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Effect of postharvest microwave heat treatment of pomegranate on carob moth, *Ectomyelois ceratoniae*, control and quality parameters

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ABSTRACT

Purpose: In some orchards in the harvest season, the pomegranates are suspected to carob moth infestation. Visible infested fruits are removed but there is a possibility of hidden infestation. The effect of microwave heating on this hidden infestation was investigated in this study. **Research Method:** The mature pomegranate fruits were artificially infested with the eggs or larvae (1st, 2nd and 3rd) of the carob moth inside the crown. Microwave radiation was focused on the crown zone of infested pomegranate with a novel setup and its effect on pest mortality was studied. The experiments were carried out at three microwave powers (540, 720 and 900 W) and three heating times (4, 6 and 8 minutes). In order to study the effect of microwave heating treatments on pomegranate, quality parameters including appearance, weight loss, total soluble solids (TSS), pH, titratable acidity (TA) and taste index (TSS/TA) of the samples were investigated after 60 days' storage. **Findings:** The egg and larval mortality rose with microwave power and heating time. The mortality was reduced with increasing the age of pest. There were no significant differences among values of quality factors except for titratable acidity. Because of 100% mortality with no significant differences on quality parameters, 6 min treatment time with 720 microwave power was selected as the optimum treatment. **Research limitations:** Energy consumption in microwave heating limits the application of this method in practice. **Originality/Value:** Microwave local heating of the pomegranate crown is an effective novel method to remove carob moth in hidden infestation.

INTRODUCTION

The scientific name of pomegranate, *Punica granatum*, is derived from the name *Pomum* (apple) *granatus* (grainy) or seeded apple. Pomegranate is native to Persia (Iran) and surrounding areas where, it spread to the rest of the world (Teixeira da Silva et al., 2013; Fadavi et al., 2006). Iran is one of the most important pomegranate producers and exporters in the world (Khajehei et al., 2015; Khodabakhshian et al., 2016).

The carob moth, *Ectomyelois ceratoniae*, is a worldwide polyphagous destructive pest witch attacks pomegranate, pistachio, date, fig and some other fruits (Khodabakhshian et al., 2016; Sobhani et al., 2015). This herbivorous insect is the major field and storage pest of the pomegranate causing 30-80% yield loss (Hosseini et al., 2017). The infestation begins in the orchard. The adult female lays its eggs in the crown (calyx) of the pomegranate. After egg's hatching, the first-instars larvae (1st, 2nd and 3rd) feed inside the crown and then burrow into the fruit and feed on internal parts (Hashemi Fesharaki et al., 2011). The pest infestation and saprophytic fungi contamination result from larvae penetration to the fruit cause the internal hidden decay of pomegranate. The degree of fruit decay grows until reaching the rind where symptoms are readily visible. In the harvest season, visibly rotted fruits are removed but the hidden infested ones not detectable. After harvesting (during handling, shipping, storage and marketing) the hidden decayed fruits grow and the symptoms become visible. Non-destructive tests (NDT) have been used to detect hidden rotted pomegranate fruits (Jamshidi et al., 2019; Khodabakhshian et al., 2016).

Postharvest heat treatments of fruits (hot water dips, vapor heat and hot air) are being widely used for quality maintenance of stored fruit and disease/pests control (Fallik & Ilic', 2019; Ferguson et al., 2000; Hansen et al., 2011; Lurie, 1998; Tang et al., 2007). For the pomegranate fruits in cold storage (90 days at 2 or 5 °C), one day intermittent warming at 20 °C, every 6 days, or curing at 33 °C for 3 days before storage, reduced decay and chilling injury symptoms (Artés, et al., 2000). Juice characteristics (soluble solids content (SSC), titratable acidity (TA) and pH) and visual appearance show the advantage of the pomegranates heat treating rather than conventional cold storage (Artés et al., 2000). Heat treating of pomegranates by hot water dip at 45 °C for 4 min, and then stored at 2 °C for 90 days significantly reduced chilling injury symptoms (Mirdehghan et al., 2007). In Iran, there are some local publications (in Persian) that confirm the effect of pre-storage thermal heating of pomegranate on quality characteristics improvement after storage. They find that the pre-storage hot water treatment of pomegranate at 45-50 °C for 1-5 minutes has a significant effect on decay control during storage.

Heat treatment has been used for carob moth control on harvested dates. Hot air (55, 60 and 65 °C for 20-40 minutes) and hot water (50, 55 and 60 °C for 0-30 minutes) had a significant effect on pest mortality (Ben-Amor et al., 2016a; Ben-Amor et al., 2016b; Zouba et al., 2013).

Microwave heating is a useful technique for postharvest thermal treatments of fruits and vegetables (Marsaioli Jr et al., 2009). Accordingly, it is an effective method for pest control (García-Mosqueda et al., 2019; Ling et al., 2015; Nelson & Trabelsi, 2016). Microwave treatment for postharvest control of the date infested by carob moth showed significant mortality of pests (Zouba et al., 2009). In the microwave, due to volumetric heating, thermal gradient on the product is lower rather than conventional heating (Datta, 2001; Feng et al., 2012; Mirzabeigi Kesbi et al., 2018).

Integrated pest management (IPM) is the careful consideration of all available pest control techniques for the economic control of pests. For carob moth, applying postharvest techniques in combination with the field approach will be useful. In this study, postharvest heat treatment was applied to control hidden carob moth infestation on pomegranate fruit. In hidden infestation, there are eggs and/or first-instars of pest inside the crown (calyx). Therefore, local heating around the crown zone was applied by microwave radiation focus with a novel setup. By the crown zone local heating, there is low temperature increasing on the edible part of the pomegranate (arils) that causes lower unwanted changes in fruit quality.

MATERIALS AND METHODS

Samples Preparation

Mature pomegranate fruits, Galubarik variety were harvested on 15 October 2018 in a commercial orchard in Varamin, Tehran Province, Iran (Latitude 35°N and Longitude 51° E) and immediately transported to the laboratory. Fruits with defects (crack, bruise, and decay) were removed. The fruits with sound appearance were kept 10 days at room temperature in order to detect the hidden naturally infestation by carob moth in the orchard. Naturally infested samples were discarded and the sound pomegranates were selected for tests. Fruits were artificially infested with two eggs or larvae (first-instars, 1^{st}, 2^{nd} and 3^{rd}) of carob moth inside the crown (calyx). To simulate hidden infestation, eggs and first-instars larvae of carob moth were used. The larvae were put into the crown after four hours of starvation. Larvae infested samples were kept at room temperature for 24 hours prior to heat treatments. This allows the larvae to embed in the fruit crown. The crown opening was closed with a fabric net piece to prevent the larvae from leaving the fruit (Fig. 1).

Experimental design

A novel setup based on a domestic 2450 MHz microwave oven (Fig. 2) was used to focus microwave radiation on the pomegranate crown zone (Mirzabeigi Kesbi et al., 2018). Focusing the radiation makes local heating of the crown and the carob moth mortality without unwanted heating on other parts of the fruit.

The power of the oven was set to values of 180, 360, 540, 720 and 900W in the control panel. The maximum output power supply (nominally 900W) was measured by two liters water load method (IEC, IMPI, (Buffler, 1993)). The mean of three measurements was 581 W. The microwave was generated by the magnetron and was fed into the chamber with an aperture in the sidewall. For preventing random reflection in the chamber, 1.2 liters' water load was used. The water load container (made from Teflon, microwave transparent) was placed in the chamber, adjacent to the sidewall. There is a cylindrical path (30 mm diameter) in the water container in front of the aperture. The propagating waves from the aperture were guided into the cylindrical path. In the oven chamber, infested pomegranate samples were placed on the sample holder and the crown was guided into the cylindrical path (Fig. 2). The waves parallel to the path axes radiated on the sample crown zone, whereas the unparalleled (random) waves entered into the water load and damped (for further information, refer to Mirzabeigi Kesbi et al., 2018). This causes local heating of the pomegranates crown and carob moth mortality.

Preheating of the chamber was carried out prior to the tests. During preheating, the water container with full capacity (1.2 liters) was placed in its location and the microwave oven was operated with 900 W power level for two minutes (without sample). After each test, the water container was taken out from the chamber and the water load replaced with room temperature water.

Experimental Procedure

Carob Moth Mortality

The carob moth infested samples (24 hours after infestation) were treated by the microwave setup. In each test, one infested pomegranate was placed in the setup (Fig. 2) and the experiments were carried out at three microwave powers of 540, 720 and 900 W, three heating times of 4, 6 and 8 minutes and three pest's age treatments of egg, 1st-2nd and 3rd instar (due to difficulty of 1st and 2nd larva instars separation, both of them were placed in one treatment). Treated samples were kept in the room temperature for 48 hours. After 48 hours, the eggs and larvae were separated by cutting the crown of fruits. In larval infested samples, live and dead pest stage were manually counted. The live larvae were fed and after four weeks, the larvae with incomplete life cycles were considered as the perished pests. Total dead and incomplete life cycle larvae were included as final mortality. In eggs infested samples, unhatched eggs were considered as final mortality and used to calculate the percentage of mortality. The microwave heat treatments were analyzed using a completely randomized design in factorial layout (3×3×3) with 10 replications. Analysis of variance (ANOVA) procedure was performed to determine the significant effects of the experimental factors on carob moth mortality. The analysis was carried out in SAS statistical software (SAS Institute Inc., NC, USA). Mean values were compared using Duncan's multiple range test to find significant differences among treatments and interactions between factors ($p<0.05$). Infested samples by three pest's age with no heating treatment were considered as control treatments. The mortality of control treatments was compared with treated samples (final mortality) using the least significant difference (LSD) test ($p<0.05$).

At the end of the heating process, the surface temperature on the crown zone of samples was measured with a non-contact infrared thermometer (ST350, Thermometer superstore, UK).

Quality Parameters

In order to study the effect of microwave heating treatments on pomegranate quality, the sound pomegranate fruits were treated in the separate microwave heating tests with the same condition of carob moth mortality investigations (at 540, 720 and 900 W microwave powers and 4, 6 and 8 minutes heating times).

The appearance of fruits was observed 48 hours after heat treatment. Damaged samples, because of overheating, were removed and others were stored at 5±0.1°C and 85±3% relative humidity. After 60 days' storage, quality factors (weight loss, total soluble solids, pH and titratable acidity) of samples were measured. The results were compared with control (without heat treatment before storage) and fresh samples using a completely randomized design with a factorial arrangement of variables.

All sample weights were measured before (w_1) and after (w_2) storage using a digital balance (KERN, KB, Germany). Weight loss was calculated by Eq (1).

$$Weight\ loss\ (\%) = \frac{w_1(g) - w_2(g)}{w_1(g)} \times 100 \quad (1)$$

After storage, the fruits were cut, peeled and arils were separated. The arils were manually pressed and the extracted juice was filtered. Sugar content or total soluble solids (TSS) of samples' juice were determined by a handheld refractometer (A. Krüss Optronic, Germany). 10 ml of sample juices were diluted with 90 ml distilled water and the pH was measured using a pH meter (Sartorius, PB-11, Germany). The diluted juices were titrated with 0.1 N NaOH (to pH 8.2) and the titratable acidity (TA) were expressed as the percent of citric acid (Elyatem & Kader, 1984; Fadavi et al., 2005; Sadler & Murphy, 2010). The ratio of

TSS/TA (taste or maturity index) as a numerical value that represents the taste condition of pomegranates was calculated for samples. Low and high values of taste index indicate sour and sweet taste respectively (Fadavi et al., 2005; Martinez et al., 2006).

RESULTS AND DISCUSSION

Carob Moth Mortality

Table 1 displays the mortality of carob moth in the microwave heat treated pomegranates. It shows the mortality of the larvae after 48 hours and the final mortality of three ages of pest against the control treatments. As mentioned, in pomegranate samples the pest was embedded inside the crown, but penetration ability of microwave radiation causes considerable mortality of carob moth.

According to Table 1, for larvae, the final mortalities are higher than the values after 48 hours. It means that 48 hours after treatments, there are some live larvae that cannot complete their life cycle. It demonstrates that microwave heating makes a persistent effect in addition to immediate mortality. Larva with incomplete life cycle is the result of persistent effect while larva death due to overheating is the result of immediate effect. In some cases, overheating makes the larva’s body disintegrating (Fig. 3).

According to the LSD test (Table 1), the final mortality of all heat treated samples is significantly higher than control treatments. It shows the satisfactory effect of microwave heating on carob moth mortality in pomegranate samples.

Analysis of variance (ANOVA) showed significant differences in carob moth mortality among simple factors (pest’s age, treatment time and microwave power) and no significant differences among interactions. Non-significant interactions mean that the change in each factor has a similar effect on others.

Table 1. Carob moth mortality of heat treated pomegranates.

	Mortality after 48 hours (%)		Final mortality (%)		
Treatments	1st-2nd instars	3rd instar	Egg	1st-2nd instars	3rd instar
Control	20	20	15	35	30
540 W-4 min	50	30	95	85	60
540 W-6 min	85	65	95	90	85
540 W-8 min	80	90	100	95	95
720 W-4 min	80	45	95	90	80
720 W-6 min	100	70	95	100	100
720 W-8 min	100	100	100	100	100
900 W-4 min	40	50	100	100	100
900 W-6 min	95	95	100	100	100
900 W-8 min	100	100	100	100	100
LSD*	---	---	11	19	23

†Least significant difference test for comparing the values of mortality with control treatments ($p<0.05$).

Table 2. Effect of pest’s age, treatment time and microwave power on carob moth final mortality after microwave heat treatments.

	Age			Treatment time (min)			Microwave power (W)		
	Egg	1st-2nd instars	3rd instar	4	6	8	540	720	900
Mortality (%)	97.8^{a}	95.6^{ab}	91.1^{b}	89.4^{a}	96.1^{b}	98.9^{b}	88.9^{a}	95.6^{b}	100^{b}

†Mean values were compared using Duncan's multiple range test ($p<0.05$). Similar letters are not significantly different.

The mean values of simple factors display in Table 2. As shown, the mortality of pest decreases with age growth. It demonstrates the higher susceptibility of pest's egg and lower age larvae to the microwave heating. These results are in accordance with the findings of Zouba et al. (2009). Since the microwave radiation creates more heating in the larvae body due to the aqueous tissue, it seems that the microwave treatment causes higher mortality in larvae rather than eggs but the susceptibility of pest in lower age is the dominant factor. As expected, the mortality has risen by treatment time and microwave power increase. The higher level of power and time cause a higher temperature in samples and increase carob moth mortality. Increasing temperature in a higher level of microwave power and time, confirmed by the measured values (Table 3). In some other studies, increasing the mortality at the higher temperature were noted in hot water, hot air and microwave heat treatment of carob moth infested samples (Ben-Amor et al., 2016a; Ben-Amor et al., 2016b; Zouba et al., 2013; Zouba et al., 2009).

According to the data in Table 2, increasing microwave power from 540 to 720 W caused significant and 720 to 900 W caused non-significant differences in mortality. Also in treatment time, increasing from 4 to 6 min made significant effects and 6 to 8 min made non-significant differences in mortality. Hence, applying 720 W microwave power with 6 min treatment time caused considerable mortality with energy saving.

Quality Parameters

In pomegranate quality tests, 48 hours after heat treatments the appearance of samples was investigated. The observations showed severe heat damage of samples in four treatments (from 9 treatments). There were severe damages in all 8 min (540, 720 and 900 W) treatments and 900 W-6 min. In all damaged samples the temperature has reached above 47 °C after heat treatment (Table 3). It demonstrates that the critical temperature of pomegranate samples is between 42 to 47 °C while higher values caused over heating damage.

Table 4 shows the quality parameters of stored pomegranates (after removing the damaged samples). As shown, there is no significant difference in weight loss, TSS and pH values. Except for 900 W-4 min, TA values of treated samples and control treatment are very close to each other and placed in one group. In stored pomegranates, no considerable differences among treated and control samples demonstrated that heat treatment has no significant effect on pomegranate quality (unless for the removed treatments). In all stored samples, pH increased and consequently, TA decreased rather than fresh pomegranates while the significant difference has occurred only in 900 W-4 min. In treated pomegranates with 900 W, a high level of microwave power caused the chemical reactions to speed up and makes a great change at TA level. pH rise and TA reduction during conventional cold storage of pomegranate have been reported by other researchers (Artés et al., 2000; Ehteshami et al., 2021; Fawole & Opara, 2013). With approximately a constant amount of TSS among all treatments, TA values were used to determine the variations of taste index (TSS/TA). Except for 900 W-4 min, all TSS/TA values were minimal together and represented sour-sweet taste. In 900 W-4 min, lower values of TA caused a higher taste index and appeared to have a sweet taste. Depending on customer demand, both sour and sweet tastes are marketable.

Table 3. The surface temperature on the crown zone of pomegranates at the end of the heating process.

Treatments	540 W 4 min	540 W 6 min	540 W 8 min	720 W 4 min	720 W 6 min	720 W 8 min	900 W 4 min	900 W 6 min	900 W 8 min
Temperature (°C)*	33.7	42.5	47.7	36.3	40.9	47.6	40.2	49.4	57

†Average of 30 replications (10 replications for each pest's age).

Table 4. Quality factors of fresh, control and microwave heat treated samples after storage.

	Weight loss (%)	TSS (%)	pH	TA (%)	TSS/TA
Fresh sample	---	15.3	3.18	1.72[a]	8.90[a]
Control	11.56	15.1	3.57	1.54[ab]	9.81[a]
540 W-4 min	11.08	15.5	3.39	1.42[b]	10.92[a]
540 W-6 min	10.80	15.5	3.48	1.40[b]	11.07[a]
720 W-4 min	11.83	13.9	3.35	1.32[b]	10.53[a]
720 W-6 min	10.69	15.5	3.29	1.34[b]	11.57[a]
900 W-4 min	11.06	14.0	3.47	0.55[c]	25.45[b]

†Mean values were compared using Duncan's multiple range test ($p<0.05$). Similar letters are not significantly different.

Fig. 1. Carob moth infested pomegranate sample.

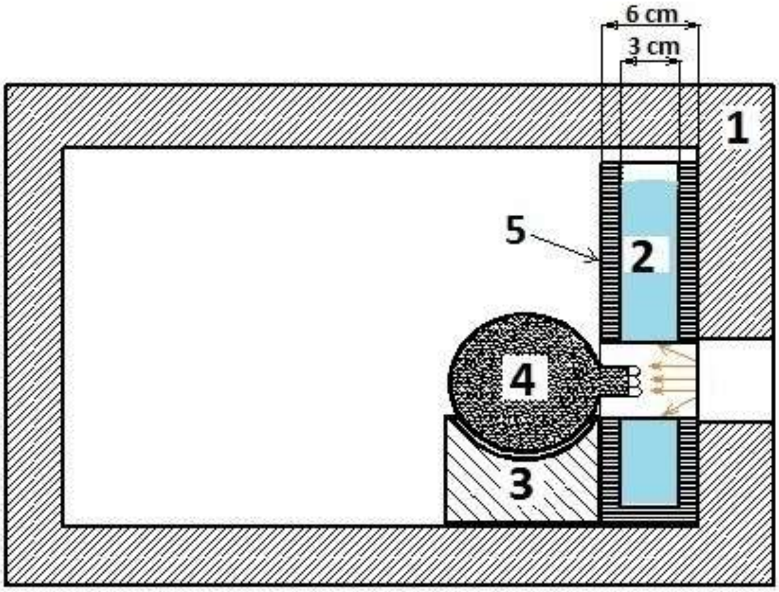

Fig. 2. Cross-sectional view of the microwave heating setup: (1) oven chamber, (2) water load, (3) sample holder, (4) sample, (5) water container.

Fig. 3. Immediate effect of microwave heating on carob moth larva body (3rd instar, 900 W, 4 min).

CONCLUSION

In this study, the significant effect of microwave heat treatment on carob moth mortality in artificially infested pomegranate fruits was studied. The mortality of 720 W-6 min treated sample is about 100 % while quality parameters have no significant differences in comparison with storage control treatment. Higher level of microwave power and treatment time creates higher temperatures and more carob moth mortality but may cause over heating damages.

Conflict of interest

The authors have no conflict of interest to report.

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