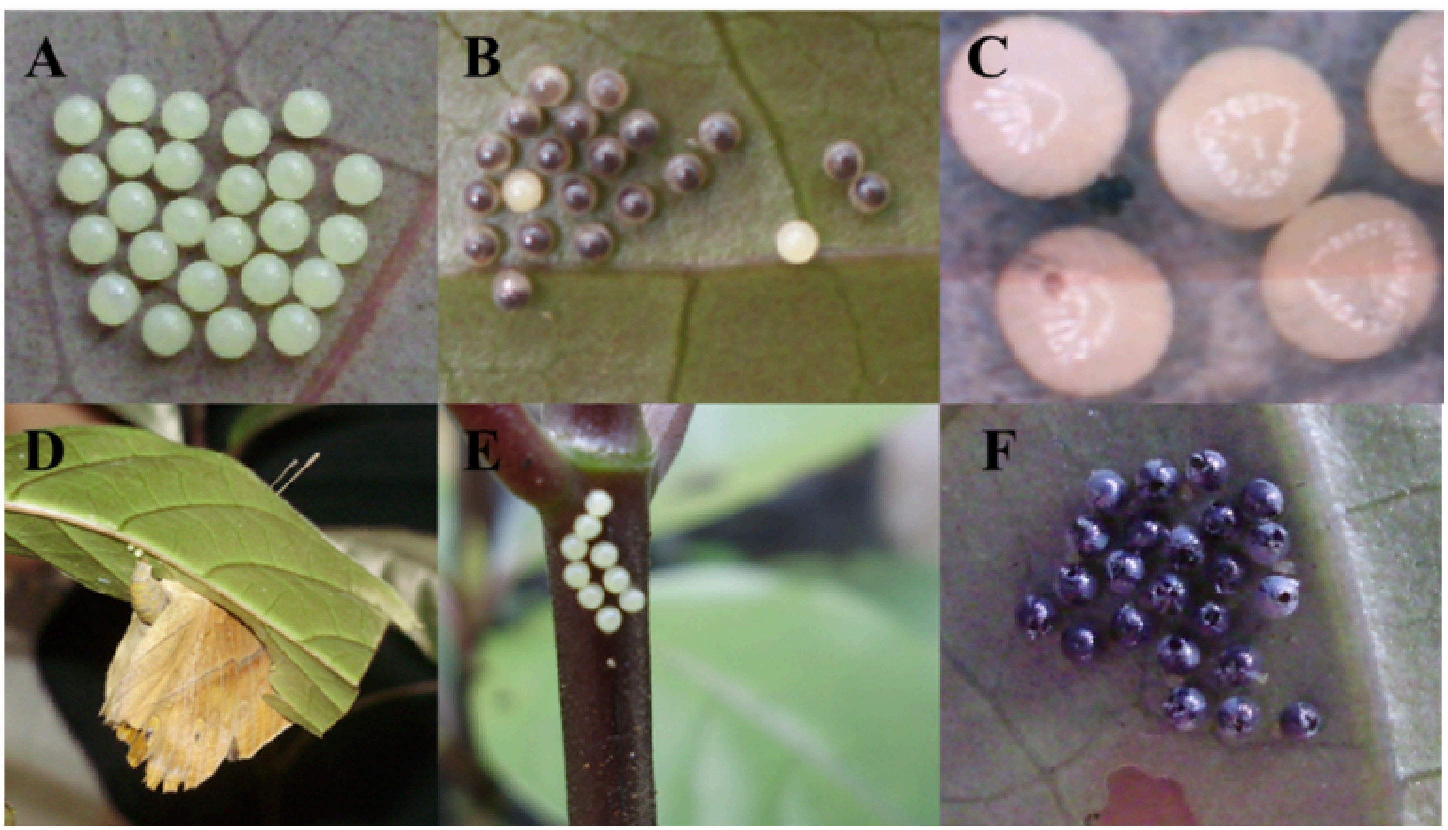
# Figures

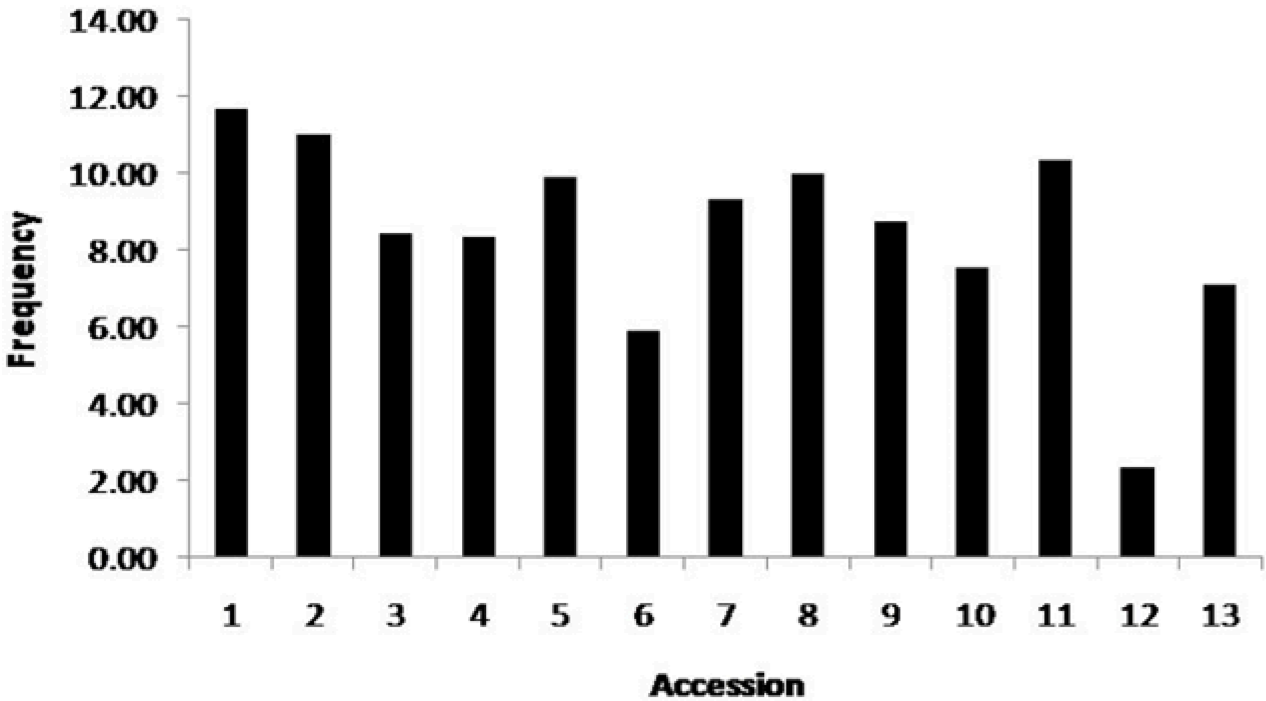

### Figure 1

Characteristics of the adult *D. bisaltide*. Female (B) and male (B) adult of *D. bisaltide*. The arrow indicates the white spots, a characteristic of males that differentiates them from females




### Figure 2

Characteristics of *D. bisaltide* eggs. Newly laid eggs (A), microscopic characteristics of eggs (B), eggs about to hatch (C), a female adult was laying eggs on the undersurface of leaves (D), a cluster of eggs laid on stems (E), and eggs that failed to hatch (F)




## Figure 3

Frequency of *D. bisaltide* visits in 13 *G. pictum* accessions


## Figure 4

Development of *D. bisaltide* larvae and pupae. Neonate (A), first instar larvae (B), second instar larvae (C), third instar larvae (D), fourth instar larvae (E), fifth instar larvae (F), prepupa (G), early pupa (H), pupa (I), and newly adult came out from pupae (J) of *D. bisaltide*

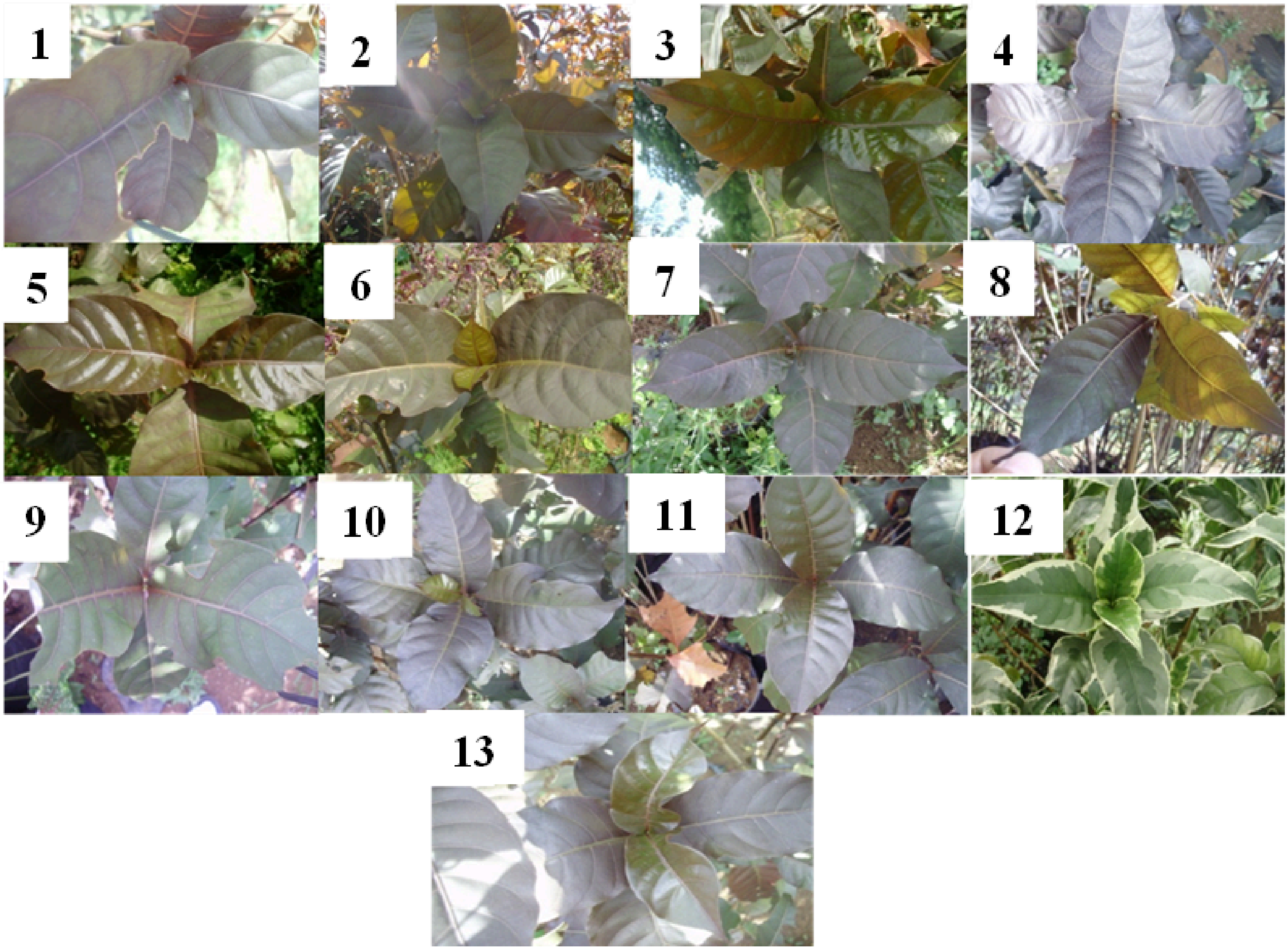



**Figure 5**

Leaf morphology of *G. pictum*. Numbers 1-13 mean *G. pictum* accession à angka pada gambar diganti fonnta dg Helvetica/Arial