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ORIGINAL ARTICLE

The Effect of Ascorbic Acid on the Formation of the Separator Layer of Walnut Fruit from the Pedicel and Acetylene Gas as Ethylene Synergism in Accelerating Fruit Ripening

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K E Y W O R D S

Acetylene gas;
Ascorbic acid;
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Walnut

A B S T R A C T

With the aim of facilitating the harvest and accelerating the formation of the separator layer of the fruit with the peduncle, an experiment was conducted with ascorbic acid as one of the synergists of ethylene. This experiment was conducted in the form of randomized complete blocks in 3 replications and 8 treatments based on the concentration of ascorbic acid. The present study showed: a significant difference was observed between the design blocks. However, in statistical calculations, ascorbic acid did not show a significant effect on the separation of fruits, but according to observations, the use of ascorbic acid accelerated and increased the production of ethylene in the plant, so that at a concentration of 2500 ppm, the lowest force to separate the fruit from the branch we experienced. In other words, the direct use of ascorbic acid on the fruit-bearing branch with the appropriate concentration was not ineffective in facilitating the separation of the fruit from the tree, and it requires more repeated experiments. To facilitate the separation of mesocarp from endocarp, acetylene gas was used as one of the synergisms of ethylene. The experiment was conducted as a randomized complete block design with 3 replications and 4 treatments based on acetylene concentration. This study showed that acetylene gas has a significant effect on the ease of walnut mesocarp separation by accelerating fruit ripening. As a result, by using this gas as an ethylene synergy, a higher percentage of quality nuts and walnut kernels with a bright color are obtained.

Introduction

Persian walnut (*Juglans regia*) is the most important genus of the Juglandaceae family from an economic point of view and is cultivated in different climatic regions of the world (Barrueto *et al*., 2018; Jahanban-Esfahlan *et al*., 2019; Shigaeva and Darr, 2020; Thapa *et al*., 2021). According to FAO data, Iran is third producer of walnut with production of 9.10% and fifth country for harvesting it (FAO, 2017; Farsi *et al*., 2018). It has significant vlaues owing to their compounds, such as a high level of unsaturated fatty acids, protein (16-24%), carbohydrates (12-21%), fiber (1.5-2%), folic acid, potassium, vitamin E (Chatrabnous *et al*., 2018;

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Jahanbani *et al.*, 2018; Sarikhani *et al*., 2021), tannins and polyphenols (Khodadadi *et al*., 2016; Khodadadi *et al*., 2020; Sarikhani *et al*., 2021). It was also reported that a significant part of walnut fruit is oil (52-70%) that is containing linolenic (18:3), oleic (18:1) and linoleic (18:2) fatty acids (Akça *et al.*, 2020; Sarikhani *et al.*, 2021).

The increased oxidation of the polyunsaturated fatty acids causes unpleasant odors and flavors (Chatrabnous *et al*., 2018). However, nutrional and commercial values are preserved during utilization of appropriate methods for preservation (Habibi *et al.*, 2023). Prevention of browning in fresh walnut kernels and uniformity in fruit ripening has significant effects on nutritional values of walnut (Habibi *et al.*, 2022; Habibie *et al.*, 2021)

In addition, artificial ripening is applied to meet timely distribution and consumer excpectations in the commercial bazzars (Maduwanthi and Marapana, 2019). Various materials are utilized for acclerating ripening and preserving fruits. Ascorbic acid (AsA) is an antioxidant structre with cyclic and enediol-lactone resonant that scavenges free radicals (Habibi *et al.*, 2021; Kim *et al*., 2015). For this reason, it plays a role in physiological processes control for stress tolerance in plants (Ikhajiagbe *et al*., 2023). It has been utilized with other agents for decreasing lipid oxidation in corn oil (Kim *et al*., 2015) and also preventing the polyphenol oxidase enzyme and surface browning in some horticultural crops (Ali et al., 2015). It was also reported that ascorbic acid prevented the polyphenol oxidase enzyme activity in fresh walnut kernels stored in cold temperature condition (Habibie *et al*., 2019). Ethylene is known as a gaseous hormone which influences maturation and senescence in plants and in combination with other materials is utilized to prevent fruit browning (Ye *et al*., 2021). Acetylene gas is utilized for accelretaing fruit ripening (Maduwanthi and Marapana, 2019). In addition to ripening and preservation of nutrient value, the green walnuts mesocarp is a valuable source for phenolic compounds which can be utilized as an alternative natural antioxidant in the food industry (Kamali *et al*., 2018).

Based on mentioned statments, ripening and preservation of nutritient value and separation of mesocarp in walnut is important. We hypothesized that acetylene gas could have significant effects for accelerated ripening and ascorbic acid could facilitate separation of walnut mesocarp. Thus, the current study was conducted to investigate the effects of acetylene gas on ripening and and ascorbic acid on separation of walnut mesocarp. Our findings can help to solve some of the problems of walnuts in the country, such as kernel browning, delay in the green husk maturation and difficult harvesting and also help to explore ways for separating the mesocarp from the endocarp.

Materials and Methods

Materials

Ascorbic acid was prepared from Sigma-Aldrich Company (St. Louis, USA). Acetylene gas was also purcahsed from Sigma-Aldrich Company (St. Louis, USA).

Ascorbic acid treatments

This study was conducted in Damavand walnut orchards (Tehran-Iran). This study was conducted in a completely randomized block design arrangment with eight treatments of ascorbic acid (0 ppm, 1000 ppm, 1500 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm) and three replications for each treatment. The group treated with 0 ppm was considered as control. Ascorbic acid was sprayed on four frutitiful branches in four different directions. Spraying was conducted on 16th September because the average harvest was 1st- 21st Octobor. We selcted branches with one fruit and trees in a row for decreasing error. Each branch was marked by a band. A maximum of 5 leaves was kept on each of the selected fruit-bearing branches and the rest were removed in order to determine the complication of leaf fall during fruit harvest and to investigate better control the leaf shedding. Different concentrations of ascorbic acid were prepared and sprayed on the leaves and fruit-bearing branches in the next step. This work was conducted until covering the entire surface of the fruit and leaves. The fruit-bearing branches were cut off and investigated for falling leaf and then graded foe separation ease of fruit as follows; the leaves of the fruit-bearing branches were cut off, and then the branch was attached to a base clip still bearing the fruit. A quadrangular fabric was placed on the fruit attached to the container under the fruit by four threads. Slight weights were hanged from these threads to determine how much weight it would take for the walnut to be separated from the tree and fall into the container. Eventually, the weights were determined with a sensitive scale (0.1 g sensitivity). After the test, the weights in the container and the respective fabric were investigated, and the weight of the contents of the container was added. The resulting weights were categorized and statistically analyzed.

Acetylene gas treatments

The effects of acetylene gas on fruit ripening was investigated in a completely randomized block design arrangment with four treatments of acetylene gas (0 Oxygen 100%, 50% acetylene + 50% oxygen, acetylene 75% + oxygen 25% and 95% acetylene + 5% oxygen) and three replications for each treatment. Plastic coatings were used to perform this experiment. Each tree was considered as a block. Fruits were harvested from different directions of each tree. Then, healthy fruits were selected and divided into four equal batches. Each batch was placed inside a plastic cover and the selected gas composition was injected into it. Thus, each plastic cover was considered a test unit.After ten days, the walnuts were removed from the plastic wrap and graded based on the ease of mesocarp peeling.

Data analyses

The data were nalayzed as a completely randomized block design arrangment by ANOVA proc of SAS software and based on below model:

$$Y_{ij}= \mu + T_i + B_j + \varepsilon_{ij}$$

Where Y_{ij} is any observation, μ is mean, T_i is the effect for being in treatment i and Bj is the effect for being in block j. Where differences were significant, the mean comparisons in this experiment were analyzed by Duncan test.

Results

The effect of ascorbic acid on leaf shedding and ease of harvest

Table 1 presents the results for analysis of varinace of the effects of ascorbic acid on leaf shedding and ease of harvest. The results showed significant effects of block [F (2, 21) = 99810.387, *P*<0.05] and non-significant effects for ascorbic acid [F (7, 21) = 17530.367, *P*>0.05] for fruit separation from the fruit-bearing branch. The results for falling leaves showed that leaves did not fall in all the concentrations.

Table 1. Analysis of variance of experimental data for the effect of ascorbic acid on fruit shedding.

Sources of variation	Degree of freedom	Sum of squares
Block	2	99810.387*
Treatment (ascorbic acid)	7	17530.367^{ns}
Error	1	55051.23767
Total	21	

Table 2 depicts the results for the effects of ascorbic acid on fruit shedding. The results showed that required force for seperation of fruit from branch was increased with increasing ascorbic acid concentration from 0-20000 ppm (316 g-460.287 g) while it was decreased on 25000 ppm (286.187 g). It was then increased from 30000 ppm to 50000 ppm (382.01 g-415.61 g). Thus, the most optimum concentration of ascorbic acid for fruit shedding is 25000 ppm.

Table 2. The average force needed to separate the fruit from the branch.

Treatment based on AA concentration (ppm)	Average force (g)
T_1 (control sample)	316.93
T_2: 10000 ppm	320.057
T_3:15000 ppm	376.876
T_4: 20000 ppm	460.287
T_5: 25000 ppm	286.187
T_6: 30000 ppm	382.013
T_7: 40000 ppm	362.737
T_8: 50000 ppm	415.613

The effect of acetylene gas on fruit ripening

Table 3 shows the analysis of varinace for the effects of acetylene gas on fruit ripening. The results showed that effects of block F (2, 11) = 11.72, P<0.05] and acetylene gas [F (3, 11) = 4.62, P<0.05] on fruit ripening were significant.

Table 3. Analysis of variance the effects of acetylene gas on fruit ripening

Sources of variation	Degree of freedom	Sum of squares
Block	2	11.724^{*}
Treatment (gas composition)	3	4.627^{+}
Error	6	1.143
Total	11	

Fig. 1 depicts the effects of various concentrations of acetylene gas on fruit ripening. The results showed significant differences between treatments of 75% acetylene + 25% oxygen and 95% acetylene + 5% oxygen with 100% oxygen. The results showed that fruit ripening was significantly higher in 75% acetylene and 95% acetylene compared with the treatment lack of acetylene. We did not observe significant differences between 50.00% acetylene treatment with other treatments. Fig. 2 confirms the effects of acetylene on fruit ripening.

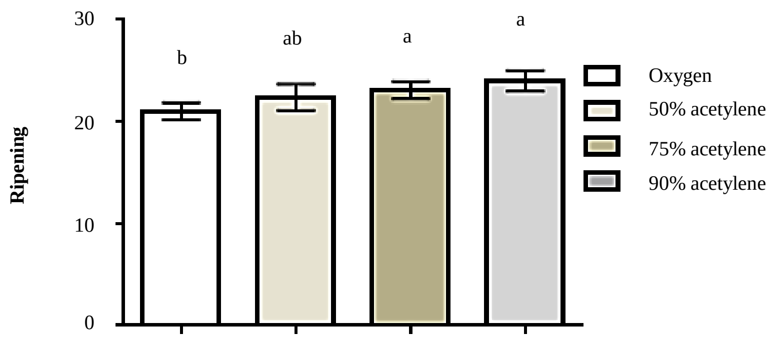

Fig. 1. The effects of acetylene gas on fruit ripening. The data are reported as mean ± SD. Non-similar superscripts show significant differences between groups at *P*<0.05.

1 2 3 4

0% 50% 75% 95%

Fig. 2. Samples of fruits treated with different concentrations of acetylene

Discussion

We investigated the effects of ascorbic acid on fruit shedding and showed that although ascorbic acid had not significant effects on fruit shedding but concentration of 25000 ppm ascorbic acid could significantly decrease force for fruit shedding. The results are partly in agreement with previous studies that showed ascorbic acid decreased fruit shedding in cotton (Kassem, 2009). Based on earlier studies, ascorbic acid in low concentrations decrease sheeding while in greater concentrations increases shedding in cotton (Vakma, 1976). Based on our findings, in concentrations lower than 20000 ppm, ascorbic acid increased force for shedding while we observed 25000 ppm as an optimum concentration for shedding. Concentrations higher than 25000 ppm decreased shedding. It can be concluded that ascorbic acid shows its effects on fruit shedding in a narrow range in walnut and higher and lower concentrations have not significant effects. Indeed, shedding process has three steps comprising stimulation, development, and fall phases. The used materials for shedding must be utilized during stimulation phase and we used it during falling phase that could be a certain reason for inefficiency of ascorbic acid on fruit shedding. We observed numerical differences that must be considered in future studies.

Our findings also showed that acetylene had significant effects on fruit ripening. Greater concentrations of acetylene could increase walnut ripeneing. The results are in agreement with previous studies (Abdulwahab *et al*., 2018; Maduwanthi and Marapana, 2019, 2021) and is in contrast with other studies for banana (Thompson and Seymour, 1982). The difference between our findings and others could be attributed to the studied species. Acetylene, an ethylene analogue, increases fruit ripening (Maduwanthi and Marapana, 2019). Studies have not reported the mechanism of action of acetylene in fruit ripening. However, it is a ethylene analogue and may show its effects in way similar with ethylene. A review article reported that ethylene activates a complex network of interacting signaling components including ripening-related transcription factors, epigenetic regulation, and multiple hormones signaling (Liu *et al*., 2020). Following the treatment with ethylene, hormonal and developmental factors are activated and show the effects with targeting fruit ripening (Liu *et al*., 2015). Based on our findings, treatments with 50% acetylene did not have significant effects on walnut ripening that shows more requirement of walnut to acetylene for ripening. It is clear a dependent-dose manner for ripening with increased the concentration of acetylene.

Conclusions

In sum, we used ascorbic acid for fuit shedding and acetylene for fruit rippening in walnut and showed that ascorbic acid did not have significant effects on fruit sheeding but lowest required force was seen in concetration of 2500 ppm. The results for the effects of acetylene showed that concentrations of 75% and 95% acetylene significantly increased walnut ripening. Acetylene can be utilized for unifrom ripening in walnut.

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Conflict of interests

There is no conflict of interest in this research.

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