

Investigating the Effects of Bamboo Vinegar as An Organic Pesticide on Insect Pests and the Nutrient Content of Harumanis Mango (MA128), *Mangifera indica* L.

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ABSTRACT

Chemical pesticides have been widely used in plantations, and their effects have more disadvantages to the environment as well as to humans. Therefore, this study tries to implement organic pesticides using bamboo vinegar. Bamboo vinegar is one of the organic pesticides to control insect pests in plantation crops. The role of this organic pesticide on the insect pests of Harumanis mango is still unknown. Hence, this study aims to determine the impact of applying bamboo vinegar on the insect pests, quality, and nutrient content of Harumanis mango (MA128). Bamboo vinegar was applied in February 2021 during the flowering phase at the Harumanis plot in the Plantation Unit, Universiti Teknologi MARA Perlis. This study used a randomized complete block design with three treatments (spraying intervals) and three replications: T1 (3-day interval), T2 (5-day interval), and T3 (no

bamboo vinegar). Sticky traps were installed to determine the population of insect pests in Harumanis mango. Matured fruits have been harvested, and the standard procedure of Harumanis mango postharvest handling has been followed. Then, the fruits were determined for total phenolic content (TPC), total flavonoid content (TFC), vitamin C, and sugar content. Bamboo vinegar significantly reduced the abundance of insects in Harumanis mango. Additionally, the highest TPC was observed from a 3-day interval of bamboo vinegar application. A

ARTICLE INFO

Article history:

Received: 24 August 2023

Accepted: 23 November 2023

Published: 22 July 2024

DOI: <https://doi.org/10.47836/pjtas.47.3.01>

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similar trend was indicated for improving TFC with bamboo vinegar application. Similarly, there was a significantly higher vitamin C and sugar content after bamboo vinegar application compared to the control group. In conclusion, applying bamboo vinegar to Harumanis mango reduced insect pests and increased the quality and nutrient content of the Harumanis mango.

Keywords: Bamboo vinegar, flavonoid, nutrient content, sticky trap, vitamin C

INTRODUCTION

Demand for healthy fruit has promoted farmers to produce products free from any chemical synthetics such as fungicides, pesticides, and weedicides that may affect the environment and human health (Meena, 2015). The use of organic pesticides to replace synthetic chemicals is an initiative to minimize the use of chemical products in agriculture management (Al-Ani et al., 2019). Bamboo vinegar is an organic product with various functions that may help farmers control insect pests. It can also be used as an organic foliar fertilizer. The liquid form of bamboo vinegar is extensively utilized in organic farming, forestry, floristry, horticulture, animal husbandry, and human healthcare. It contains a variety of organic elements, including organic insecticides effective at preventing pest infestations, fostering plant growth, improving nutrient uptake, lowering fertilizer use, and encouraging compost generation (Alias et al., 2020).

Due to rising demand and bamboo's adaptability as a source of wood products, production has expanded today. In Malaysia, *Gigantochloa albociliata*, sometimes referred to as Buluh Madu, is a bamboo species that frequently reaches its highest size (Zhu et al., 2021). This bamboo is typically pyrolyzed to make charcoal, vinegar, tar, and other goods. This bamboo helps produce organic products such as vinegar for pest control and fertilizer (Yusoff et al., 2021). Bamboo vinegar is a by-product of condensed acidic bamboo carbon liquid obtained during the manufacturing of bamboo charcoal. It has a distinct smoky aroma and a pale yellow to brown tint. Bamboo vinegar contains over 200 chemical components, such as organic acids, phenolic, alkane, alcohol, and ester compounds (Mu et al., 2006).

Agriculture-related sectors are crucial for supplying food for human and animal consumption. In Malaysia, Harumanis mangoes (MA128), scientifically named *M. indica* L., is one of the most famous mango varieties due to its aromatic, sweetness, fragrance, and price. The Department of Agriculture Malaysia has registered this mango as an MA128 variety clone (Mahmood et al., 2011). Additionally, this crop has been commercialized in Perlis, Malaysia, where the geography is ideal for fruit production in a hot and dry climate. Uda et al. (2020) state that hot weather and precipitation will impact flowering patterns, fruit formation, and fruit development. Harumanis mango will only bear fruit once a year as a seasonal crop with proper planning

and maintenance. The skin is bright green with prominent light-yellow specks, and the fruits are still green, even though they are matured (Peng & Christian, 2005). It has thick yellow pulp with no fiber, is soft, and has high water content. The weight of Harumanis fruit can be found from 300 to 700 g, with Brix readings ranging from 14° to 18°Bx (Mahmood et al., 2011). The Harumanis tree is cultivated for its edible flesh fruit and is a good source of polyphenols, vitamin A, and vitamin C (Talib et al., 2020).

Aziz et al. (2020) reported that the fruit has a high total soluble solids (TSS) value based on the sugar solution in mango juices. The effects of bamboo vinegar on plant growth and phytochemical content were widely evaluated on other agricultural plants but lacking on mango, especially on Harumanis mango. Besides, according to Meena et al. (2015), organic products increase the nutritional value of vitamin and mineral content. Furthermore, the vitamin C content of tomatoes was enhanced after spraying with bamboo vinegar (Yao et al., 2012). In addition, the root length, plant height, living biomass, and chlorophyll content of tomatoes were increased with bamboo vinegar application compared to without spraying. Although research has indicated bamboo vinegar improves growing media properties and plant growth on crops, the effects of bamboo vinegar on the nutrient content of Harumanis mango have not been reported. Thus, this study was conducted to evaluate the effect of bamboo vinegar on insect pests, nutrient content, and quality of Harumanis mango (MA128).

MATERIALS AND METHODS

Sampling Procedures

This experiment was conducted at the Harumanis mango planting site at the Plantation Unit, Universiti Teknologi MARA, Perlis. Harumanis crop was planted in 2015 and is 6–7 years old. Bamboo vinegar treatment has been applied during the flowering season, from 14th February 2021 until 1st April 2021. This study used a randomized complete block design (RCBD) with three treatments (spraying intervals), three replications, and four experimental units. Harumanis mango trees were labeled with three different colors of tape to differentiate them according to their treatment: green (3-day intervals), red (5-day intervals), and yellow for control. The yellow sticky trap was installed a day before and after spraying to observe the insect abundance after being treated with bamboo vinegar. Yellow color has been chosen for this study due to the color preferences of insects (Bae et al., 2019). The population of insects has been identified up to order levels by referring to Borrow and Delong's book (Luqman et al., 2018).

Bamboo Vinegar Preparation and Application

The concentrated bamboo vinegar (100%) from Tadam Eco Living Sdn. Bhd. (Malaysia) has been used in this study. The 20% bamboo vinegar concentration was prepared by diluting it with water in a ratio of 1:5 for this experiment. A total of 200 ml bamboo vinegar concentration was

prepared for 10 L of water. The spraying process was conducted early morning using a knapsack sprayer, sprayed fully on Harumanis trees. The treatments have three different time intervals of bamboo vinegar application: 3 days (T1), 5 days (T2), and without applied bamboo vinegar (control). After treatment, the fruits were covered with carbon wrapping bags to protect them until harvest. After fruits fully matured, they were harvested and underwent postharvest treatment. Then, the fruits were analyzed for their nutrient content.

Harumanis Mango Extraction

The ripened Harumanis mango have been selected for nutrient analysis following Oviasogie's method (Rahman et al., 2007). The fruits were analyzed for TPC, TFC, vitamin C, and TSS. Mango fruit has been cleaned, peeled, and cut into slices for extraction to separate a particular chemical from a larger complex compound. The fruit samples of each treatment were homogenized using mortar and pastel, recorded weight, and fruit samples required to be macerated in methanol for 24 hr at room temperature. The extracts were filtered under vacuum conditions, and the residue was repeatedly extracted with the same solvent until colorless. A rotary evaporator was used to remove methanol from the extract solution.

Determination of TPC

The TPC of fruits was evaluated by drawing a calibration curve using gallic acid as the standard of reference. A test tube containing

9 ml of deionized water was filled with 1 ml of sample and 1 ml of a standard gallic acid solution (R&M Chemicals, Malaysia). Deionized water was also used to prepare the blank. The Folin-Ciocalteu reagent (R&M Chemicals, Malaysia) has been added to the solution and stirred with a value of 1 ml. The solution was then mixed with 10 ml of 7% sodium carbonate (R&M Chemicals, Malaysia) and incubated for 2 hr. A UV-visible spectrophotometer with a wavelength of 760 nm has been used to determine the absorbance of the TPC (Rahman et al., 2007).

Determination of TFC

TFCs were determined using a quercetin standard curve following the method of Mahmood et al. (2011). Flavonoid content has been determined using a UV-visible spectrophotometer. A sample with a value of 1 ml was added and mixed with 3 ml of 95% ethanol (R&M Chemicals, Malaysia) in a test tube. Then, 0.2 ml of 10% aluminum chloride (R&M Chemicals, Malaysia) was mixed and left for 5 min. Then, 0.2 ml of potassium acetate (R&M Chemicals, Malaysia) and 5.6 ml of deionized water were added and made up to 10 ml. A UV-visible spectrophotometer was used to measure the solution's absorbance at 415 nm after it had been incubated at room temperature for 30 min.

Determination of Vitamin C Content

All samples of fruits were properly washed with deionized water before the

extraction procedure to remove any cling impurities. Five (5) g of material was carefully weighed and pulverized in a mortar and pestle after being treated with 10 ml of 4% oxalic acid (R&M Chemicals, Malaysia). The mixture was then crushed and strained four times through muslin fabric. In a standard flask, the extract was prepared up to 25 ml with 4% oxalic acid (R&M Chemicals, Malaysia), and all the samples were processed similarly. The 2, 6-dichlorophenol indophenol (DCPIP) titration method calculated the ascorbic acid content of mangoes. In preparation for the standard solution, 5 ml of ascorbic acid (R&M Chemicals, Malaysia) working standard (500 µg/5 ml) and 10 ml of 4% oxalic acid (R&M Chemicals, Malaysia) were pipetted into a tube bottle. The contents of the tube bottle were titrated against the dye solution (DCPIP) V1 until a faint pink color appeared, which lasted a few minutes. Similarly, 5 ml of the test sample was titrated against the dye solution (DCPIP) V2 (Sani et al., 2018). The ascorbic acid content present in the test samples was determined using the formula:

$$\text{Amount of ascorbic content (mg/100 g)} \\ = \frac{500 \times V2 \times 25 \times 100}{V1 \times 5 \times 5}$$

where, V1 = Amount of dye consumed by 500 µg of standard; V2 = Amount of dye consumed by 5 ml of a test sample; 25 = Total volume of the extract; 100 = Ascorbic acid content/100 g of the sample; 5 = Weight of sample taken; 5 = Volume of the test sample taken for titration.

Determination of Sugar Content

The TSS, or sugar content, measures the fruit's carbohydrates, organic acids, proteins, lipids, and minerals. The sugar content of Harumanis fruits was determined using Sani's method (Mahmood et al., 2011). It is determined by measuring the fruit's Brix degrees. A volume of 1 ml from homogenized fruits was determined for TSS value using a refractometer.

Statistical Analysis

The data were analyzed using a paired *t*-test to determine the significant effect of bamboo vinegar on the insect pests of Harumanis mango. Analysis of variance (ANOVA) and mean comparison Tukey's test at $P \leq 0.05$ were done to quantify the nutrient content of Harumanis mango after being treated with bamboo vinegar. All the analyses were done by using SPSS software (version 26).

RESULTS AND DISCUSSION

Insect Abundance

Figure 1 shows the total insect abundance before and after being treated with bamboo vinegar. A total of 508 individuals were collected in this study, comprising 5 orders and 11 families. Diptera was the highest insect order, with 229 individuals, and Orthoptera was the least, with 28 individuals. *Ceratitis cosyra* (Walker), *Ceratitis silvestrii*, *Ceratitis quinaria* (Bezzi), and *Bactrocera invadens* are four fruit fly species most commonly found in this study. Vayssieres et al. (2008) reported a comparable outcome with mango trees in

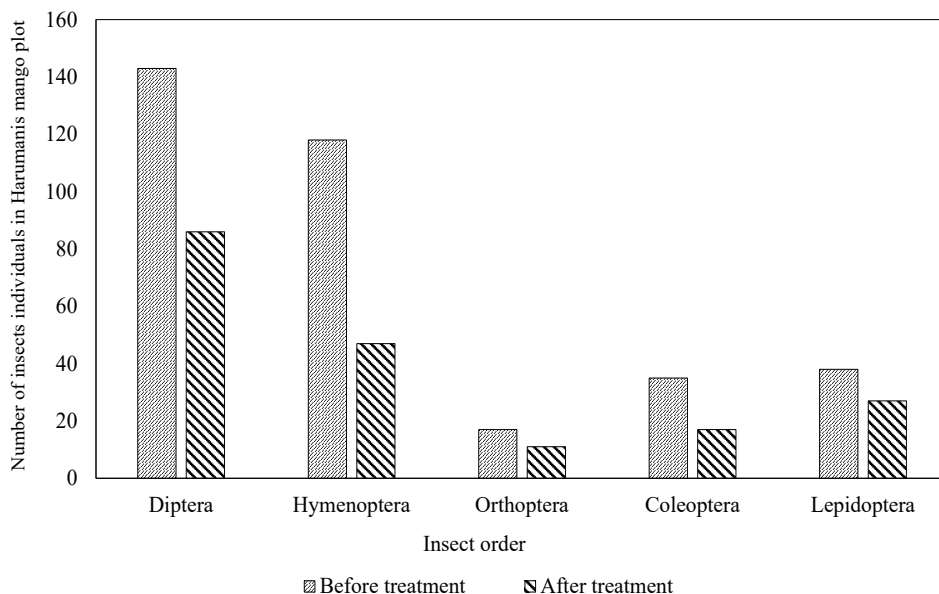


Figure 1. Number of insects collected before and after spraying with bamboo vinegar

Benin, which are affected by temperature, relative humidity, and rainfall. Among 508 individuals, 351 individuals were collected before bamboo vinegar treatment, while 188 individuals were after being sprayed with bamboo vinegar.

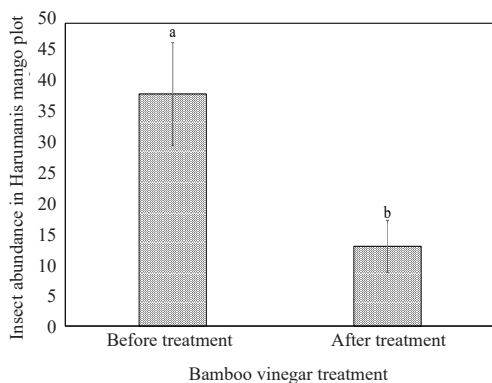


Figure 2. Insect abundance before and after spraying with bamboo vinegar

Note. The a and b letters represent the significant differences using a paired *t*-test at $p < 0.05$. Error bars indicate the standard error of means

Paired *t*-test results indicate that there was a significant difference between sprayed and unsprayed bamboo vinegar on insect abundance in the Harumanis mango plot ($t = 3.56, p < 0.05$) (Figure 2). Peng and Christian (2005) reported that organic treatment using organic insecticides can kill more fruit flies than biological treatment, which is the weaver ant treatment.

Spraying Intervals of Bamboo Vinegar

The 2-sample *t*-test result demonstrates that there was no significant relationship between spraying intervals of bamboo vinegar and insect abundance on Harumanis plot ($t = -1.50, p > 0.05$) (Figure 3). However, 3-day spraying intervals have lower insect abundance even though there was insignificant in which the bamboo vinegar had a better effect on shorter intervals compared to long intervals.

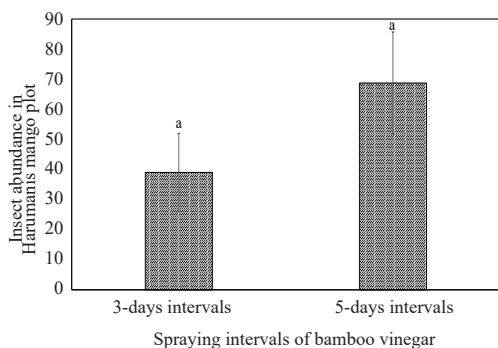


Figure 3. Insect abundance on 3- and 5-days spraying intervals

Note. The letters represent there is no significant difference using a paired *t*-test at $p > 0.05$. Error bars indicate the standard error of means

Nutrient Content of Harumanis Mango Fruit

Total Phenolic Content (TPC)

The Folin-Ciocalteu reagent assay method was used to determine the TPC, and that amount is equivalent to the calibration curve ($y = 0.1159x + 0.1232$, $R^2 = 0.95$).

The TPC of Harumanis mango affected by bamboo vinegar application is shown in Figure 4. In this study, bamboo vinegar application at a 3-day interval (T1) shows significantly higher TPC in Harumanis mango as compared to other treatments. TPC was 13% higher with 3-day intervals (486.02 mg GAE/100 g) than without bamboo vinegar application (422.32 mg GAE/100 g).

The results of TPC obtained from this experiment are within the range (400–700 mg GAE/g) of the previous study conducted by Agatonovic-Kustrin et al. (2018). According to previous studies, the cultivar, crop, and ripening stage all influence the content and properties of phenolic acids (Burton-Freeman et al., 2017). Bamboo vinegar has a beneficial effect on the nutrient content in Harumanis mango fruits after showing improvement

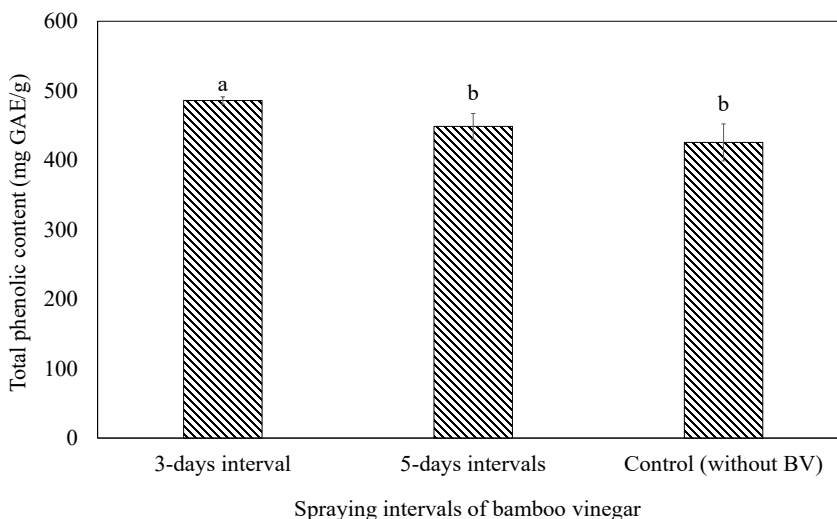


Figure 4. Total phenolic content of Harumanis mango as affected by bamboo vinegar application

Note. The different letters represent the significant differences using one-way ANOVA at $p < 0.05$. Error bars indicate the standard error of means

in TPC. In this study, Harumanis mango harvested from the tree without bamboo application shows significantly lower TPC because the pyroligneous acid (PA) in bamboo vinegar has been shown to increase crop growth, enhance soil quality, and lessen the impact of insect pests and plant diseases (Léchaudel & Joas, 2007). In addition, more than 200 chemical compounds have been found in bamboo vinegar, with organic acids, phenolic chemicals, carbon substances, alcohol, neutral materials, and base acidic substances (Oviasogie et al., 2009). This treatment may affect the growth of Harumanis mango trees and influence the amount of nutrient content in mangoes, where a tree treated with bamboo vinegar shows higher TPC than an untreated tree.

Antioxidants, structural polymers (lignin), attractants (carotenoids and flavonoids), UV filters (flavonoids), signal molecules (flavonoids and salicylic acid), and defensive response substances (tannins and phytoalexins) are all examples of plant phenolic compounds. Phenolics are plants' most prevalent secondary metabolites and the entire metabolic process. These phenolic substances are frequently connected to the defense mechanisms of plants. Increased pollination, color for camouflage, defense against herbivores, and antibacterial and antifungal properties are some additional phenolic metabolite-related functions (Mu et al., 2004).

TFC of Mangoes

The aluminum chloride colorimetric test method was used to calculate the TFC and

the total flavonoid equivalent from the calibration curve ($y = 0.0069x + 0.0149$, $R^2 = 0.98$). As shown in this study, the application of bamboo vinegar has a positive effect on TFC (Figure 5). The highest amount of TPC (36.79 $\mu\text{g}/\text{mg}$ QAE) was obtained from T1 at 3-day intervals after being treated with bamboo vinegar. However, there is no significant difference between the interval of bamboo vinegar application. As from Figure 5, T3 (without bamboo vinegar application) shows a significantly lower amount of TFC (25.37 $\mu\text{g}/\text{mg}$ QAE) compared to other treatments. The total amount of flavonoid was higher by 31% from T1 (3-day interval) than without bamboo vinegar application.

Plant vinegar and wood vinegar are becoming more popular as organic products. These organic products share the same production process as bamboo vinegar. The previous study shows that wood vinegar may prevent stimulating pathogenic fungus growth (Chuaboon et al., 2016). The 2-phenyl-benzopyrene or flavan nucleus, made up of two benzene rings connected by a heterocyclic pyran ring, is flavonoids' most fundamental structural component. Antifungal activity is one of these substances' biological and pharmacological properties (Lashari et al., 2013). The effect of vinegar will enhance phytochemical change and simultaneously become an organic fungicide for plants. This change has affected flavonoid content to ensure plants have an excellent defense to prevent disease in crop growth.

Flavonoids are the polyphenols that are most prevalent in human diets. Flavonoids

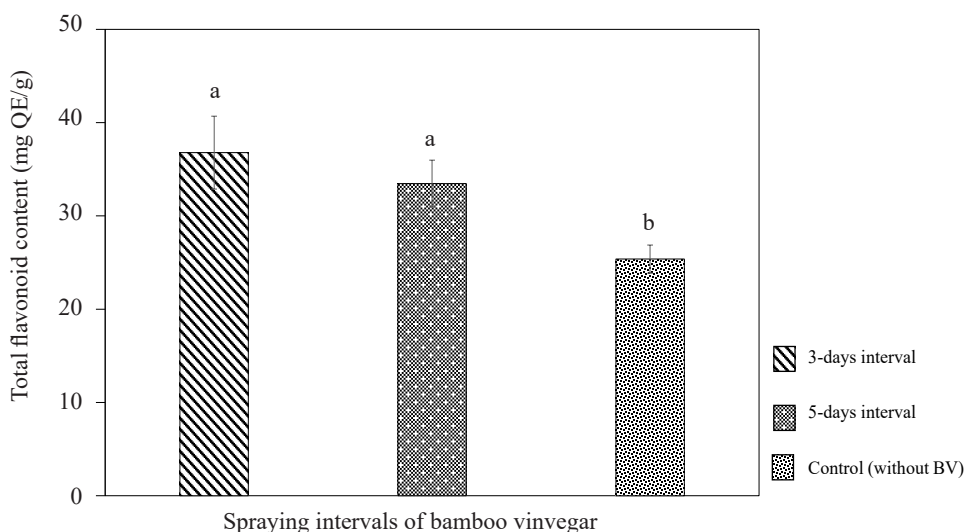


Figure 5. Total flavonoid content of Harumanis mango as affected by bamboo vinegar application

Note. BV = Bamboo vinegar; the different letters represent the significant differences using one-way ANOVA at $p < 0.05$. Error bars indicate the standard error of means

were once considered vitamins and were known by names like vitamin P and vitamin C. Many fruits and vegetables contain quercetin, the most prevalent flavanol in the human diet. Catechin, epicatechin, quercetin, isoquercetin (quercetin-3-glucoside), fisetin, and astragalins are all flavonoids present in mangoes. Quercetins and other flavonoids significantly influence the coloring of various fruits, flowers, and vegetables during ripening. Furthermore, mango peels have more flavonoids than mango pulps (Masibo & He, 2008).

Vitamin C Content

Applying bamboo vinegar increased the vitamin C content in Harumanis mango (Figure 6). A similar trend was observed with TFC, where there was a significantly higher vitamin C content for T1 (3-day interval) and T2 (5-day interval) as compared to

control (without bamboo vinegar). The result shows that vitamin C content in Harumanis mango of T1 (53.33 mg/100 g) was increased by 17% as compared to without bamboo vinegar (45.55 mg/100 g). In addition, there is no significant difference between 3- and 5-day intervals of bamboo vinegar application on TFC in Harumanis mango.

The most significant dietary sources are fruits and vegetables because they contain phytochemicals, including phenol, vitamin C, and flavonoids, which can help maintain good health. These phytochemicals are necessary nutrients that are also found in fruits and vegetables. In this study, the amount of vitamin C in Harumanis mango increased after treatment with bamboo vinegar. This result is supported by a previous study where bamboo vinegar positively increases vitamin C content and

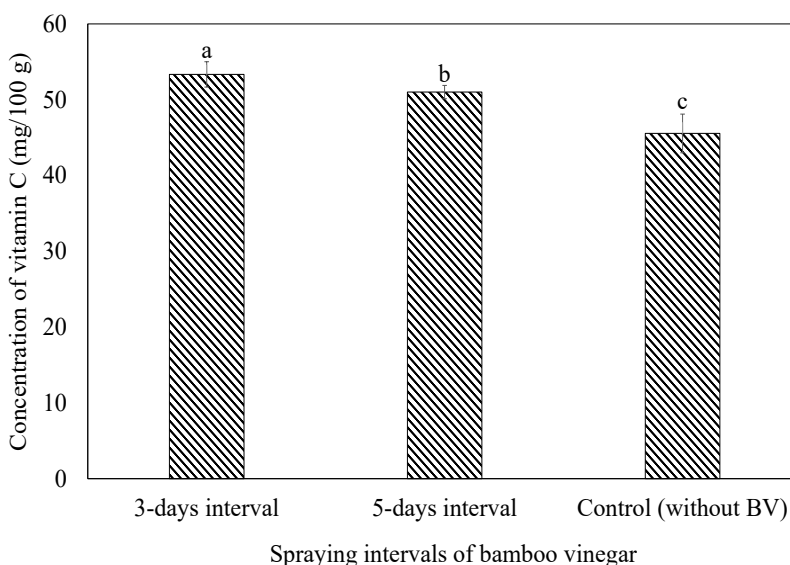


Figure 6. Vitamin C of Harumanis mango as affected by bamboo vinegar application

Note. BV = Bamboo vinegar; the different letters represent the significant differences using one-way ANOVA at $p < 0.05$. Error bars indicate the standard error of means

plant yield. The concentration of bamboo vinegar also plays an important factor, as higher concentrations provide a higher change value of vitamin C (Mun & Ku, 2010). It also stated that bamboo vinegar helps increase plant quality and yield. Plant variety, weather and climate conditions, cultivation technique, production factor, plant growth and health, maturation stage, fruits handled, and storage all influence the quantity of ascorbic acid in fruits (Dar et al., 2016). The benefit of bamboo vinegar is that it promotes plant growth and, at the same time, increases nutrients in Harumanis mango crops.

Sugar Content

The application of bamboo vinegar shows a positive effect on total soluble sugar in

Harumanis mango (Figure 7). Sugar content from T1 (3-day interval) shows a significant difference ($p \leq 0.05$) compared to control (without bamboo vinegar). However, there is no significant difference between T2 (5-day interval) and control. The mean sugar content for Harumanis mango for T1 reaches up to 14.8%, T2 with a value of 14.3%, and T3 with a value of 13.1°Brix (Figure 7).

Mangoes are rich in carbohydrates, with 60% of the fruit containing sugar and acids. Mangoes have a major component that contributes to their sweetness and acidity. The quantity of carbohydrates trees provide is determined by the number of trees created by photosynthetic leaves (Lin et al., 2016). Bamboo vinegar has been used to develop crops, vegetables, and forest plants, and it has been examined. A healthy plant will

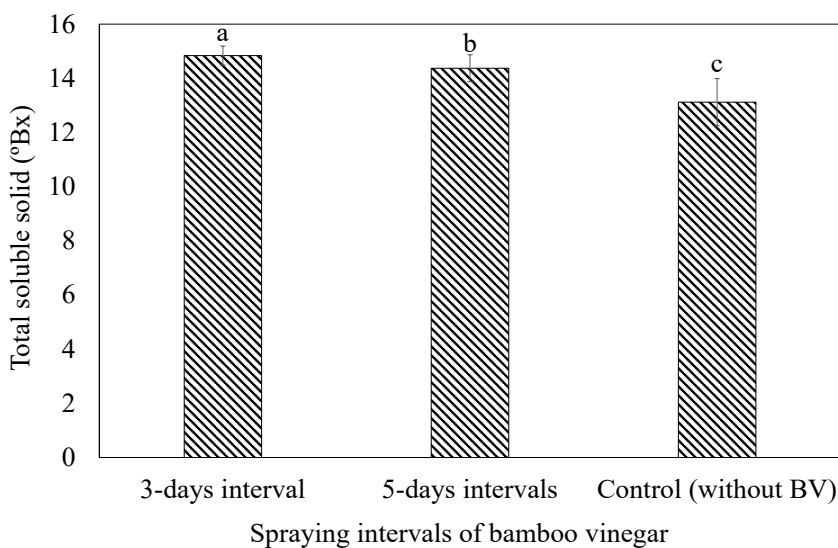


Figure 7. The sugar content of Harumanis mango as affected by bamboo vinegar application

Note. BV = Bamboo vinegar; the different letters represent the significant differences using one-way ANOVA at $p < 0.05$. Error bars indicate the standard error of means

provide a high process of photosynthesis to increase its mechanisms and, at the same time, can increase the sweetness of fruits.

An investigation has been done into the effects of wood vinegar only and a mixed combination of wood vinegar with different treatments, such as gibberellin (T1), sodium D-gluconate (T2), and melatonin (T3), on the rapeseed plant. As a result, the net rate of photosynthesis increased during the seedling and blooming periods. The net photosynthetic rates with treatment M, T1, T2, and T3 treatments at the seedling stage were 9.40%, 26.96%, 19.07%, and 14.92%, respectively, greater in two years than the control (water). Over two years, the blooming stages grew by an average of 12.81%, 21.29%, 19.07%, and 22.32% (Vayssieres et al., 2008).

CONCLUSION

In this study, the bamboo vinegar treatment with 3-day intervals (T1) is the most suitable treatment for reducing the insect abundance and enhancing the nutrient content and quality of Harumanis mango (MA128), *M. indica* L. Applying bamboo vinegar affected the quality and nutrient content of Harumanis mango positively. It has shown a positive effect on the nutrient content of Harumanis mango compared to other treatments. Bamboo vinegar application improved the TPC, TFC, vitamin C, and sugar content in Harumanis mango. According to several studies have shown the effectiveness of plant development after being treated with bamboo vinegar. The increasing mechanism could be that the major components in bamboo vinegar cause the plant to produce hormones in

trace amounts or increase photosynthesis in the leaves, which regulates the plant's development. Overall, the increased effect could be a synergistic effect of bamboo vinegar on plant growth.

ACKNOWLEDGEMENTS

This research was financially supported by the Dana Pembudayaan Penyelidikan Dalaman (DPPD) Grant [600-TNCPI 5/3/DDN (09) (009/2020)]. We sincerely thank the students from the Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA Perlis Branch, Arau Campus, Malaysia, academic and non-academic staff for their ongoing assistance, counsel, and encouragement since the start of this research and throughout the fieldwork. A special thanks to Mr. Zulkifli Aswan and the staff for assisting in sampling insect pests from Harumanis mango plantations.

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