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Preliminary in Vitro Tests for Microwaves as a Post-harvest Disinfestation Treatment on Fruit Flies

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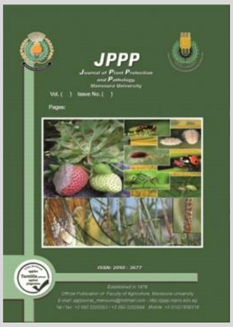

ABSTRACT

In vitro preliminary tests executed to evaluate the possibility of using microwave to disinfest fruits as a postharvest quarantine treatment using a cocking microwave oven. Subjecting the eggs, 1st, 2nd and 3rd larval instars of both medfly and peach fruit fly for 10, 15, 25 and 30 seconds and estimate the mortality percentage. Results revealed that there were a gradual increase in mortality percentage for the larval instars for both flies, eggs and larval instars of peach fruit flies have a higher heat tolerance than medfly stage consequently, lower mortality percentage for the same time period microwave treatments. Also, revealed that eggs of fruit flies (medfly and peach fruit fly) have a higher resistance to heat than larval instars. Thus, Microwave can be a very efficient tool for disinfesting treatments of fruit flies since it can give a very fast rise in temperature in very short time which is a very important parameter in qualifying postharvest quarantine method to avoid fruit damage, still a challenges are presents for developing microwave processes to be applicable as a postharvest quarantine treatment.

Keywords: *Ceratitis capitata*, *Bactrocera zonata*, Diptera: Tephritidae, Microwave, postharvest and quarantine treatment.

INTRODUCTION

Due to their high polyphagia, great adaptation to various climatic regions, and fast reproduction, many species of fruit flies (Diptera: Tephritidae) are a serious threat pests of horticultural crops worldwide, (Sarwar, 2015). They cause indirect damage to fruits and vegetables (female oviposition punctures) and direct damage (larval activities inside the fruit) (Aluja, 1994). Leading to high reduction in the trading and exportation of horticultural crops and losses of 40% up to 80% of the yield (Kibira *et al*., 2010). And make importing countries to force a strong quarantine restrictions thus limits access to international markets (Lanzavecchia *et al*., 2014). Also, exporting countries spend too much money yearly on control of these pests, and has strict trade penalties for applying these quarantine restrictions (Dhami *et al*., 2016). larvae destruct fruits and drop to pupate in the soil, thus protected from insecticides applied to fruit surface making management of fruit flies challenging (Heve *et al*., 2016). Strong restrictions on insecticides usage and increasing demand for healthy food around the world, a broad-spectrum of insecticides are removed from the market, leading to increased difficulty of fruit flies control (Böckmann *et al*., 2014), and consequently, arising environmentally friendly new techniques for control of fruit fly (Navarro-Llopis *et al*., 2011). including: fumigation with fumigants (MeBr) (Australian Government, 2014), dipping in pesticide solutions (fenthion), storage in controlled atmosphere, cold treatments (Amstrong & Mangan, 2007), Heat treatments (hot water dips, vapour heat or hot dry air) (Corcoran *et al.* 1993), ionizing radiation (ISPM No 18, 2003), and treatment with electromagnetic energy (Tang *et al.* 2000) also, combinations of some of these treatments (Varith, Sirikajornjaru, & Kiatsiriroat, 2007). it is needed to determine the thermal exposure necessary to kill fruit fly stages to meet quarantine security levels, this is by determine times and temperatures used in commercial heat treatments in vitro, then, testing infested fruits (Gazit *et al.* 2004). The temperatures used in the heat treatment procedure have been determined by : 1-testing in vitro the thermal level necessary to kill fruit flies stages. 2 - test the infested fruits to determine probit values (99.9968% mortality) required to meet levels of quarantine security. Temperatures above 40C° are needed against fruit flies larvae (Heard *et al.* 1991; Mangan and Ingle 1992, 1994; Nascimento *et al.* 1992; Sharp and Hallman 1992; Sharp and Gould 1994). Gamage *et al.* 2015 proved that microwave heat is effective for insect disinfestation without any negative impact on total soluble solids, flesh or peel firmness of the treated apples.. Jones 2008 described microwave heating as the use of the electromagnetic waves and it involves heat transfer to beam up the material exposed to its radiation. Its frequency range is from 300 MHz to 300 GHz and it lies between radio frequencies and infrared radiation. The rapidly varying electric and magnetic fields lead to the occurrence of heating. Electric field induced by the microwave field will cause dipolar molecules such as water and fat to oscillate back and forth and lead to heat dissipation in the material exposed (Kueon 2008). heating by RF or microwave is suggested as an alternative successful method for post harvesting quarantine protocol (Tang *et al.* 2000). RF and microwave heating in which direct interactions between dielectric materials and electromagnetic waves generate heat. because of the dielectric properties of insects, heat larvae inside the fruit before the surrounding plant tissue. Microwave heating is most efficient on water and much less on sugars and fats those have less molecular dipole moment (Sutar and Prasad 2008). Sharp *et al.* 1999; Tang *et al*., 2000

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and Ekman & Pristijono, 2010, used non-ionizing radiation in the form of electromagnetic waves (radio frequency and microwaves) for faster heating of fruits. Microwaves have been applied to a wide range of products, from soil and museum artifacts to fresh fruits specially, pests of grain and stored products (Nelson 1973; Roseberg and BÖgl 1987; Nelson *et al*. 1998; Wang and Tang 2001). (Wang and Tang, 2001) proposed an promising technique using radio frequency and microwave heating treatments as an alternative quarantine treatment. (Andreuccetti *et al*. 1994; Hallman and Sharp 1994; Nelson 1996; Tang *et al*. 2000; Wang *et al*. 2001a, 2002, 2007a, b) showed in their work that using of (RF) can present the required homogeneous and fast heat treatment against insect pest. The use of non-ionising radiation in the form of electromagnetic waves such as radio frequency and microwaves for faster heating of fruits has been the subject of several recent studies (Robertson & Preisler, 1999; Sharp *et al.* 1999; Tang *et al*., 2000 and Ekman & Pristijono, 2010). Electromagnetic waves penetrate the fruit and the energy is internally dissipated into thermal energy volumetrically heating the fruit. despite of heating of the whole fruit is theoretically possible, but uneven heating during the treatment often caused due to some practical hindrances such as, heterogeneous fruit structure and its varying thermal and dielectric properties, non-uniform electric fields in microwave cavities, among other microwave inherent factors (Ikediala *et al*., 2000; Knoerzer, Regier, & Schubert, 2008). An amazing event of using the microwave energy to control fruit flies is the insects are faster rate heating than the fruit they infest due to high moisture content of insects. So, it is possible to heat the invading stage to a lethal temperature while leaving the drier foodstuff unaffected or slightly warm (Vadivambal *et al*. 2006).

The current work aim to Initial tests in the laboratory to evaluate the possibility of using microwaves to eliminate any fruit flies *Ceratitis capitata* and *Bactrocera zonata*, as a quarantine measure as part of the post-harvest treatment.

MATERIALS AND METHODS

Fruit flies strains

Mediterranean fruit fly lab strain (Horticultural Crop Insects Research Department - plant protection research institute) that reared upon an artificial larval diet composed of Sugar (as a source for carbohydrate) 8.45 %, Dried sterile yeast (as a source for protein) 8.45 %, Wheat bran (as a bulking agent) 33 %, Sodium benzoate 0.3 %, Citric acid 0.3 % and Water 50 %. The adult flies feed normally upon water, sugar and hydrolyzed protein (4:1).

Microwave oven specifications :

GMS cooking microwave oven
Model number: ANS – 20E
Rated voltage : 220 V – 240 V
Rated frequency: 50 Hz
Input power : 1100W
Output power : 700 W

Egg treatments:

A 100 eggs placed in tab water (80 ml^3) were subjected to microwave beam for periods of 10, 15, 25 and 30 seconds and left for 3 days to ensure egg hatching (if still life), then examined for hatching and estimate mortality percentage.

Larval treatments

A 100 larvae of the examined instar placed in tab water (80 ml3) were subjected to microwave beam for periods of 10, 15, 25 and 30 seconds, then examined and estimate mortality percentage.

Statistical analysis

Results subjected to statistical analysis using IBM SPSS statistics version 23 one-way ANOVA test and charts executed by Microsoft excel 2010 .

RESULTS AND DISCUSSION

Results

1- Medfly

Data in Table (1), clarified that, the percent mortalities of medfly including (eggs, 1st, 2nd and 3rd larval instars) subjected to microwave beam for 10, 15, 25 and 30 seconds**.**

Table 1. Percent mortalities of medfly subjected to microwave beam for 10, 15, 25 and 30 seconds

	Mortality percent of medfly			
time	**Eggs**	**1st instar**	**2nd instar**	**3rd instar**
10 sec	42.4±0.374 k	54.6±0.812 h	50±0.547 i	44.6±0.510 j
15 sec	51.2±0.374 i	65.4±0.510 f	61.8±0.374 g	60±0.707 g
25 sec	61.8±0.583 g	76.2±0.374 c	73.4±0.245 d	68.2±0.374 e
30 sec	89.6±0.510 b	100±0.000 a	100±0.000 a	99.4±0.400 a

Eggs:

Results obtained in Table (1), revealed that percent mortality of eggs subjected to beam of microwaves was 42.4 %, 51.2 %, 61.8 % and 89.6 when subjected to 10, 15, 25 and 30 seconds respectively with a significant differences among the four treatments.

1st instar:

In case of 1st instar larvae, results gave a similar trend, since there were a gradual increase in the mortality percentage with a significant differences among the four treatments beginning with a mean of 54.6 % for 10 sec treatment, then 65.4 % by 15sec, raised to 76.2 % when subjected to 25 seconds and get 100 % mortality in 30 sec. (Table 1).

2nd instar

Also in case of 2nd larval instar, also, a gradual increase in the mortality percentage with a mean of 50 % for 10 sec treatment, 61.9 % by 15sec, 73.4 % when subjected to 25 seconds and get 100 % mortality in 30 sec. this with a significant differences among the four treatments (Table 1).

3rd instar

Data presented in Table (1), showed that for the 3rd larval instars, percent of mortality due to 10 seconds of microwave treatment give a mean of 44.6 %, 60 % with 15 seconds, 68.2 % by 25 seconds and 99.4 % mortality when subjected for 30 seconds with a significant differences among the four treatments.

Also data obtained in Table (1), revealed that resistance for heating by microwave increased gradually from 1st to 3rd larval instars, but eggs (with 42.4 % mortality) have a higher resistance for heating than larval instars (54.6 % for 1st, 50 % for 2nd & 44.6 % for 3rd instars) when subjected to 10 seconds. The same in case of 15 seconds treatments, eggs (with 51.2 % mortality) have a higher resistance for heating than larval instars (65.4 % for 1st, 61.8 % for 2nd & 60 % for 3rd instars) and for 25 seconds, eggs with 61.8 %

mortality, higher resistance for heating than larval instars (76.2 % for 1st, 73.4 % for 2nd & 68.26 % for 3rd instars) also for 30 seconds, eggs have a higher resistance for heating than larval instars with 89.6 % mortality but 100 % for 1st, 100 % for 2nd & 99.4 % for 3rd instars.

2- Peach fruit fly

Data in Table (2), clarified that, the percent mortalities of peach fruit fly including (eggs, 1st, 2nd and 3rd larval instars) subjected to microwave beam for 10, 15, 25 and 30 seconds.

Table 2. Percent mortalities of medfly subjected to microwave beam for 10, 15, 25 and 30 seconds

Mortality percent of medfly				
time	**Eggs**	**1st instar**	**2nd instar**	**3rd instar**
10 sec	43.6±0.510 i	54.2±0.374 g	51.8±0.490 g	47.88±0.392 h
15 sec	60.2±0.860 f	74.2±0.800 c	67.8±0.860 d	65±0.548 e
25 sec	64±0.548 e	82.4±0.872 b	74.6±0.510 c	70.6±0.510 d
30 sec	84.2±0.374 b	100±0.000 a	100±0.000 a	98.2±0.374 a

Eggs:

Results obtained in (Table: 2) revealed that percent mortality in eggs subjected to beam of microwaves was 43.6 %, 60.2 %, 64 % and 84.2 when subjected to 10, 15, 25 and 30 seconds respectively with a significant differences among the four treatments.

1st instar:

In case of 1st instar larvae, results gave a similar results, since there were a gradual increase in the mortality percentage with a significant differences among the four treatments beginning with a mean of 54.2 % for 10 sec treatment, then 74.2 % by 15sec, raised to 82.4 % when subjected to 25 seconds and get 100 % mortality in 30 sec. (Table 2).

2nd instar

Also in case of 2nd larval instar, there were a gradual increase in the mortality percentage with a mean of 51.8 % for 10 sec treatment, 67.8 % by 15sec, 82.4 % when subjected to 25 seconds and get 100 % mortality in 30 sec. this with a significant differences among the four treatments (Table 2).

3rd instar

Results in Table (2), cleared that for the 3rd larval instars, percent of mortality due to 10 seconds of microwave give a mean of 47.8 %, 67.8 % with 15 seconds, 70.6 % by 25 seconds and a 96.2 % mortality when subjected for 30 seconds with a significant differences among the four treatments.

Also data obtained in Table (2), indicated that resistance for heating by microwave increased gradually from 1st to 3rd larval instars, but eggs (with 43.6 % mortality) have a higher resistance for heating than larval instars (54.2 % for 1st, 51.8 % for 2nd & 47.88 % for 3rd instars) when subjected to 10 seconds. The same in case of 15 seconds treatments, eggs (with 60.2 % mortality) have a higher resistance for heating than larval instars (74.2 % for 1st, 67.8 % for 2nd & 65 % for 3rd instars) and for 25 seconds, eggs with 64 % mortality, higher resistance for heating than larval instars (82.4 % for 1st, 74.6 % for 2nd & 70.6 % for 3rd instars) also for 30 seconds, eggs have a higher resistance for heating than larval instars with 84.2 % mortality but 100 % for 1st, 100 % for 2nd & 98.2 % for 3rd instars.

3- Comparison between Medfly and Peach fruit fly

Collective histograms (Fig 1, 2, 3 & 4) showed that percent mortality of medfly treated stages (eggs, 1st, 2nd and 3rd instar larvae) have a higher mortality percentage than the corresponding stages of the peach fruit fly, i.e. peach fruit fly have a higher resistance to heating than medfly.

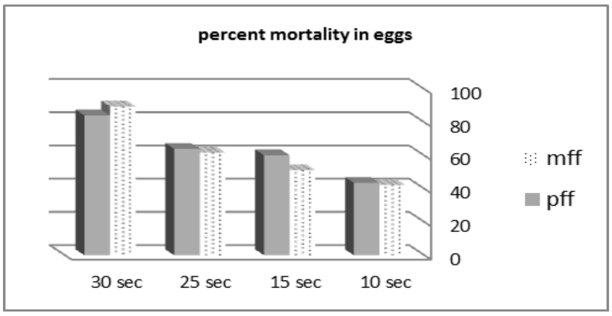

Fig. 1. Percent mortalities of fruit flies eggs subjected to microwave beam for 10, 15, 25 and 30 seconds

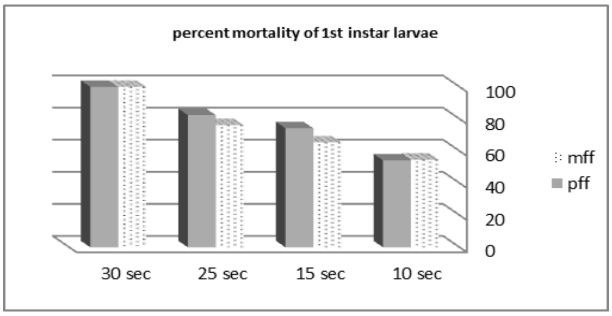

Fig. 2. Percent mortalities of fruit flies 1stlarval instars subjected to microwave beam for 10, 1525 and 30seconds

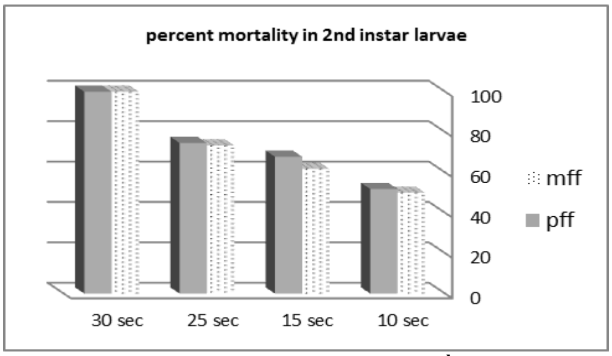

Fig. 3. Percent mortalities of fruit flies 2nd larval instars subjected to microwave beam for 10, 15, 25 and 30 seconds

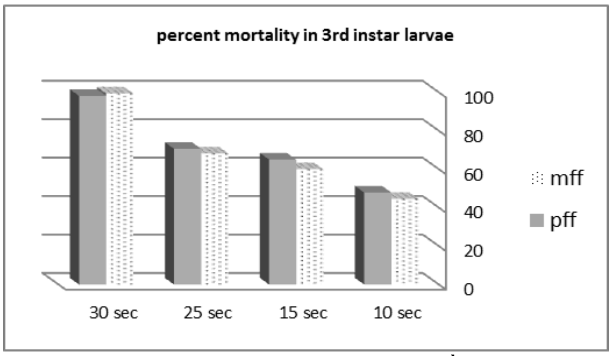

Fig. 4. Percent mortalities of fruit flies 3rd larval instars subjected to microwave beam for 10, 15, 25 and 30 seconds

Egg stage of peach fruit fly have a mortality means of 42.4 %, 51.2 %, 61.8% & 89.6% for treatment of 10, 15, 25 & 30 seconds while means of medfly were 43.6 %, 60.2 %, 64 % & 84.2 % for the same time periods respectively. 1st larval instars for PFF also have a higher heat resistance than medfly, 54.6 %, 65.4 %, 76.2 % and 100 % for PFF and 54.2 %, 74.2 %, 82.4 % and 100% for medfly, in case of 2nd larval instar 50 %, 61.8 %, 73.4 % and 100 % for PFF and 51.8 %, 67.8 %, 74.6 % and 100 % For medfly and for 3rd larval instars, 44.6 %, 60 %, 68.2 % and 99.4 % for PFF and 47.88 %, 65 %, 70.6 % and 98.2 % for medfly.

CONCLUSION

It can be concluded from the obtained results throughout the whole experiments important events:

1- Microwave can be a very efficient tool for disinfesting treatment soft fruit flies since it can give a very fast rise in temperature in very short time which is a very important parameter in qualifying postharvest quarantine method to avoid fruit damage.
2- Eggs of fruit flies (medfly and peach fruit fly) have a higher resistance to heat than other larval instar (may be due to presence of egg shell).
3- There were gradual increases in mortality percentage for the larval instars for both flies.
4- Eggs and larval instars of peach fruit flies have a higher heat tolerance than medfly stage consequently, lower mortality percentage for the same time period microwave treatments.
5- Still challenges are presents for developing microwave processes to be applicable as a postharvest quarantine treatment.

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إختبارات معملية أولية لإستخدام أفران الميكروويف كعلاج لتطهير ذباب الفاكهة بعد الحصاد

طارق عبد العاطى عبد الحفيظ ، عبدالله على الذوق و أكرم شوقى عبد المعبود
معهد بحوث وقاية النباتات ـ مركز البحوث الزراعية ـ الدقى ـ الجيزة ـ مصر

تم إجراء اختبارات أولية في المختبر لتقييم إمكانية استخدام الميكروويف للتخلص من أي اطوار لذباب الفاكهة كأجراء للحجر الزراعي ضمن معاملات ما بعد الحصاد. و قد تم تطبيق اقل مستوى طاقة لجهاز الميكروويف (ازالة الجليد) لمدة ١٠ ، ١٥ ، ٢٥ و ٣٠ ثانية على البيض ، الطور الأول ، الثاني ، والثالث ليرقات كل من ذبابة ثمار الخوخ وذبابة فاكهة البحر المتوسط و قد أوضحت النتائج أن هناك زيادة تدريجية في نسبة موت اليرقات لكلتا الذبابتين كذلك وجد ان البيض والأعمار اليرقية لذباب ثمار الخوخ تتحمل الحرارة بقدر أعلى من ذبابة فاكهة البحر المتوسط وبالتالي انخفاض نسبة الموت في نفس الفترات الزمنية من معاملات الميكروويف. كما اظهرت النتائج أيضًا أن بيض ذباب الفاكهة (ذبابة ثمار الخوخ وذبابة فاكهة البحر المتوسط) يتمتع بمقاومة أعلى للحرارة من عمر اليرقات. وبالتالي ، يمكن أن يكون الميكروويف أداة فعالة للغاية للتخلص من اصابات ذباب الفاكهة لأنه يمكن أن يؤدي إلى ارتفاع سريع جدًا في درجة الحرارة في وقت قصير جدًا وهو عامل مهم جدًا في معاملات الحجر الصحي لما بعد الحصاد لتجنب تلف الفاكهة ، ولا تزال هناك تحديات. لتطوير عمليات الميكروويف لتكون قابلة للتطبيق كمعاملات للحجر الزراعي و ما بعد الحصاد.