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Short communication

Effects of Hydrogen Peroxide and Methyl Eugenol on Fruit Growth, Yield and Fruit Fly Infestation of Syzygium Samarangense

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Abstract

A study was conducted to investigate the effects of hydrogen peroxide (H_2O_2) and methyl eugenol (ME) on fruit growth, yield and fruit fly infestation of wax apple (*Syzygium samarangense* var Jambu madu). The selected experimental tree branches were either left untreated or sprayed with 20 mM H_2O_2, 20 mM H_2O_2 plus ME or ME alone under field condition. The results showed that combine treatment of H_2O_2 and ME increased the net photosynthetic rate, CO_2 assimilation, stomatal conductance and internal CO_2 concentration of wax apple leaves. In addition, fruit weight, fruit firmness and TSS content of wax apple fruits significantly improved with H_2O_2 and ME treatment. The application of Methyl eugenol alone or combined with H_2O_2 reduced the number of fruit fly in wax apple fruits. There was positive correlation between fruit fly infestation and fruit size and between fruit fly infestation and fruit sweetness (TSS content) in H_2O_2 and Methyl eugenol treated fruits. Besides, we recorded negative correlation between the fruit fly infestation and firmness of treated wax apple fruits. It was concluded that application of hydrogen peroxide and methyl eugenol stimulated fruit growth and reduced the fruit fly infestation on wax apple under field condition.

Keywords: *Hydrogen peroxide, physiological activities, Syzygium samarangense, fruit fly infestation*.

1. Introduction

Syzygium samarangense (Blume) Merr. & L.M. Perry (Myrtaceae), known as wax apple or wax jambu usually cultivated and grown in Malaysia, Thailand, Indonesia, Taiwan and other Asian countries [1]. This plant are generally fruit-bearing species with fleshy fruit. The wax apple fruit rich in polyphenolic and antioxidant compounds which represents a potential benefit for human health [2]. It has been reported that wax apple fruits are a potential source of phenol, flavonoid and antioxidant [1, 3]. The fruits can grow any time of the year, but the peak period is from February to April and October to December [4]. The peel color of the fruits is pink, light red, red green, sometimes greenish-white or cream-colored [4]. The sweet taste and aromatic flavour of the wax apple fruit would attract many insect pests such as female fruit flies to lay eggs. The infestation by the fruit fly not only reduce the quality of the fruits by leaving puncture mark on their pericarp [5], it also may cause the fruit drop [6], thus will reduce the fruit production especially when there are no regular precaution on fruit fly infestation and poor management on wax apple trees. The infestation by fruit fly also encourage secondary attack by other pest such as fungal pathogen when the spores of pathogenic fungal grow on the injured cell cause by the fruit fly [7].

The fruit fly, from family Tephritidae, are known to have obligate relationship with fruit trees especially those bearing soft fleshy fruits [8]. It is well known that infestation by the fruit flies on fruits may cause pre-mature and ripening fruit drop. However, to date, there is no information available about the tephritid fruit fly infestation on wax apple, thus this research is crucial to find those information. In tropical and subtropical countries, the oriental fruit flies (*Bactrocera spp*) are the most important pests of fruit crops which cause severe damage and economic loss [9]. In Malaysia, a total of 52 species of fruit fly were recorded [10]. Out of 52 species, 26 species were belonged to the genus *Bactrocera* (*Bactrocera*), 11 species to *Bactrocera* (*Zeugodacus*), two species each to *Bactrocera* (*Paradacus*), *Bactrocera* (*Parazeugodacus*), *Bactrocera* (*Sinodacus*), *Dacus* (*Callantra*) and *Monacrostichus*, and one species each to *Bactrocera* (*Asiadacus*), *Bactrocera* (*Bulladacus*), *Bactrocera* (*Gymnodacus*), *Bactrocera* (*Javadacus*) and *Bactrocera* (*Paratridacus*) [10]. Each species of fruit fly can attack either single or various types of host plants [11]. Methyl eugenol is the most effective of attract-and-kill systems against male oriental fruit flies [12]. They also stated that this chemical can attract male fruit flies from about 800 m radius distance). This male annihilation method is considered the most economic and effective method for oriental fruit fly control [13].

The growth of wax apple tree and its fruit can be enhanced with the application of hydrogen peroxide [14]. Hydrogen peroxide (H_2O_2) is an environment friendly compound that is predominantly produced in plant cell during photosynthesis and photorespiration as well as in plant respiration [15]. It plays a crucial role as signaling molecule in various physiological processes and these will lead to the increment of crop yield and productivity [14]. While exogenous application of gibberellin and auxin increased the morph physiological and biochemical properties of wax apple fruits [16], the application of H_2O_2 increased the germination percentage of seeds, enhance early seedling growth and root establishment [17]. It also has been reported that suitable concentration of hydrogen peroxide stimulates the plant growth and develop-

ment under different growing conditions [14]. The application of exogenous hydrogen peroxide reduced the bud and premature fruit drop, increased fruit set, fruit size and yield of wax apple fruits under field condition [18]. It also stated that exogenous hydrogen peroxide increased the K^+, anthocyanin and carotene content of fruits. However, to date, there is no information available in the literature on the effect of hydrogen peroxide and methyl eugenol on plant physiological activities, fruit drop and their indirect effect on fruit fly infestation of wax apple fruits. Our research was designed to find those answer.

2. Materials and Methods

2.1. Study Area and Plant Meterials

The study was conducted between 2015 and 2016 to investigate the effects of hydrogen peroxide and methyl eugenol on the photosynthetic characteristics, fruit growth and fruit fly infestation on wax apple fruits. Experiments were carried out at a commercial farm located at Banting, Selangor, Malaysia under hot humid and tropical climate. The experimental trees were received the same pruning, training and fertilizing as well as other horticultural techniques. The selected branches from the experimental plants were tagged before the treatment application. In this study, there are four treatments viz; control (T0), 20 mM H_2O_2 (T1), 20 mM H_2O_2 + ME (T2), and ME (T3). The branches were spray treated five times weekly during the experimental period. All treatments were applied in five replicates and a single tree was used as an experimental unit.

2.2. Measurement of physiological parameters

The data for chlorophyll content were taken by using the SPAD 502 Plus meter (Konica Minolta; Opti-science, USA). Chlorophyll fluorescence and photosynthetic yield of randomly selected leaves of wax apple plants were measured using the plant efficiency analyzer (Hansatech Instrument Ltd., England). The clip of this meter was clamped over the leaf for about ten minutes. The readings, as represented by lower fluorescence (Fo), higher fluorescence (Fm), relative variable fluorescence (Fv) and photosynthetic yield (Fv/Fm), were observed on the meter screen and the result was recorded. A portable leaf porometer (Model SC-1, USA) was used for the measurement of stomatal conductance of the treated and untreated leaves. The leaf chamber of leaf porometer was kept in an ambient temperature around 10 to 15 minutes to maintain the light adaption before the measurement started. After light adaptation, the leaf chamber was attached with the leaf and the stomatal conductance was recorded from the screen of the porometer. The photosynthetic rate (*Pn*) was determined by using portable photosynthesis system CI-340 Handheld Photosynthesis System (CID Biosciences, USA). The clip of this system was clamped over the leaf for each treatment. All the readings were taken three times for each replicate. All data of physiological parameters viz; net photosynthetic rate, stomatal conductance, transpiration rate and internal CO_2 concentration were taken at the fruit growth and developmental stage.

2.3. Measurement of harvested fruits

Total number of bud was counted manually from the treated branch of experimental trees. Bud drop (%) was calculated using the following formula: Bud drop (%) = Total number bud before anthesis-Number of bud after anthesis/Total number of bud before anthesis. The percentage of fruit set and fruit drop were determined in a similar manner. After harvested, fruits from each treatment were labeled, kept separately in the paper bag and brought to the laboratory where the measurements of length and diameter as well as weight of the fruits were taken. The fruit length was measured using the vernier caliper. The fruit yield was measured by weighing the fruits per treatment using digital balance (EK-4100i/Japan). Digital hand held penetrometer (Model KM-1, Fijiwara, Japan) was used to determine the fruit firmness and the data value was expressed in pound. Digital hand refractometer was used to measure the TSS content (sweetness) of fruits and the data value was expressed in unit of °Brix.

2.4. Fruit fly rearing procedure

The dropped fruit (ten fruits per treatment) that suspected infested by the fruit fly were collected and brought to the plant physiology laboratory at the University of Sultan Zainal Abidin Terengganu. The infested fruits were placed individually in the rearing plastic containers (Fig. 1) and kept in the laboratory condition (1000 lux, 75% RH and 23 ± 2°C). The size of plastic containers are varies since there is no strict requirement of the cage size to rear fruit flies. A piece of cloth was used to cover the "mouth" of the container. The cloth prevents condensation and allows air circulation in the container, as well as preventing the insect from escaping. The containers were lined with tissue papers on top and crumbled newspapers at the bottom. Both tissue and newspapers were used to absorb liquid from the rotting fruits and acted as a medium for the maggots to pupate. The rearing containers were checked daily for any excess condensation and to observe for the emergence of fruit fly. Fruit flies were identified using natural image processing. Condensation was wiped clean with tissue paper to avoid fungal growth which would affect the development of the fruit fly larvae.

2.5. Statistical analysis

Treatments were arranged according to the Completely Randomized Design (CRD) with five replicates. Data obtained were pooled and analyzed by using SPSS statistical software. One way ANOVA was performed to evaluate the significance differences in the studied parameters within the treatments at 5% level of significance.

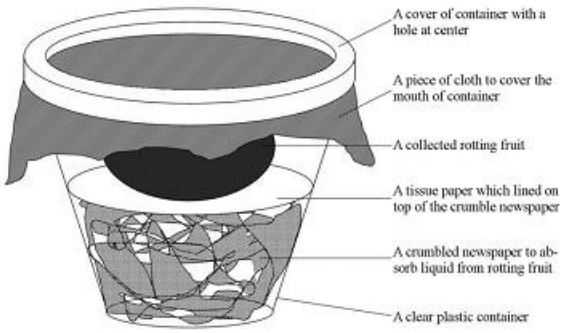

Fig. 1: The modified container for rearing fruit fly larvae [19].

3. Results and discussion

Effect of H_2O_2 on physiological growth of wax apple: The physiological activities of wax apple showed significantly different between treatments. The chlorophyll content of wax apple leaves was significantly affected by exogenous hydrogen peroxide and methyl eugenol (Table 1). The highest chlorophyll content was recorded in 20 mM H_2O_2 treatment with a value of 38 SPAD, while the lowest amount of chlorophyll content was found in control treatment (Table 1). However, the different treatment did not produce any significant effect on lower florescence of wax apple leaves (Table 1). Similar positive effect of H_2O_2 on chlorophyll content was reported by Butcher et al. [20], who reported that the application of hydrogen peroxide increased the chlorophyll content in *Pelargonium tomentosum*.

The highest value for the higher fluorescence (Fm) was detected on the leaves treated with methyl eugenol at 3334, followed by hydrogen peroxide treatment at 3274 (Table 1). While, the lowest

reading of higher fluorescence was as shown in control treatment with value 2959 (Table 1). Similar result was recorded for the variable fluorescence. Kautsky et al. [21] stated that chlorophyll fluorescence yield changed with the reduction of electron acceptors, downstream of photosystem, plastoquinone and QA in photosynthetic pathways. Chlorophyll fluorescence gives the information about the state of photosystem II and stomatal density in the leaves [22]. Plant physiologists and ecophysiologist widely used this measurement to determine the plant health status grown in different environmental condition. The chlorophyll fluorescence is a signal of fate of excitation energy in the chloroplast that used to detect the physiological injury in the leaves of plant under different growing condition [22].

Photosynthetic yield is positively correlated with photosystem II and chlorophyll fluorescence. Both of these parameters were used as an indirect measurement of plant health status at different growth environments where values of 8 indicate the plant is highly efficient in photochemical process [23]. Meanwhile, the photosynthetic yield below 0.65 is a sign of plant severe stress and may hamper the plant growth and development [24]. Adel et al. [25] stated that photosynthetic yield of different cultivars of wax apple range from 0.73 to 0.80 and the highest photosynthetic yield was recorded in Jambu madu cultivar. The results for photosynthetic yield shown the highest value in methyl eugenol treatment at 0.82, followed by 20 mM H_2O_2 treatment with value of 0.81. The lowest value was recorded in control plant (Table 1). Our results were supported by the findings of Saifuddin et al. [26], who reported that growth regulators application increased the photosynthetic yield of Bougainvillea plant.

In this study, the photosynthetic activity was measured to investigate the effects of H_2O_2 and ME application on the wax apple. Result obtained shows hydrogen peroxide produced significant effects on net photosynthetic rate (Table 2).

Table 1: Effects of Hydrogen peroxide (H_2O_2) and Methyl eugenol (ME) on the chlorophyll fluorescence of wax apple leaf.

Treatment	Leaf chloro	(Fo)	(Fm)	(Fv)	(Fv/Fm)
T0	19 ±3 b	599±20a	2959±47b	2360±54b	0.7±0.b
T1	38 ± 4a	632±30a	3274±65a	2642± 57a	0.8±0.1 a
T2	23 ± 4a	595±25a	3208±58a	2613±70a	0.8±0.5 a
T3	20 ± 5b	611 ±35a	3334±75a	2723±60a	0.8±0.4 a
LSD	0.04	0.001	0.31±2	0.324	0.43

Control (T0), 20 mM H_2O_2 (T1), 20 mM H_2O_2+ ME (T2), and ME (T3). Different letter in same column represent the significant different at 5% level of significance.

Based on our results, it clearly shows that leaves treated with hydrogen peroxide has the highest photosynthetic rate as compared with the untreated and methyl eugenol treated leaves. The lowest photosynthetic rate was recorded in leaves treated with methyl eugenol. This finding is double confirmed by the result of Khandaker et al. [18], who stated that hydrogen peroxide increased the net photosynthetic rate of wax apple plants. Transpiration rate was also affected by the combine application of hydrogen peroxide and methyl eugenol and control treatment (Table 2). The highest transpiration rate was recorded in combine application hydrogen peroxide and methyl eugenol followed by control treatment. The lowest transpiration rate was found in methyl eugenol treatment (Table 2).

Result obtained also showed that leaves with methyl eugenol treatment had the lowest stomatal conductance (21.53) (Table 2). This was followed by hydrogen peroxide and control treatment with the values of 57.42 and 23.63, respectively. The highest stomatal conductance was recorded in combine treatment of hydrogen peroxide and methyl eugenol treatment with a value of 125.6 (Table 2). Similar as above, Khandaker et al. [18] have reported similar positive effects of hydrogen peroxide on stomatal conductance of wax apple leaves. The results of this study showed that different treatments did not produce any significant effect on internal CO_2 concentration of wax apple leaves (Table 2). It has been also stated that exogenous hydrogen peroxide increased the photosynthesis characteristics of melon plants [27].

Growth promoting chemical H_2O_2 plays numerous important roles in physiological processes of plants and mitigates the negative effects of environmental stresses. Peng et al. [28] reported that hydrogen peroxide regulates photosynthesis, stomatal conductance, plant growth and development.

Table 2: Effects of H_2O_2 and ME of net photosynthesis, transpiration, stomatal conductance and internal carbon dioxide (CO_2) of wax apple leaves.

Treatment	Net pho (*Pn*)	Transpiration rate (E)	Stomatal conductance	Internal CO_2
T0	4 ± 0.3b	2.02± 0.3b	24±3.0c	222± 10a
T1	11± 0.8a	0.95 ± 0.1c	57± 5.0b	211±12a
T2	5± 0.8b	3.78± 0.4a	125 ±10.0a	190±10a
T4	2 ± 0.2c	0.91± 0.1c	21± 3.0c	202±13a
LSD	0	0	0	0.366

All the data are mean of five replications. Different letter in same column represent the significant different at 5% level of significance. T0: Control; T1: Treatment of 20 mM H_2O_2; T3: Treatment of 20 mM H_2O_2 with ME; T4: Treatment with ME only.

It has been reported that removal of young leaves and growth regulators application stimulated the flower bud formation of bougainvillea plants [29]. In this experiment, we found the hydrogen peroxide alone or combine with methyl eugenol increased the number of wax apple bud (Table 3). The highest number of bud was recorded on branches treated with 20 mM H_2O_2 followed by the branches treated with the combination of hydrogen peroxide and methyl eugenol. The control branches produced the lowest number of flower bud (Table 3). Our result was in agreement with the findings by Peng et al [28], who reported that hydrogen peroxide treatment enhanced the plant growth and development. The result shows that hydrogen peroxide treatment significantly reduced the bud drop (33 %) as compared to others. The lowest bud drop (58 %) was recorded in untreated branches of wax apple plants. Our findings were supported by the results of Webber et al. [30], where the exogenous treatment of H_2O_2 to potted grown nasturtium flowers enhanced bud growth and finally increased the total number of flower. We speculate that the applied treatments increased the photosynthetic rate and enhanced the accumulation of carbohydrates in the treated leaves that's correlated with bud drop [31]. In addition, the data in Table 3 showed that fruit set was increased almost 1.56 times on the 20 mM H_2O_2 treated branches compared to the control. The findings of our study showed in harmony with the observations of Souza et al. [32], who reported that hydrogen peroxide application increased the floral receptivity and increased the fruit set in passion fruits. Bryce et al. [33] reported that H_2O_2 application with irrigation water can increase the fruit size and dry matter content in the tomato fruits. We found the hydrogen peroxide alone or combined with methyl eugenol produced larger fruit size as compared to control (Table 3). The fruit produced by the plant treated with 20 mM H_2O_2 (6 cm) is larger than the fruit produced by the plant treated with the combination of hydrogen peroxide and methyl eugenol (5.4 cm) and control plant (5 cm) (Table 3).

Several cultural and environmental factors such as insect infestation, disease, abiotic stress, unhealthy foliage and poor nutrition during the early growth and development of plant accelerate bud and premature fruit drop. We found that the application of H_2O_2 at 20 mM concentration or combination with methyl eugenol have reduced the bud and premature fruit drop of wax apple under field condition. The highest fruit drop of wax apple (42 %) was found in untreated branch, while the lowest premature fruit was recorded with 20 mM H_2O_2 treatment (Table 3). Khandaker et al [14] reported that optimum concentration of H_2O_2 can reduce the premature bud and fruit drop of wax apple fruits. Different treatment

produced significant effect on fruit yield of wax apple. The results showed that branch treated with hydrogen peroxide or methyl eugenol exhibited a higher fruit yield (Table 3). The yield calculated on the basis of fresh fruit weight was almost 1.57-fold on the 20 mM H_2O_2 treatment than on the control. Ozaki et al. [27] reported that exogenous treatment of hydrogen peroxide increased the physiological and biochemical characteristics, fruit yield and dry matter accumulation in the leaves of melon plants.

Table 3: Effects of hydrogen peroxide (H_2O_2) and Methyl Eugenol (ME) on fruit growth and yield characteristics of wax apple fruit under field condition.

Treatment	No of Bud/ Branch	Bud drop (%)	Fruit Set (%)	Fruit growth length (cm)	Fruit drop (%)	Fruit yield (kg)/ Branch
T0	40b	58a	25b	5.0b	42a	0.19c
T1	60a	33c	39a	6.0a	25b	0.30a
T2	52a	35c	33a	5.4a	27b	0.27a
T3	45b	45b	30a	5.2b	30b	0.23b

All the data are mean of five replications. Different letter in same column represent the significant different at 5% level of significance. T0: Control; T1: Treatment of 20 mM H_2O_2; T3: Treatment of 20 mM H_2O_2 with ME; T4: Treatment with ME only.

Relationship of fruit fly with fruit weight, fruit firmness and fruit sweetness

Total number of fruit fly emerged from the fruits of control (T0) and 20 mM H_2O_2 (T1) treatment were higher compared to the hydrogen peroxide combine with methyl eugenol treatment (T3) (Table 2A), while low number of emerged fruit flies was recorded in methyl eugenol treatment (T4) (Fig. 2A). In this study, we recorded a positive correlation between the wax apple fruit firmness and the number of emerged fruit fly (R^2= 0.95) (Fig. 2B). However, Singh and Vashishta [35] reported that there is no correlation between the fruit firmness and fruit fly infestation in Indian jujube. Likewise, the weight of wax apple fruit and the number of emerged fruit fly was also positively correlated (R^2= 0.75) (Fig. 2C). Our findings are concunrrent with the results of Singh and Vashishta [35], who reported that fruit fly infestation positively correlated with fruit size and fruit weight of jujube. It has been also reported that number of fruit flies positively correlated with the percentage of infested fruits, level of ripens and fruit drop of citrus fruits [34]. However, the fruit fly population varied according to the seasonal changes and internal fruit quality [35]. In addition, number of reared fruit fly positively correlated (R^2= 0.99) with fruit sweetness (TSS) of wax apple (Figure 2D). Our results are supported by the findings of Haldhar et al. [37], who reported that fruit fly infestation as well as larval density depends on fruit sugar content of muskmelon and fruit fly infestation increased with the sugar content of fruits.

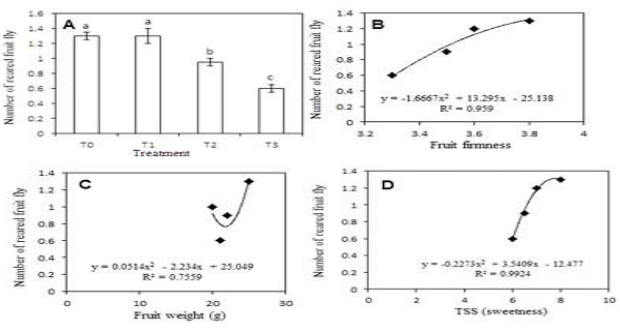

Fig. 2: Number of emerged fruit flies per treatment (A). Correlation between fruit fly with fruit firmness (B), fruit weight (c) and sweetness (D) between four different. Control (T0), 20 mM H_2O_2 (T1), H_2O_2combined with ME (T2) and ME (T3).

4. Conclusion

Result obtained from this experiment shows the application of hydrogen peroxide improved the plant physiological activity of wax apple plants. Fruit growth, yield and quality of wax apple fruits were significantly affected with the application of 20 mM H_2O_2. The application of Methyl eugenol alone or combination with hydrogen peroxide indirectly reduced the fruit fly infestation. Additionally, our results also showed that number of emerged fruit fly positively correlated with fruit weight, fruit firmness and fruit sweetness of wax apple. We conclude that the application of hydrogen peroxide and methyl eugenol treatments are promising for fruit growth and yield and have a potential to reduce the fruit fly infestation under field condition.

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