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EFEFCT OF DIASPIDIDAE (HEMIPTERA) PEST ON CHLOROPHYLL CONTENT AND PLANT HEALTH IN VANILLA

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ABSTRACT

Vanilla plants are one of the crops that produce expensive spice and vanilla extract, or vanillin. A study of insect populations on vanilla plants was carried out at Kairos Plantation, Penang, Malaysia to determine the influence of the insect scale of the family Diaspididae on the photosynthetic pigment such as chlorophyll content in the vanilla leaves. A systematic collection of vanilla leaves to ensure that each leaf measured between 19 and 21 cm in length across two different vanilla species, (*Vanilla tahitensis* and *V. planifolia)*. The leaves were meticulously gathered from the lower, middle, and top parts of the plant, followed by a detailed recording of the insects found on each leaf. The family Diaspididae also known as scale insects was identified as the primary herbivorous pest damaging the vanilla leaves. The lower part of *V. tahitensis* and *V. planifolia* leaves had more than 100 individuals of diaspidid and infestation on all studied leaves (level 5) and the severity was the greatest. There was a significant negative correlation between the height of vanilla plants and with infestation intensity of scale insects, r=-0.776, *P*=0.00. Vanilla plants that have been infested with scale insects showed loss of chlorophyll and carotenoids at different parts of the plant's height. Chlorophyll a, b and total chlorophyll content at the lower part of the leaves recorded the lowest values compared to the upper part of both vanilla species with significant results of chlorophyll a (F=22.87, *P*=0.002) and total chlorophyll (F=14.32, *P*=0.000). The photosynthetic pigments content in vanilla plants, namely chlorophyll a, chlorophyll b, and total chlorophyll, was highly influenced by the level of infestation and plant height. This shows that scale insect infestation had given negative impact on chlorophyll loss of the vanilla leaves in response by the plants to survive.

Keywords: Bugs, pests, scale insects, vanilla plant

ABSTRAK

Tanaman vanila adalah salah satu tanaman yang menghasilkan rempah yang mahal dan ekstrak vanila atau vanillin. Kajian populasi serangga pada tumbuhan vanila telah dijalankan di Ladang Kairos, Pulau Pinang, Malaysia untuk menentukan pengaruh serangga teritip famili Diaspididae terhadap pigmen fotosintesis seperti kandungan klorofil pokok vanila. Daun vanila dikutip secara sistematik dengan memastikan setiap daun berukuran antara 19 dan 21 cm panjang untuk kedua-dua spesies vanila (*Vanilla tahitensis* dan *V. planifolia*) Daun dikumpulkan dengan teliti dari bahagian bawah, tengah dan atas tumbuhan, diikuti dengan merekodkan secara terperinci serangga yang terdapat pada setiap daun. Serangga daripada famili Diaspididae yang juga dikenali sebagai serangga teritip dikenal pasti sebagai perosak herbivor utama yang merosakkan daun vanila. Bahagian bawah daun *V. tahitensis* dan *V. planifolia* menunjukkan lebih daripada 100 individuals dan kesan serangan pada semua daun (tahap 5) dan tahap keterukan adalah yang paling teruk. Terdapat korelasi negatif yang signifikan antara ketinggian pokok vanila dengan intensiti serangan serangga teritip, r=-0.776, *P*=0.00. Tumbuhan vanila yang telah diserang serangga teritip menunjukkan kehilangan klorofil dan karotenoid pada bahagian ketinggian tumbuhan yang berlainan. Keterukan serangan Diaspididae berkorelasi negatif dengan ketinggian tumbuhan (*P*<0.01). Klorofil a, b dan jumlah klorofil di bahagian bawah daun mencatatkan nilai terendah berbanding bahagian atas kedua-dua spesies vanila dengan keputusan ketara klorofil a (F=22.87, *P*=0.002) dan jumlah klorofil (F=14.32, *P*=0.000). Kandungan pigmen fotosintesis dalam tumbuhan vanila iaitu klorofil a, klorofil b dan jumlah klorofil sangat dipengaruhi oleh tahap serangan dan ketinggian tumbuhan. Ini menunjukkan bahawa serangan serangga teritip memberi kesan kepada kehilangan klorofil daun vanila akibat tindak balas tumbuhan untuk terus hidup.

Kata kunci: Pepijat, perosak, serangga teritip, tumbuhan vanila

INTRODUCTION

Vanilla is one of the most popular spices in the world, second only to saffron. The quality and quantity of vanilla plants are important for the global market. Vanilla is a worldwide trade and brings impacts economically on vanilla-producing countries. Madagascar has been the primary vanilla bean producer for the past years, followed by Indonesia, and Mexico (FAO 2020). Malaysia had its first smart vanilla farm that utilized the greenhouse concept in 2019 by Kairos Agriculture (Kairos Agriculture 2020). Vanilla farming presents certain difficulties and restrictions. A study conducted by Borbolla-Pérez et al (2017), revealed that both external and internal factors played a role in influencing the quality and quantity of vanilla production. These farmers, who are relatively new to the industry, often lack the necessary experience and training to optimize crop yields, encounter processing issues, and struggle with limited networking systems, which in turn hinder their ability to market their products effectively.

One of the problems faced by the vanilla farmers is insect infestation. The infestation of vanilla plants by Diaspididae scale insects, acting as herbivorous pests, is influenced by various factors. Vanilla plants, from the Orchididae family's susceptibility to scale insect attacks, where they feed on phloem sap by puncturing plant leaves, facilitate their establishment in areas rich in nutrients in vanilla plantations (Volf 2020). Diaspididae, the tough-shelled scale insects, exhibit high reproductive rates, making it difficult to control their population (Camacho & Chong 2015; Kakoti et al. 2023).

Plant overcrowding and the mobility of scale insects as crawlers promote their colonization of plant hosts, especially when plants are densely clustered (Kakoti et al. 2023). These crawlers can passively travel considerable distances via wind currents, landing on new plants and initiating infestations in various locations (Camacho & Chong 2015). Family Diaspididae is known to infest 36 plant families, including citrus, ornamental plants, and families like Moraceae, Asparagaceae, Arecaceae, and Rutaceae (Martins et al. 2022). Due to their significance as pests, there is a substantial interest in studying this group of insects, especially in nations involved in the export and import of unprocessed agricultural products (Martins et al. 2022). One critical factor influencing the interaction between these insects and host plants is the amount of chlorophyll present in the plant tissues (Golan et al. 2015). These herbivorous insects are known for their ability to feed on a wide range of agricultural and ornamental plants (Martins et al. 2022). They use their piercing mouthparts to extract sap from various parts of the host plant (Lakshmishree et al. 2019). This feeding activity can result in both direct damage to the plant and indirect harm, as it involves the secretion of honeydew and the potential transmission or promotion of the growth of plant pathogens (Golan et al. 2015). Pests in vanilla plants have caused low production due to diseases and a lack of knowledge of insect fauna. In a study by Borbolla-Pérez et al. (2017), the red bug (*Tentecoris confusus*) is the most frequently encountered pest on vanilla plants as this bug creates wounds on leaves that make them more susceptible to fungal diseases. This contributes to the farmer's struggle to control pests without disturbing beneficial insects and lack of proper pest management plans. This factor contributes to the quality and quantity of the vanilla product that is being produced.

This study was conducted to determine the influence of the insect scale of the family Diaspididae on the photosynthetic pigment such as the chlorophyll content of the infested vanilla plant. The information offers insights into plant-insect interactions, aiding for future effective management planning for the vanilla plantation, and ultimately supporting its growth and expansion in our country.

MATERIALS AND METHODS

Study Area

Kairos Vanilla Plantation was chosen for its availability as the only registered vanilla plantation in the northern part of Malaysia. This plantation is located in Kubang Semang, Penang, Malaysia covers six acres of land with its primary crop being vanilla (*Vanilla tahitensis* and *Vanilla planifolia).* These two vanilla plants aged five years old were planted individually with a 2-meter PVC act as the pillar for support inside the shade house. The plant substrates used for this plant were coconut husk and soil.

Pest Infestation on Vanilla Leaves

Leaves with lengths of 19 to 21 cm were collected for each vanilla species. Three leaves were collected at the lower, middle, and upper parts of the plant, respectively. The leaf in the lower part was collected from the mulch until 0.5m in height, and the middle part was set from the end of the lower part stretched up until 0.5m, while the upper part continued from the end of the middle part up until its shoot, approximately 0.5 m. This collection was repeated on five vanilla plants. A total of 30 pieces of leaf samples (*V. tahitensis* and *V. planifolia*) were collected for each sampling visit and placed inside a plastic container for examination at a laboratory. The sampling was repeated for three consecutive months.

The leaves were examined for any infestation signs such as the exuviae and any biting marks of scale insects. The frequency of pit or exuviae was counted using the quadrat method (1 cm x 1 cm) on the leaf. The characteristics of the exuviae/ pit then were used to identify the infested pest by referring to Makale et al. (2020). The infestation intensity percentage formula was as follows (Al-Egaili et al. 2020):

$$\frac{\text{Number of infested leaves}}{\text{Total number of leaves}} X\ 100$$

The density of Diaspididae infestations on vanilla leaves was categorized based on the number of individuals present on a single leaf (Table 1) and the grade of occurrence and infestation level (Table 2).

Table 1. Level of Diaspididae density infesting vanilla leaves (Golan et al. 2015)

Level	Range (Individual)	Indicator
5	>100	Severely infested
4	51-100	Highly infected
3	31-50	Moderate infested
2	11-30	Low infested
1	1-10	Non-infested
0	0	Control

Insect Species Identification

Collected vanilla leaves were placed in plastic jars, and brought to the laboratory for further identification. Insects were observed under a compound microscope and the pest population found was recorded and counted during the observation. The insects obtained were identified using taxonomic keys referring to Johnson and Triplehorn (2005) and Makale et al. (2020). Observation of attack symptoms was carried out directly by observing the presence of pests on plant parts followed by Ginting et al (2023).

Sample Preparation for photosynthetic pigments

The values of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid were measured from the vanilla leaves followed by Uddain (2016). A leaf segment weighted 0.1 gm was collected from lower, middle and upper vanilla plant species (*Vanilla tahitensis* and *Vanilla planifolia*) and ground with one gm of calcium carbonate ($CaCO_3$). Twenty-five ml of 80% acetone was added and evenly mixed. Then the mixture was filtered using filter paper and the collected filtrate was used to determine the photosynthetic pigment content. Samples were measured at wavelengths of 649 nm and 665 nm for chlorophyll a and b content, respectively and at 440 nm for carotenoid content using the Shimadzu UV mini–1240 spectrophotometer. Total chlorophyll and carotenoid were determined and following these equations:

C_a= Chlorophyll a (µg/mL) = 11.75 (Abs_{665})- 2.35 (Abs_{649})
C_b= Chlorophyll b(µg/mL) = 18.61 (Abs_{649}) – 3.96 (Abs_{665})
Total chlorophyll = $C_a + C_b$

The equation of carotenoid content was determined by using the absorbance at 440 nm.

Carotenoid content (µg/g) = $\frac{A \times V\ (mL) \times 10^4}{A_{1\ cm}^{1\%} \times P(g)}$

A= Absorbance
V= Total volume of extract
P= Sample weight

The same method was repeated on leaves from vanilla plants (both species) that are non-infested with insects as control.

Data Analysis

One-way ANOVA with Tukey's test was used to analyse the Diaspididae infestation with chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content. Pearson correlation was used to investigate the relationship between the Diaspididae infestation level, plants' height, and chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. All statistical analyses were accomplished by using the Statistical Package for Social Science (SPSS) version 27.

RESULTS

All vanilla plants reported Diaspididae infestation, with a higher occurrence observed in the lower parts of the plants. Family Diaspididae primarily affected the lower section of *Vanilla planifolia* plants, where 14 leaves exhibited infestations exceeding 100 individuals (Level 5) on the leaves. In the middle part of these plants, seven leaves showed infestations, but none were detected on the upper leaves (Table 2). In *V. tahitensis*, 15 leaves displayed infestation exceeding 100 individuals on the leaves, indicating the highest level of infestation severity (Table 3). Among these samples, nine had over 100 infestation exuviae, four leaves had infestations ranging between 51 and 100 individuals (Level 4), and one leaf had infestations ranging from 11 to 30 individuals (Level 3). In contrast, another leaf had infestations ranging from 1 to 10 individuals (Level 1).

Table 2. Infestation level of Diaspididae insect on *Vanilla planifolia*'s leaves based on its division (lower, middle, upper)

Plant Part	**Infestation level**					
Lower / No. of leaves	0	1	2	3	4	5
	0	0	0	0	1	14
Plant Part	**Infestation level**					
Middle / No. of leaves	0	1	2	3	4	5
	0	3	3	0	2	7
Plant Part	**Infestation level**					
Upper / No. of leaves	0	1	2	3	4	5
	15	0	0	0	0	0

Table 3. Infestation level of Diaspididae insect on *Vanilla tahitensis*'s leaves based on its division (lower, middle, upper)

Plant Part	**Infestation level**					
Lower / No. of leaves	0	1	2	3	4	5
	0	0	0	0	0	15
Plant Part	**Infestation level**					
Middle / No. of leaves	0	1	2	3	4	5
	0	0	1	1	4	9
Plant Part	**Infestation level**					
Upper / No. of leaves	0	1	2	3	4	5
	15	0	0	0	0	0

In *V. tahitensis,* photosynthetic pigments such as chlorophyll a, b and total chlorophyll recorded the lowest value on leaves at the lower part of the plant followed by middle and upper leaves (Table 4). A similar pattern of results was also recorded from *V. planifolia* (Table 5). However, the middle leaves of the *Vanilla planifolia* showed the greatest values of carotenoids. One-way ANOVA was conducted to analyse the mean photosynthetic pigments value at three different parts of the plant. Results showed there was a significant effect on the chlorophyll a [F= 22.837, P = 0.00], and total chlorophyll [F = 14.319, P = 0.000] only by the scale insects. Statistical analysis continues using Pearson correlation analysis to see the relationship between the level of scale insects attack and the photosynthetic content. Results showed a significant negative correlation between the Diaspididae with chlorophyll a (r = -0.849), Chlorophyll b (r= -0.542) and total chlorophyll at r = -0.800 for both vanilla species.

Table 4. Mean (±SE) of photosynthetic pigments content of *Vanilla tahitensis*

Plant Part	**Photosynthetic Pigments**			
	Chlorophyll a	**Chlorophyll b**	**Total Chlorophyll**	**Carotenoid**
Lower	0.519±0.139	0.556±0.279	1.074±0.143	24.557±4.986
Middle	1.316±0.094	0.744±0.021	2.060±0.115	48.631±3.972
Upper	1.964±0.240	1.078±0.461	3.042±0.654	56.204±6.942
Control	2.0147±0.125	1.046±1.410	3.204±1.520	59.474±4.147

Table 5. Mean (±SE) of photosynthetic pigments content of *Vanilla planifolia*

Plant Part	**Photosynthetic Pigments**			
	Chlorophyll a	**Chlorophyll b**	**Total Chlorophyll**	**Carotenoid**
Lower	0.615±0.144	0.743±0.084	1.358±0.221	31.533±17.452
Middle	1.591±0.19	1.240±0.190	2.831±0.509	50.269±5.542
Upper	1.766±0.223	1.460±0.140	3.226±0.206	36.253±7.885
Control	2.012±1.210	1.541±0.222	3.314±1.710	37.517±3.140

DISCUSSION

A few variables affected the effectiveness of Diaspididae's colonization as a herbivorous pest on vanilla plants. First off, vanilla plants are native to Mexico and are members of the Orchididae family. Jennifer et al. (2012) stated that plants in the Orchididae family are susceptible to being attacked by the scale insects, which puncture the leaves of the plant to feed on the phloem sap (Volf 2020), which aids in the insects' effective establishment at vanilla. According to Camacho and Chong (2015), scale insects of the Diaspididae family are hard-scale insects with great fecundity. Because of their fast rate of reproduction, which results in several overlapping generations annually, it isn't easy to manage the population (Kakoti et al. 2023). According to Martins et al. (2022), the Diaspididae have been discovered to infest 36 host plants including the Rutaceae, Asparagaceae, Arecaceae, citrus, and ornamental plant families. The presence of scale insects from the family Diaspididae on vanilla plants and its high prevalence levels indicate that the insect was probably introduced some years before this report. This scale insect incidence is quite similar to the presence of mealybugs, *Rastrococcus tropicasiaticus* (Hemiptera: Pseudococcidae) reported in Bengkulu, Indonesia by (Zarkani et al. 2021).

Results from this study showed the lower part of the plants has the most intense Diaspididae infestation, whilst the upper part of the plants is free of infestation. This preference may be attributed to varying nutrient levels in different plant regions (Al-Egaili et al. 2020). The lower parts have more sucrose (sugar), as this part accumulates higher sugar content through phloem transport, which stores sucrose in the roots (Hennion et al. 2019) and it is the preferred diet by the scale insects (Volf 2020). The higher humidity near the coconut mulch and soil makes them more prone to scale insect infestation (Chang et al. 2008). As plants grow taller, the relative humidity decreases due to increased sun exposure. This supports the hypothesis that herbivorous damage may reduce photosynthesis. The number of insects on vanilla plants varies depending on favourable conditions and adequate food with insects consuming nutrients in optimal amounts to maintain a balanced diet and enhance their fitness for survival (Córdoba-Aguilar et al. 2018). Furthermore, Diaspididae deposit their eggs beneath their bodies, and when they hatch, the young nymphs disperse to locate their feeding spot (Gullan et al. 2003). The upper part of vanilla plants typically has more abundant foliage compared to the middle and lower parts. Under specific circumstances, plants can withstand pests by activating their defence mechanisms (Golan et al. 2015). As plants grow, their photosynthesis becomes more intense, allowing them to counteract the adverse effects of Diaspididae's plant feeding (Golan et al. 2015).

Plants can withstand the presence of pests under some circumstances and due to induced defense (Golan et al. 2015). According to Golan et al. (2015), as plants mature, their intensity of photosynthetic processes rises, allowing them to counteract the detrimental impacts of phytophagy by Diaspididae. Assessing the amount of carotenoid and chlorophyll can be a useful way to assess pest or disease infestations that affect plant health and productivity (Cárdenas & Gallardo 2016). The infestation was observed in indirect methods by analyses of the photosynthetic pigments such as chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content since the photosynthetic tissue isn’t removed from the plant. This is following the research done by Golan et al (2015), and Huang et al (2014). Both reported sap-sucking herbivorous insects cause damage that was associated with chlorophyll loss, and the intensity depends on the abundance and life stage of insects present on the plants. Damage caused by herbivores has decreased the photosynthetic area of a plant resulting in a reduction of the overall pool of resources that plants have for growth and reproduction (Jacobsen & Raguso 2021). The results show that the high intensity of Diaspididae’s infestation had caused a significant decline value in the content of chlorophyll a, chlorophyll b and total chlorophyll compared to the middle part and upper part of the plants. One of the primary factors regulating the relationship between insects and their host is thought to be the amount of chlorophyll in plant tissue (Golan et al. 2015). Golan et al. (2015) also reported that scale insects have caused a great decrease in chlorophyll b content in ferns as well.

CONCLUSION

The research concluded that scale insects (family Diaspididae) have been identified as the most herbivorous pest that causes injury to *V. planifolia* and *V. tahitensis* leaves. Scale insect infestation is significantly correlated with plants' height; as the height of the vanilla plants increases, the severity of infestation by the scale insect decreases. Scale insect's presence had affected the content of photosynthetic pigments in vanilla plants, particularly chlorophyll a, chlorophyll b, and total chlorophyll. However, the intensity of infestation doesn't impact carotenoid levels. Lower content of chlorophyll in vanilla leaves was observed at the greatest density of the scale insect. The data recorded in this study provide valuable insight into the insect fauna associated with vanilla plants and enhance our understanding of insect pest management.

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My manuscript has no associated data.

Authors' Contributions
Suhaila Ab Hamid (SAH) created this research and designed experiments; Nur Hanani Zolkifli (NHZ) and SAH participated in the design and interpretation of the data; NHZ performed experiments and analysis. All authors read and approved the final manuscript after critical review.

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