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Hot water as an effective post harvest disinfestant for the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) on mango

ABRAHAM VERGHESE, D. K. NAGARAJU and K. SREEDEVI

Division of Entomology and Nematology
Indian Institute of Horticultural Research, Bangalore - 560089, India
E-mail: avergis@iihr.ernet.in

ABSTRACT: Oriental fruit fly (OFF), *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is an important quarantine pest affecting a wide variety of fruits and vegetables and the loss in mango due to fruit fly is about 25 percent. For exports of mango, the treatments for the management of OFF should ensure 99.99683% control (by 'probit 9' standards). Heat treatment of infested fruits is known to ensure this standard. However, an optimum temperature-time regimen needs to be worked out for different varieties under Indian conditions. To achieve this, a series of studies was conducted at the Indian Institute of Horticultural Research (IIHR), Bangalore, India ($12^0$58'N; $77^0$35'E) from 2003 to 2008. The results revealed that, eggs showed total mortality at temperature-time regimes of 45^0C- 60 min and 48^0C- 45min & 60 min. The first instar maggot was completely susceptible only at 48°C-60 min. The second instar maggots succumbed to 100% mortality at 45^0C & 48^0C for 45min & 60min. Whereas, third instar recorded 100% mortality at 48°C for 45 and 60 minutes. These results were further supported by regression analysis between temperature and duration of exposure. Further, the temperature regimen 48°C – 60 minutes was validated on different varieties viz., Alphonso, Banganapalli, Neelum and Totapuri at different location under farmers' conditions yielded cent per cent disinfestations of OFF on mango. Organo-leptic tests showed that the fruit quality in terms of colour at ripening and taste was not affected.

Keywords: *Bactrocera dorsalis,* disinfestation, fruit fly, hot water, mango.

INTRODUCTION

The Oriental Fruit Fly (OFF), *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is an important pest on fruits and vegetables in India. The mean average crop loss due to OFF on mango is 25 percent with maximum loss occurring in South India (Verghese *et al.,* 2004). Besides the loss, fruit fly infestation has become a major export barrier, as importing counties like USA, New Zealand, Australia, Japan, etc. are wary of OFF being introduced to their countries. As mango is consumed fresh as a table fruit, toxic residues are not desirable. Therefore, non-chemical treatments are desirable and one of these is the heat treatment.

Our objective was to standardize a temperature-time regime for Indian variety of exportable mango Alphonso, and a processing variety Totapuri, using leads from the earlier available literature for a temperature ranging between 45 to 52°C, at appropriate time intervals. This study assumes significance as certain states of America has experienced interest in thermal susceptibility of *Bactrocera* spp.

MATERIALS AND METHODS

A series of laboratory studies was conducted from 2003 to 2005 to evaluate the effects of hot water as a post harvest disinfectant for the OFF. The results were validated during 2006-2008 at farmers' level. Two types of experiments were conducted in the fruit entomology laboratory of the Indian Institute of Horticultural Research (IIHR), Bangalore, South India ($12^0$58'N; $77^0$35'E). In the first type, the known stages of the fruit fly were placed into the fruit, sealed and treated (Table 1). In the second type, the fruits were exposed to caged gravid females for oviposition, and were treated at different temperature –time regimes (Table 2).

For the study OFF was mass reared on banana (cv *Elakki*) as standardized by Jayanthi and Verghese (2002) at the insectary at 28±1°C. The stock culture was from fallen infested fruits of mango in the month of March of each year. Second generation onwards age-wise collection of fruit flies was maintained in separate cages, with each cage having at least 100 flies in 50♀: 50♂ ratio. Gravid females from these were exposed again to semi ripe banana. The immature stage were then collected after oviposition at intervals of 1 day, 3 days, 7 days and 10 days to obtain eggs, first, second and third instar maggots, respectively.

Experiment I

The effect of temperature-time on different stages of the fruit fly was evaluated in five trials (Table 1). Different stages of *B. dorsalis* were collected and introduced into mature unripe fruits of cv. Totapuri, 24 hr after harvest. Ten eggs or larvae were placed in each

fruit by making scoops and the scoop was later closed. Each fruit with ten immature stages formed a replicate. Each replicate had a parallel control and each fruit with 10 immature stages was placed in water at ambient room temperature (25±2^{0}C). Thus, each replication had 20 eggs (including parallel control). There were 8 treatments (Table 1) with ten replications, with a parallel control for each replication.

Each fruit with the inserted immature stages was placed in a glass beaker (1000 cc) having hot water of required temperature. A 'L' shaped thermometer kept inside the beaker was used to monitor the temperature. Using a glass rod stirrer the water was gently agitated to ensure uniform distribution of hot water throughout the beaker.

After the known time interval, the fruits were removed from the beaker and allowed to cool. Then the eggs/maggots were removed from the fruits and placed on a tissue paper provided with water soaked cotton beneath. The eggs were observed for hatching up to a day after the control eggs hatched. Larvae were observed for mortality.

Experiment II

The studies were conducted for two years on two commercially cultivated varieties namely, Alphonso and Totapuri. For the study an orchard consisting of 25 trees of Alphonso and 50 trees of Totapuri was selected. Pre harvest IPM practices for the management of fruit flies namely, collection and destruction of fallen fruits, male annihilation using methyl eugenol, and a single cover spray of deltamethrin 0.0028% at 20 days prior to harvest were carried out (Verghese and Jayanthi, 2001) to get infestation-free fruits.

At harvest, four trees of each variety were randomly selected and the fruits were harvested. From the harvested fruits, 180 fruits were randomly selected from each variety, and grouped into six sets of 30 fruits each. Of these five sets were exposed to caged gravid females under laboratory conditions for 48 hr. The sixth group was used (without exposing to laboratory adults) to assess the extent of infestation in the IPM treated fields. A set not exposed to hot water served as control.

For laboratory exposure, each of the set (of 30 fruits) was placed in cage (1 m^3) having 20 gravid females for 48 hours. Of the five sets of exposed fruits, four were subjected to hot water treatment in a fabricated hot water bath (1000 l capacity) at different temperature- time regimes (Table 2). The remaining group was used to assess the extent of induced infestation under laboratory conditions and served as control. For every kilogram of fruit five litres of water was used. Fruits were weighed and proportionate volume of water was preheated to the required temperature. Once the water showed constancy of temperature, fruits were introduced for the required time-regimes.

After the known time interval, the fruits were shifted to cages at a room temperature of 24 - 27^{o}C. At the optimum ripening, each fruit was cut and carefully examined for the presence of larvae. The larvae present were counted and per cent infestation worked out and tabulated.

Quality acceptability quotient (QAQ)

Healthy fruits from each of these treatments were presented to a panel of 15 'fruit tasters'. They were asked to evaluate colour and taste on a scale of 1-5, 1=Excellent, 2=Very good, 3=Good, 4= Bad and 5=Very bad. Number of respondents to different categories was recorded as percentages (Table 3). Further the TSS in ^{0} Brix was also measured from 5 randomly selected fruits in each treatment.

Validation under farmers' field condition

The temperature time regimen of 48^{0}C for 60 minutes was further validated during 2006-2008 under farmers' conditions on varieties viz., Alphonso (Rayagada, Orissa state), Neelum (Trinvandrum and Trichur, Kerala state), Aphonso, Banganpalli and Totapuri (Bangalore, Karnataka state).

RESULTS AND DISCUSSION

There was 100 per cent mortality of eggs at the temp.-time regimes of 48^{0}C –60 min., 48^{0}C- 45 min., 48^{0}C –60 min., 52^{0}C-10 min, 52^{0}C-20 min. At 30 min duration the mortality was between 14 and 70 per cent for a temperatures 45 and 48^{0}C. The thermal mortality ranged between 60 and 70 per cent at 45^{0}C –45 min. Zero per cent mortality was recorded in control at all the temp-time regimes (Table 1).

First instar maggots recorded 100 per cent mortality of at the temp.-time regimes of 52^{0}C-10 min., and 52^{0}C-20 min. At 30 min. duration the mortality was between 8 and 40 per cent for temperatures 45^{0}C and 48^{0}C. Second instar maggots recorded 100 per cent mortality at temp.-time regimes of 45^{0}C-45 min., 45^{0}C-60 min and 48^{0}C-45 min., 48^{0}C –60 min. At 30 min. duration the mortality was zero percent for 45^{0}C and 48^{0}C. Zero per cent mortality was recorded at 52^{0}C – 10 min. At temp.-time regimes of 48^{0}C- 45 min. and 60 min. third instar maggots showed 100 per cent mortality. Zero per cent mortality was recorded at temp.-time regimes 48^{0}C-30 min, 45^{0}C for 30min, 45 min, and 60 min. and 52^{0}C

Table 1. Mortality (%) of *B. dorsalis* stages at different temperature-time regimes*

1.1. Eggs

Temperature	Time	2003				2004					
		Trial 1	Control	Trial 2	Control	Trial 1	Control	Trial 2	Control	Trial3	Control
45	30	44.00	0.00	58.00	0.00	50.0	0.00	54.00	0.00	52.30	0.00
	45	66.00	0.00	70.00	0.00	62.0	0.00	60.00	0.00	68.00	0.00
	60	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00
48	30	40.00	0.00	36.00	0.00	24.00	0.00	36.00	0.00	14.00	0.00
	45	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00
	60	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00
52	10	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00
	20	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00
CD (p=0.05)		**13.49**	-	**11.40**	-	**8.37**	-	**7.21**	-	**4.90**	-

*Mortality of eggs at all time intervals was zero in control

1.2. I instar maggots

Temperature	Time	2003				2004					
		Trial 1	Control	Trial 2	Control	Trial 1	Control	Trial 2	Control	Trial3	Control
45	30	14.00	0.00	16.00	0.00	18.00	0.00	12.00	0.00	8.00	0.00
	45	30.00	0.00	24.00	0.00	32.00	0.00	24.00	0.00	18.00	0.00
	60	38.00	0.00	42.00	0.00	44.00	0.00	36.00	0.00	28.00	0.00
48	30	40.00	0.00	36.00	0.00	24.00	0.00	36.00	0.00	14.00	0.00
	45	66.00	0.00	70.00	0.00	62.00	0.00	60.00	0.00	68.00	0.00
	60	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00
52	10	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00
	20	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00
CD (p=0.05)		**9.27**	-	**9.59**	-	**10.68**	-	**10.10**	-	**11.05**	-

*Mortality of I instar at all time intervals was zero in control

1.3. II instar maggots

Temperature	Time	2003				2004					
		Trial 1	Control	Trial 2	Control	Trial 1	Control	Trial 2	Control	Trial3	Control
45	30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	45	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00
	60	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00
48	30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	45	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00
	60	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00
52	10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	20	16.00	0.00	20.00	0.00	20.0	0.00	18.00	0.00	16.00	0.00
CD (p=0.05)		**2.45**	-	**3.16**	-	**6.32**	-	**3.74**	-	**2.45**	-

*Mortality of II instar at all time intervals was zero in control

1.4. III instar maggots

Temperature	Time	2003				2004					
		Trial 1	Control	Trial 2	Control	Trial 1	Control	Trial 2	Control	Trial3	Control
45	30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
48	30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	45	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00
	60	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00	100.0	0.00
52	10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	20	8.00	0.00	6.00	0.00	10.0	0.00	8.00	0.00	8.00	0.00
CD (p=0.05)		**2.00**	-	**2.45**	-	**3.16**	-	**2.00**	-	**2.00**	-

*Mortality of III instar at all time intervals was zero in control
All the values are mean of 5 replications

Table 2. Percentage *B. dorsalis* infestation at different temperature-time regimes

Temperature (°C)	Time (minutes)	Alphonso		Totapuri	
		2004	2005	2004	2005
46	60	0.00	0.00	0.00	0.00
48	60	0.00	0.00	0.00	0.00
48	75	0.00	0.00	0.00	0.00
48	90	0.00	0.00	0.00	0.00
Pre harvest IPM		1.11	2.22	4.30	1.11
Control		33.33 (287)	13.33 (122)	40.00 (316)	3.33 (26)

Figures in the parenthesis are number of larvae found in 30 fruits

Table 3 : Quality acceptability quotient (QAQ)

Alphonso

	Taste		Colour	
	Control	Treated	Control	Treated
Excellent	33.33	46.67	33.33	66.67
Very good	53.33	33.33	33.33	26.67
Good	13.33	20.00	33.33	6.67
Bad	0.00	0.00	0.00	0.00
Very Bad	0.00	0.00	0.00	0.00

Totapuri

	Taste		Colour	
	Control	Treated	Control	Treated
Excellent	13.33	20.00	60.00	80.00
Very good	26.67	40.00	40.00	20.00
Good	40.00	33.33	0.00	0.00
Bad	20.00	6.67	0.00	0.00
Very Bad	0.00	0.00	0.00	0.00

Values are percentages

for 10 min. At 20 min duration the mortality was 16-20 per cent at 52^{0}C. All the three instar larvae recorded zero per cent mortality under control (Table 1).

The regression analysis between the time of exposure and mortality at 45^{0}C and 48^{0}C for eggs and first instars maggots are presented in Figs. 1-4. From the linear equations, time required to kill 99.99683% of eggs or first instar maggots were worked out. The result showed that to obtain probit 9 security, eggs need an exposure of 62.19 and 55 minutes at 45 °C and 48°C, respectively. The same, for first instar maggots were 137.99 and 59.97 minutes, respectively at 45 °C and 48°C.

The fruits exposed to gravid females had several folds more of infestation of 3.33 to 40% in untreated control. These fruits treated at 46^{0}C for 60 minutes and 48^{0}C for 60, 75 and 90 minutes had no fruit fly infestation (Table 2).

Quality acceptability quotient (QAQ)

The fresh fruit quality acceptance as graded by consumers showed that in both the varieties, the treated fruits tasted better with higher acceptable colour. It was observed that heat-treated fruits had uniform ripening and colour, while untreated fruits had tinges of green patches, especially in Alphonso. In Alphonso, the reddish tinge on shoulder was more pronounced in treated fruits.

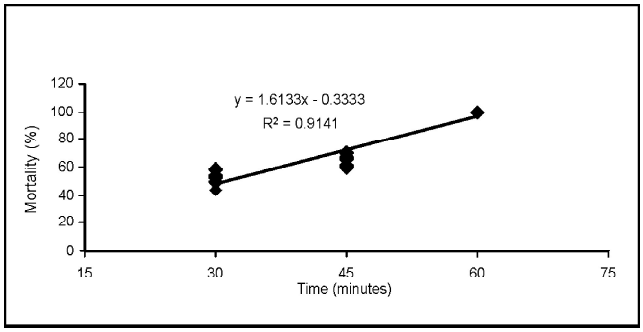

Fig. 1 : Mortality (%) of eggs at different time intervals at 45°C

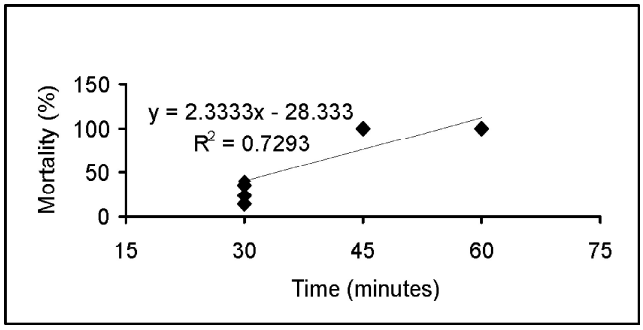

Fig. 2 : Mortality (%) of eggs at different time intervals at 48°C

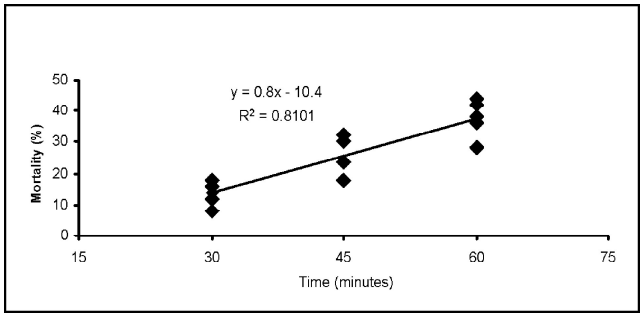

Fig. 3. Mortality (%) of first instar larvae at different time intervals at 45°C

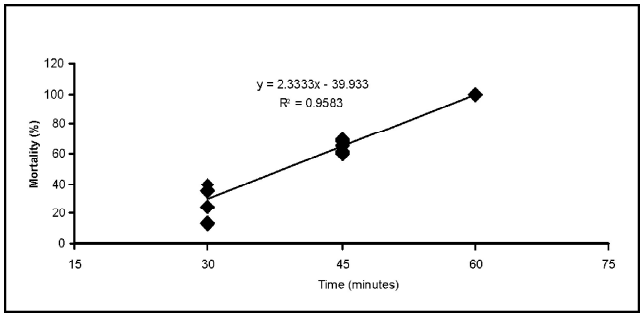

Fig. 4. Mortality (%) of first instar larvae at different time intervals at 48°C

The TSS was not affected by treatmenting the fruits at 46 °C for 60 cm and 48 °C for 60, 75 and 90 minutes. This study clearly showed that fruits treated between 46-48°C for 60-90 minutes were not affected qualitatively.

Validation under farmers' field condition

The results were further validated during 2006-2008 under farmers' conditions on varieties viz., Alphonso (Rayagada, Orissa state), Neelum (Trinvandrum and Trichur, Kerala state), Aphonso, Banganpalli and Totapuri (Bangalore, Karnataka state). It was successful in all the places.

The first set of experiments was conducted on the immature stages of the fruit fly, *B. dorsalis*. The eggs showed total mortality at 45°C and 60 minutes 48°C for 45 and 60 minutes. However, the trend was different with respect to first instar and they showed total mortality at only 48°C-60 minutes, which was equally efficacious for the eggs. The regression analysis showed that to obtain 'probit 9' security, eggs and first instar maggots needed an exposure of 62.19 and 55 minutes at 45 and 48°C, respectively. The same for first instar maggots was 137.99 and 59.97 minutes, respectively at 45 and 48°C (Figs. 1-4). Thus, at 48°C, a time regime of 60 minutes fell within 'probit 9' security.

Armstrong and Couey (1989) opined that the heat treatments function independently of the host. The results obtained with the immature stages in the present study using cv. *Totapuri* as host, should be relevant to different varieties of mango. The results are further confirmed by the experiment II, where the extent of disinfestations obtained was similar in the two varieties *viz, Alphonso* and *Totapuri.*

So, the ideal temperature –time regimes was 48°C-60 minutes. Interestingly, the second and third instars had similar thermal response. Both these stages survived at 45°C and 48°C at 30 minutes, while at and beyond 45 minutes, they were thermal susceptible.

The thermal responses of fruit fly eggs and larvae are identical to first order kinetics used to describe monomolecular chemical reactions (Jang, 1991). In the present study, first instar seemed quite tolerant to even 45 minutes at 45°C and 48°C, while the second instars succumbed at this time duration. One plausible reason is that these later stages may be sensitive, as in these stages, the maggots have active protein synthesis and tissue differentiation, which are affected by heat.

Fruit immersion, post exposure to gravid females

The temperatures of 46 and 48°C were selected for the present investigation. The temperature 45°C was not

considered for further trials with full fruits, as it was found unsuitable for first instar maggots, which are critical as there are chances of them escaping detection in an infested fruit, at the culling and sorting stage. Hot water treatment at 46.1^{0}C for 65, 75 or 90 minutes depending on size of fruits is practiced in Mexico for quarantine insects. In the present study therefore, 46^{0}C was considered along with 48^{0}C for fruit immersion study (Yahia and Pedro Campos, 2000).

In fruit immersion testing, the time factor was increased based on literature (Yahia and Pedro Campos, 2000). The third instar maggots were more thermo tolerant than second instar or they could survive at 45^{0}C even for 60 minutes. Only when the temperature was raised to 48^{0}C for 45 minutes, they succumbed to heat treatment. Therefore, for disinfestations 48^{0}C for 60 minutes seemed ideal, as at this temperature-time treatment all eggs, and first, second and third instar maggots showed 100% thermo mortality. This is expected to meet with the 'probit 9 security' and with 100% mortality (by the maximum limit concept) (Landolt *et al.,* 1984).

In the second set of experiments with whole fruits exposed to infestation the time treatments were enhanced to include 75 and 90 minutes to make allowance for cv. *Totapuri*, weighing approximately 500-700 g. However, in the present study, even at 60 minutes of 46 and 48^{0}C, 100% disinfestations were achieved. As the test was carried out within 48 hours of exposure to gravid females, the thermal effect was presumably on the eggs. Carry over of infestation, manifesting in advanced instars could be ruled out, as fruits were strictly culled for any external signs of yellowing or softening. Only healthy green and hard fruits, free of diseases or injuries were used.

It was found that the two varieties of mango could be disinfested by hot water treatment. *Alphonso* is an export variety and *Totapuri* is widely used in processing industries. At these temperature-time regimes the fruit quality was not affected (Table 3). The results are in corroboration with Sharp and Spalding (1984). In addition, heat treatment increases the shelf life of mango to 30-45 days at 9 - 10^{0}C (Armstrong and Couey, 1989). Besides, meeting quarantine standards of importing countries, this post harvest heat treatment reduces loss due to fruit fly infestation, which is about 25% in South India.

Prior to hot water treatment, all diseased, injured, yellowing or soft fruits should be culled. It is recommended that treatments should be adjusted to 48^{0}C, > 60 minutes (75-90 minutes) depending on the size of fruits, and to ensure absolute disinfestations, when fruits are treated for exports. Further, the results of the study have been validated at farmers' condition from 2006 to 2008 and have been found consistent

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