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Effectiveness and suitability of vapor heat treatment in disinfestation of export mango fruit, cultivar Abu Samaka, from fruit flies

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ABSTRACT

Sudan has a great potential for the export of mango (*Mangifera Indica* L.) but fruit flies, mainly *Bactrocera dorsalis* (Hendel), *Ceratitis cosyra* (Walker), *C. capitata* (Wiedmann) and *B. zonata* (Saunders) are threatening the export industry. The countries importing mango require disinfestation treatment against fruit flies as a quarantine regulation. Effectiveness and suitability of vapor heat treatment (VHT) for disinfestation of the Sudanase mango cultivar Abu Samaka were undertaken in this study. In the VHT, the relative humidity of the treatment chamber was maintained at 99.7% and the temperature of the fruit pulp was raised gradually to reach 46.7°C in 5 hours then kept at this degree for 30 minutes before hydro-cooling for 20 minutes. For evaluation of the effectiveness of the treatment, naturally and artificially infested fruits were examined for fruit flies after treatment and compared with their respective untreated samples. To assess suitability of the treatment with respect to quality of the mango fruit, respiration rate, peel color, weight loss, flesh firmness, ascorbic acid content, total soluble solids, titratable acidity and reducing sugars were measured in the treated and control fruits. The VHT was found effective in disinfestation of the mango cultivar Abu Samka from fruit flies and did not adversely affect the fruit market quality and increased the shelf life.

INTRODUCTION

In the Sudan, mango (*Mangifera Indica* L.) is an important fruit for local consumption and export. It is produced almost all year round in different parts of the country. It is commercially grown in South Kordofan, Sinnar, Blue Nile, Northern, Gadarif, Gezira, South Darfour, West Darfur, Kassala, River Nile, Khartoum and White Nile States (El Hag, 2014). The total area under mango and the total production were estimated at 29.9 thousand ha. and 641 thousand tons, respectively (Elhassan, 2014). About 57 cultivars were reported to exist in the country and these cultivars are categorized into three groups: True Indian cultivars, Egyptian seedling cultivars of Indian origin such as Zibda, Alphons, Malgoba and Hindibesinara, and Sudanese seedling cultivars of Indian origin of high quality including Shendi, Taimoor, Nailm, Mabroka, Debsha and Abu Samaka (El Hag, 2014; Sudan Trade Point, 2016). The varieties, Haden, Kent, Sensation and Sabrin are newly introduced ones. In the year 2011, the mean export amounts were 4625 tons during the years from 2000 to 2005 and 1466 tons during the years from 2005 to 2010 (El Hag, 2014). The main markets for the Sudanese mango are Saudi Arabia, Syria, Lebanon, Jordan, Egypt, Emirates, Qatar and the European markets (Sudan Trade Point, 2016). The decrease in the Sudanese mango export during the period after 2005 could be attributed in part to the import ban made on Sudanese mango as a phytosanitary measure against fruit flies.

The main fruit fly species of mango in the Sudan are the Mediterranean fruit fly (*Ceratitis capitata* Wiedmann), the mango fruit fly (*C.cosyra* Walker), the Asian fruit fly (*Bacterocera dorsalis* Hendel) and the peach fruit fly (*B. zonata* Saunders) .The later two species were introduced into the country during the last decade (Mohamed and Ali 2008; Salah *et al*., 2012).

Fruit flies are serious pests of fruits and vegetables. In addition to losses, they are major limiting factors in accessibility to export markets. Many countries have imposed quarantine restrictions on the import of products from countries infested with particular fruit fly species and/or require disinfestation treatments of the products before importation is allowed (JAFTA, 2009; Vargas *et al.*, 2015 ; FAO, 2016).

In recent years, either vapor heat or cold temperature is mainly used for the control of fruit flies (JAFTA, 2009), but cold temperature is unsuitable for mango treatment because of the fruit sensitivity to chilling injury (Collin *et al*., 2007). Hot water was also used for the treatment of mango against fruit flies (Anwar and Malik, 2007; Hernandez *et al*., 2012; Zhang *et al*., 2012).

Vapor heat treatment (VHT) was developed as a quarantine measure in the USA and Japan (Sinclair and Lindgren, 1955 ; JAFTA 2009). It is a method of heating fruit with air saturated with water vapor at temperatures of 40-50 °C to kill insect egg and larvae as quarantine treatment before fresh market shipment (Lurie, 1998). Temperatures higher than 45 °C kills eggs and larvae of fruit flies (Collin *et al*., 2007). Vapor heat treatment for disinfestations of mango from fruit flies is used in Philippines, Thailand, Taiwan, Australia, Hawaii and India. The treatment standards depend on the varieties (JAFTA, 2009). In general, the European Union Countries require VHT of mango either at 46.5 °C for 30 minutes or 47.5 °C for 20 minutes (Palta, 2016).

Several studies were carried out on the effects of heat treatment (VHT or Hot water treatment) on mango. Physical, physiological, and biochemical parameters were investigated (Mitcham and Mc Donald, 1992; Yahia and Perdo-Campos, 2000; Anwar and Malik, 2007; Le *et al.*,
2010; Hernandez *et al*., 2012; Zhang *et al*., 2012). In mango cultivars Tommy Attkins and Keitt, the VHT reduced the rate of fruit softening and mesocarp colour development and increased post harvest shelf life (Mitcham and Mc Donald, 1992). In a Taiwan native cultivar of mango treated with the VHT at 46.5 °C for 40 minutes and stored for 3 weeks at 3°C, the quality of the fruit was not affected (Le *et al*, 2010). The fruit variety and the degree of maturity are among the factors that influence the effect of VHT on the fruit (Sinclair and Lindgren, 1955).

This work was carried out to study the effectiveness and suitability of the VHT in disinfestation of the export mango cultivar, cv. Abu Samaka, from fruit flies.

MATERIALS AND METHODS Vapor heat treatment conditions

Five lots each 3 - 4 tons of mature green mango, cv. Abu Samaka, were treated separately in the facilities of the Sudan's Center for Sterilization of Horticultural Exports in Khartoum. The vapor heat treatment unit (EHK1000, Sanshu Sangyo Co. Ltd.,) was used. In the treatment, the relative humidity of the treatment chamber was maintained at 99.7% and the temperature of the fruit pulp was raised gradually to reach 46.7 °C in 5 hours and then kept at this degree for 30 minutes before cooling. Hydrocooling was used for 20 minutes and the treated fruits were removed to the ambient room temperature. Relative humidity in the chamber and fruit pulp temperature were monitored on screen and recorded at 5 minutes intervals by computer software. Probes of five temperature sensors (Pt100, Chino Co. Ltd.) were inserted in fruits distributed randomly at different places in the sterilization chamber to measure the innermost pulp temperatures of the treated fruits in the chamber. The sensors were calibrated before running the treatment.

Detection of disinfestation of mango fruits from fruit flies

Artificially infested fruits (12 fruits/treatment) were marked, randomly embedded within each treated lot and retrieved after treatment. The artificial infestation was made by exposure of mature fruits (30 -50% yellow) to colonies of the fruit fly (*B. dorsalis*) in the laboratory of the IPM Research and Training Center, Agricultural Research Corporation, Wad Medani, Sudan. The insect colonies were kept in 36×36×60 cm, wooden cages, with muslin cloth cover at sides and top glass cover under ambient temperature and humidity in the laboratory. The colonies were at least 10 days old and were fed on a diet of hydrolyzed mixture of pure baker´s yeast and sugar (1:3 vol./vol.) and provided with water as soaked in cotton wool on petri dishes (Ambele *et al*., 2012). The exposure time for the infestation was 24 hrs and in the 4th day from the exposure, the fruits were treated. Samples of artificially infested fruits were not treated and kept as control (sample size of 12 fruits). Besides the samples of the artificially infested fruits, pre- and post-treatment samples were taken from each treated lot for fruit fly detection (sample size of 12 fruits). In sampling for fruit fly detection, fruits with visible fruit fly oviposition punctures were considered. Some fruits from the lots were dissected and examined for fruit fly larvae immediately prior treatment and after. The fruit samples for detection of fruit flies were incubated in 25x25x36cm, wooden cages, with muslin cloth cover and top side glass cover, lined with pure sand in the bottom) under room

conditions for four weeks. Periodically the samples were examined for fruit fly larvae and living larvae were reared out to adult flies then counted and identified. Means and standard deviations (SD) of fruit fly numbers detected per treatment were calculated.

Fruit quality analysis

For fruit quality analysis, random fruit samples of 36 pieces were taken from every pre- and post- treatment of four lots. The fruit quality analysis and shelf life study were carried out in the National Food Research Center, Khartoum North. The fruit samples were stored at 13º±1 °C and 85-90% RH and the observations were taken every five days during storage. Respiration rate, peel color, weight loss, flesh firmness, ascorbic acid content, total soluble solids (TSS), titratable acidity (TA) and reducing sugars were measured. Replicates of 10 fruits/ treatment time were taken pre and post treatment for respiration rate, peel color and weight loss assessments. In analysis of flesh firmness, ascorbic acid content, TSS, TA and reducing sugars, three fruit replicates/analysis period during storage / treatment time were used. The observations on respiration rate, ascorbic acid content, total soluble solids (TSS), titratable acidity (TA) and reducing sugars covered 20 days of storage and that on peel color, weight loss and flesh firmness extended for 30 days. The methods of analysis used were as follows:

Respiration rate: The total absorption method was used (Mohamed-Nour and Abu-Goukh, 2010) and respiration rate was expressed in mg CO_2/kghr.

Peel color: Mango skin color guide (Queensland Government, Department of Primary Industries, Horticulture Australia, 2012) was used. The color scores were: 0- 10% yellow,1; 10-30% yellow,2; 30-50 yellow,3; 50-70% yellow,4; 70-90% yellow,5; and 90-100% yellow,6.

Weight loss: A digital sensitive balance was used to determine fruit weight. Weight loss percentage was determined according to the formula: $W_1 = [(W_0 – W_t)/W_0] \times 100$ where W_1 is the percentage weight loss, W_0 is the initial weight of fruits at harvest and W_t is the weight of fruits at the designated time.

Flesh firmness: Measured by Magness and Taylor firmness tester (D. Ballauf Meg. Co.), equipped with a 10 mm-diameter plunger tip. Two readings were taken from opposite sides of each fruit after the peel was removed, and expressed in kg/cm².

Total soluble solids: Measured directly from the fruit juice extracted by pressing the fruit pulp in a garlic press, using a kruss hand refractometer (model HRN-32). Two readings were taken from opposite sides of each fruit and the mean values were calculated and corrected according to the refractometer chart.

Ascorbic acid content: Determined in fruit pulp extracts using the 2, 6 - dichlorophenol-indophenol titration method of Ruck (1963).

Titratable acidity: Thirty gram of fruits pulp of the three fruits used for flesh firmness and TSS determination were homogenized in 100 ml of distilled water for one minute in a Sanyo Solid State blender (model SM 228p) and centrifuged at 10000 rpm for 10 minutes using a Gallenkamp portable centrifuge (CF- 400). The volume of supernatant, which constituted the pulp extract, was determined. Titratable acidity was measured according to the method described by Ranganna (1979) and expressed as percent citric acid.

Reducing sugars**:** Determined in the pulp extract from each treatment according to the technique of Somogyi (1952).

Statistical analysis

Analysis of variance (ANOVA), followed by fisher's protected LSD test at P≤ 0.05 were performed on the data of the fruit quality parameters (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Table 1 shows the effectiveness of the VHT on disinfestation of mango fruit cv. Abu Samaka from fruit flies. No fruit flies were detected in the naturally or artificially infested fruits after VHT in the four times of treatment. The fruit fly *B. dorsalis* was frequently detected in all untreated control samples either naturally or artificially infested. Dissection of some fruits from the treated lots immediately after the treatments showed that larvae of fruit flies were dead as a result of VHT treatments. The VHT at 46.7 °C for 30 min. disinfested the treated fruits from fruit flies. As mentioned by Collin *et al.* (2007), temperature higher than 45 °C kills eggs and larvae of fruit flies. The conditions of VHT in this experiment were similar to the standard of the European Union for disinfestation of mango from fruit flies (Palta, 2016).

Table 1. Effectiveness of vapor heat treatment in disinfestation of mango fruit, cv. Abu Samaka, from fruit flies.

Treatments	No. of fruit flies ± SD	Species of fruit fly
VHT of naturally infested fruits	0	-
Untreated naturally infested fruits	7.5 ±2.5	*B. dorsalis*
VHT of artificially infested fruits	0	-
Untreated artificially infested fruits	297±49	*B. dorsalis*

Tables 2 to 8 show the results of the quality analysis made for the vapor heat treated and untreated mango fruits. The VHT delayed the onset of the climacteric peak of respiration by five days in the treated fruits compared to that in the untreated fruits. Climacteric peak of 159.1 mg CO_2 / Kg – hr was reached in the untreated fruits in day 10 of storage while climacteric peak of 156 mg CO_2 / Kg – hr was reached in the treated fruits in day 15 of storage (Table 2). Heat treatment, depending on temperature and length of exposure, can decrease or increase the climacteric respiration peak as well as advancing or delaying it. When fruits were returned to ambient conditions, often the respiration is lower than the non-heated fruits (Lurie, 1998).

Table 2. Respiration rate (mg CO_2 / kg – hr) of vapor heat treated and untreated mango fruit, cv. Abu Samaka during storage (13±1°C and 8590% RH).

Time of storage (days)	Treated	Untreated	SE (±)	Significance
0	45.2	45.6	0.91	NS
5	89.0	95.2	0.43	***
10	142.0	159.1	4.05	*
15	156.0	136.4	1.01	***
20	125.2	83.6	1.98	***

*, *** Significant at P = 0.05 and 0.001, respectively.

The heat treatment retarded the development of the peel color. Peel color scores of 5.9 (about full yellow) and 4.21 were attained in day 20 of storage in the untreated and treated

fruits, respectively (Table 3). In further observations, peel color scores of 5 and 5.8 were reached, respectively, in 25 and 30 days of storage in the treated fruits. In vapor heat treated Keitt mango (at 46°C), the mesocarp color was reduced (Mitcham and McDonald, 1993). Also, the peel color was maintained in a Taiwan cultivar of mango when vapor heat treated at 46.5 °C for 40 min and stored at 3°C. (Le *et al*., 2010).

Table 3. Peel color of vapor heat treated and untreated mango fruit, cv. Abu Samaka during storage (13±1°C and 85-90% RH).

Time of storage (days)	Treated	Untreated	SE (±)	Significance
0	1.0	1.0		NS
5	1.6	2.8	0.18	*
10	2.4	4.3	0.43	*
15	3.4	5.2	0.34	*
20	4.2	5.9	0.44	*

* Significant at P≤0.05

Score: 1 = 0 - 10 % yellow, 2 =10 - 30% yellow, 3 =30 - 50% yellow, 4 = 50 -70% yellow, 5 = 70 - 90% yellow, and 6 = 90 - 100% yellow

Weight loss was not significantly different between treated and control mango fruits during 10 days of storage but at day 20, weight loss was higher in the control fruits (14.9%) compared to that in the treated fruits (8.17%) (Table 4). Weight loss of 13.6% was found by further observations in the treated fruits in day 30 of storage. In Kent mango treated with hot water at 52 °C for 10 min, less weight loss was recorded than in untreated fruits (Woldeselassie *et al*. 2015).

Table 4. Weight loss (%) of vapor heat treated and untreated mango fruit, cv. Abu Samaka during storage (13±1°C and 85-90% RH).

Time of storage (days)	Treated		Untreated		SE (±)		Significance
0	0		0				NS
5	2.6		2.5		0.51		NS
10	3.2		5.6		1.08		NS
15	5.0	10.4	0.57	** 20	8.2	14.9	0.31 ***

, * Significant at p = 0.01 and 0.001, respectively. NS, not significant.

Fruit softening was reduced in the vapor heat treated fruits. Flesh firmness of 0.47 and 0.23 Kg/cm² were recorded in day 20 of storage, respectively, in the treated and untreated fruits (Table 5). In 25 and 30 days of storage, firmness of 0.28 and 0.15 Kg/cm² were attained

orderly in the treated fruits. Reductions in rate of softening in mango fruits treated with vapor heat were reported by Micham and Mc Donald (1993) and Le *et al* (2010).

Table 5. Flesh firmness (kg/cm²) of vapor heat treated and untreated mango fruit, cv. Abu Samaka during storage (13±1°C and 85-90% RH).

Time of storage (days)	Treated	Untreated	SE (±)	Significance
0	2.5	1.6	0.44	NS
5	1.8	1.2	0.25	NS
10	1.4	0.6	0.05	***
15	0.9	0.3	0.08	**
20	0.5	0.2	0.05	*

*, **, *** significant at p = 0.05, 0.01 and 0.001, respectively. NS, not significant.

The treated fruits maintained higher ascorbic acid contents during the storage than the untreated fruits (Table 6). There were no significant differences between the vapor heat treated and untreated fruits with respect to total soluble solids, titratable acidity (Table 7), and reducing sugars (Table 8). When Keitt, Kent and Tommy Attkins mango varieties were treated by hot air (at 40°C for 4h) followed by hot water treatment (at 50 °C for 5min.), there were no significant differences between the treated and control fruits in total soluble solids, titratable acidity and vitamin C contents (Mansour *et al*., 2006).

Table 6**.** Ascorbic acid content (%) of vapor heat treated and untreated mango fruit, cv. Abu Samaka during storage (13±1°C and 85-90% RH).

Time of storage (days)	Treated	Untreated	SE (±)	Significance
0	33.7	33.5	0.56	NS
5	29.2	24.7	1.18	*
10	26.4	18.9	1.38	*
15	19.5	16.3	1.56	NS
20	17.1	12.9	1.04	*

* Significant at P≤0.05.NS, not significant.

Table 7. Titratable acidity (%) of vapor heat treated and untreated mango fruit, cv. Abu Samaka during storage (13±1°C and 85-90% RH).

Time of storage (days)	Treated	Untreated	SE (±)	Significance
0	3.2	2.7	0.42	NS
5	2.6	2.0	0.44	NS
10	2.1	1.3	0.23	NS
15	1.4	0.7	0.18	NS
20	0.8	0.3	0.03	*

* Significant at P≤0.05.NS, not significant.

Table 8. Reducing sugars content (%) of vapor heat treated and untreated mango fruit, cv. Abu Samaka during storage (13±1°C and 85-90% RH).

Time of storage (days)	Treated	Untreated	SE (±)	Significance
0	3.0	3.1	0.15	NS
5	3.7	4.9	0.48	NS
10	4.3	5.2	0.23	NS
15	4.9	6.4	0.42	NS
20	5.5	6.9	0.29	*

* Significant at P≤0.05. NS, not significant.

Abu Samaka mango tolerated the VHT (46.7 °C for 30 min) without any heat injury and the market quality was not adversely affected. Similar results were obtained with Irwin and Tuu Shien mangoes treated with VHT at 46.5 °C for 40 min and at 46.5 °C for 30 min. respectively (Hasbullah *et al*., 2002 ; Le *et al*., 2010).

Shelf life of the vapor heat treated fruits was prolonged by 10 days compared to that of the untreated fruits. Mitcham and Mcdonald (1992) mentioned that mild heat stress may increase postharvest shelf life by reducing the rate of softening.

In conclusion, the vapor heat treatment with the specified timetemperature and RH% regime (46.7 °C reached gradually in 5 hrs then maintained for 30 min. and RH of 99.7% throughout the treatment period) was effective in disinfestation of mango cultivar Abu Samka from fruit flies. The treatment did not adversely affect the fruit market quality and increased the shelf life.

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الخلاصة

السودان لديه امكانات كبيرة في تصدير ثمار المانجو ولكن المانجو في السودان يصاب بأنواع من ذبابة الفاكهة اهمها ، ذبلبة ثمار المانجو (Saunders *B. zonata*) ، ذبابة الخوخ (*Bactrocera dorsalis*) (Hendelذبابة الفاكهة الشرقية والدول المستوردة (Wiedman *C. capitata* وذبابة فاكهة البحر الابيض المتوسط ((Walker *Ceratitis cosyra* للمانجو تطلب تطهير الثمار من ذبابة الفاكهة كإجراء للحجر الزراعي. في هذه الدراسة اجريت التجارب لمعرفة فاعلية وملائمة المعاملة بالحرارة وبخار الماء لتطهير صادر ثمار المانجو ، الصنف ابو سمكة. تمت المعاملة برفع درجة حرارة لب الثمرة تدريجيا خلال نحو 5 ساعات الي 46.7 درجة مئوية ثم استمرار المعاملة عند هذه الدرجة لمدة 30 دقيقيه قبل التبريد المائي لمدة 20 دقيقة ومن ثم فتح غرفة المعاملة لحرارة الهواء الجوي. طيلة فترة المعاملة تم الحفاظ علي الرطوبة النسبية عند 99.7 % داخل غرفة المعاملة. للتأكد من الفاعلية والملائمة اجري الفحص الحشري مخبريا لعينات ثمار معاملة واخري غير معاملة وتمت دراسة معدل تنفس الثمرة، لون القشرة، الفقد في الوزن، الصلابة، محتوي حامض الاسكوربيك، المواد الصلبة الذائبة الكلية، الاحماض الكلية و السكر المختزل في الثمار المعاملة مقارنة بالثمار غير المعاملة وكذلك عمر الرف. اظهرت الدراسة ان المعاملة كانت ذات فاعلية في التطهير من ذبابة الفاكهة دون تغيير سالب علي جودة الثمار مع زيادة عُمر الرف للثمار.