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Field Evaluation of Slow-Release Wax Formulations: A Novel Approach for Managing *Bactrocera zonata* (Saunders) (Diptera: Tephritidae)

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Abstract: Chemical management of the peach fly, *Bactrocera zonata* has been compromised due to adverse effects of pesticide residues that not only contaminate environment but also affect non-target organisms including beneficial insects, birds, aquatic life, and soil microorganisms. They can be impacted through direct exposure or by consuming contaminated prey or plants. The present study was designed keeping in view this increasing demand of the consumers to get pesticide residue free fruit and vegetable produce because it reflects the growing consumer concern for food safety and environmental sustainability, motivating the need for alternative pest management strategies. The field experiment was conducted to determine the best slow-release formulation prepared by mixing the following five different types of waxes, including Candelilla wax (CanW), Paraffin wax (PW), Carnauba wax (CarW), Lanolin wax (LW) and Bees wax (BW) with methyl eugenol (ME) (to attract male *B. zonata*). The selection of the five different types of waxes was likely based on their biodegradability, availability, and potential for slow-release properties. The result revealed that formulations containing SRF-7[LW], SRF-9[CanW], SRF-8[BW], SRF-9[CarW] and SRF-9[PW] exhibited the maximum capture of 42.10 ± 8.14, 43.30 ± 1.76, 34.30 ± 2.96, 35.30 ± 3.18 and 22.70 ± 3.18 male *B. zonata* per trap per day, respectively. These effective formulations were further evaluated in experiment in which the comparative trapping efficiency of each wax formulation was assessed. The results demonstrated that formulation containing SRF-9[CanW] was expressed maximum capture 13.77 ± 1.26 male *B. zonata* per trap per day. These formulations were further evaluated in another experiment in which the trapping efficiency was assessed by four different application methods (simple bottle trap, simple bottle trap with water, yellow sticky trap and jute piece with sticky material). The results demonstrated that formulation containing SRF-9[CarW] applied by yellow sticky trap (YST) trapped 61.74 + 7.69 male *B. zonata* per trap per day and proved more effective. This formulation can be recommended for trapping and management of male population of *B. zonata* in fruit orchards. This study can influence eco-friendly *B. zonata* pest control policies, reducing chemical pesticide usage and promoting agricultural sustainability. Future research should study the long-term impact of slow-release formulations on agricultural sustainability, including pest control, crop yield, and agroecosystem health.

Keywords: *Bactrocera zonata*; biodegradable waxes; slow-release formulations; methyl eugenol; trapping efficiency; application techniques

1. Introduction

The peach fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is a serious polyphagous pest of fruits and vegetables [1–3], which attacks more than 50 cultivated and wild plants, mainly fleshy fruits, such as guava (*Psidium guajava* L.), mango (*Mangifera indica* L.), peach (*Prunus persica* L.), apricot (*Prunus armeniaca* L.), figs (*Ficus carica* L.) and citrus in many parts of the world [4–6] through its oviposition behavior. The female fruit fly lays eggs within the fruits, and upon hatching, the larvae feed on the fruit pulp. This feeding damages the fruit, rendering it unmarketable and susceptible to secondary infections [7].

Bactrocera zonata reproduces through sexual reproduction, involving both males and females. When male and female flies use visual clues and pheromones to locate each other, the mating process begins. The courtship behavior, such as wing display and distinct sounds, helps to attract mating partners. Once successful mating has taken place, a male transfer sperm to the female. In this case, the female is looking for appropriate host fruits and uses ovipositor to insert her eggs. Host fruit selection is of utmost importance in order to ensure the survival of offspring. Within these chosen fruits, eggs develop into larvae, which feed on the pulp as they grow. Depending upon factors such as temperature and fruit type, the duration of a larval stage may be different. After this, the maggots leave the fruits and pupate in the soil to transform into adult flies. The adult flies emerge from the soil, ready to continue their reproductive cycle by discovering a mate and laying eggs in suitable host fruit [8,9].

The fruit fly species is indigenous to Asia and is widely distributed in south-eastern countries such as India, Sri Lanka, Bangladesh, Thailand and Mauritius [4,10–13]. *Bactrocera zonata* has acquired the status of an economic and quarantine pest throughout the world. It causes 10–20% of losses in the northwestern Himalayas and up to 89.50% in Pakistan [14], and reportedly leads to 3–100% fruit loss in different fruits or vegetables [15].

Fruit flies also cause 40–80% direct damage to the export of major crops in various varieties, seasons and regions is primarily due to the strict quality and phytosanitary standards imposed by importing countries [16,17]. Due to sanitary, phytosanitary and quarantine restrictions imposed by importing countries may include requirements for fumigation, hot water treatment, or cold storage of fruits, their detection in fruits restricts the export of fruits in the international market [18–20]. Every year, millions of dollars are spent on the management of fruit flies to reduce the pre- and post-harvest losses and implement strict pre-export processing of horticultural products [21]. The management of fruit flies in pre- and post-harvest is intended to minimize losses through the implementation of a variety of control measures. These include orchard sanitation, trapping, insecticide application, and cultural practices. Development of insect resistance in fruit fly populations, as well as need to use sustainable and environmentally friendly methods for pesticide control are some of the challenges during this process [22,23].

In many countries, the management of fruit flies is becoming increasingly difficult due to their behaviors at different stages of life, their adaptability to various foods and biological conditions, and the elimination of effective broad-spectrum fruit-fly-specific insecticides from the market due to the development of resistance in fruit fly populations, environmental concerns, and the desire for safer and more sustainable pest management practices [24,25].

Extensive research on various aspects of fruit fly management strategies has been reported in the literature [26,27]. The total number of existing research literature shows that fruit fly management includes biological control (using natural predators or parasites) (29%), chemical control (insecticides) (20%), behavior control (pheromones) (18%), biological pesticides (microbial agents) (17%) and natural pesticides (plant extracts) (13%), mechanical control (trapping) (7%) and genetic control (sterile insect technique) (6%) strategies [28–33]. However, only 14% of the studies were conducted on different surveillance techniques to monitor fruit flies [25]. This gap hinders the timely detection and control of fruit fly outbreaks, leading to economic losses. A number of obstacles, including pesticide resistance, environmental concerns, and species-specific behavior prevent effective implementation of these control methods against *B. zonata*. In order to overcome these obstacles and enhance the effectiveness of control, integrated pest management strategies that combine a variety of approaches and consider local conditions are essential [25].

In developing countries, the management of *B. zonata* is completely dependent on the application of synthetic insecticides through cover sprays because of their affordability and immediate effectiveness [2,34–36]. Farmers mostly use the blind and injudicious application to suppress the population of *B. zonata* but these insecticides lead to contaminations of fruits and vegetables and cause many other serious problems, including food safety and food security, increasing the level of pesticides residues in food commodities, resistance in the pest population and destruction of beneficial and non-target fauna [37–41].

Therefore, it is necessary to explore alternative eco-friendly pest management strategies for the control of the *B. zonata*, so mating disruption with synthetic insect sex pheromones is one method of insect control that can be used as part of an Integrated Pest Management (IPM) program [42–44]. Pheromones are relatively nontoxic, are used in small quantities, and are biodegradable [45–47]. Pheromones are also easy to handle, have fewer regulatory restrictions, and result in minimal disruption of other orchard or crop operations [48,49].

Currently, most of the pheromones used for mating disruption are contained in plastic dispensers that are manually attached to trees or plants [50–52]. Within 2–3 months, the pheromone slowly diffuses through the plastic or polymeric wall of the dispenser [53]. Although the use of plastic pheromone dispensers has achieved effective control of certain pests, the application is labor-intensive and typically they must be dispensed several times per season.

The use of pheromones to disrupt mating in insects is a control technique that can result in a significant reduction in the number of synthetic pesticides used by farmers. However, it is difficult to develop a controlled release formulation that can be sprayed on the crops for an extended period and release pheromones at a constant rate over some time (6 weeks or more) due to the need to maintain stability, consistency, duration, and compatibility.

This is a factor that limits the widespread use of mating disruption as a part of pest management strategy. Spray applications generally require the active agent to be dispersed in the carrier, resulting in a decrease in the release rate over time. However, a constant (zero-order) release rate is necessary to obtain the most economical controlled release of pheromone. Although the concept of controlling insects by mating disruption is effective, to gain widespread acceptance and use requires the dispensation of pheromones at a constant rate over an extended period.

The objectives of this study included the preparation and evaluation of slow-release formulations (SRFs) using various biodegradable waxes i.e., Candelilla wax (CanW), Paraffin wax (PW), Carnauba wax (CarW), Lanolin wax (LW) and Bees wax (BW) combined with methyl eugenol (ME) for the management of male *Bactrocera zonata*. The aim of these objectives was to assess the trapping performance of such SRFs in different plant canopies, select suitable formulations and compare their effectiveness with a standard trap. In addition, the attractiveness between treatments was assessed and classified them by attractancy indices in order to determine which techniques was most effective when applied, such as simple bottle trap, bottle trap with water, yellow sticky trap and jute piece with sticky material. The study offers a promise in the field of pest control and sustainable agriculture. The aim of the study is to develop eco-friendly, slow-release wax formulas for male *B. zonata* control. Success could deliver a revolution in pest control, providing benefits to crops, the environment and agricultural sustainability. Farmers could benefit from cost effective solutions, reducing costs and increasing profits. The study has further improved the methods for controlling pests, as well as our understanding of pest behavior. The findings would have applicability to other pests, affecting policies and serving as a source of learning for farmers by promoting best practices in pest management and sustainability.

2. Materials and Methods

2.1. Preparation of Slow-Release Formulations of Biodegradable Waxes

The five biodegradable waxes including Candelilla wax (CanW) (The Nature's Store, Lahore, Pakistan), Paraffin wax (PW) (The Nature's Store, Lahore, Pakistan), Carnauba wax (CarW) (AUCHEMICALS, Lahore, Pakistan), Lanolin wax (LW) (Aroma Farmacy, Karachi, Pakistan) and Bees wax (BW) (PANSARI, Lahore, Pakistan) were used to prepare slow-release formulations (SRF) with methyl eugenol (ME) (Vantage, Lahore, Pakistan). Each wax was mixed with ME in nine ratios (Table 1). The waxes were melted inside the microwave oven (15–22 °C) for 2 min. When these melted waxes were near to cooling, ME was admixed by employing a gentle stirring method [54]. ME was slowly added while continuously stirring until a homogenous mixture was obtained. The mixture of ME and waxes was filled in glass vials with a cap and kept at room temperature till their solidification (Figure 1).

Table 1. Ratios at which biodegradable waxes was mixed with methyl eugenol to prepare slow-release formulations against *B. zonata*.

Treatments	LW	CanW	BW	CarW	PW
SRF-1	LW 90% + ME 10%	CanW 90% + ME 10%	BW 90% + ME 10%	CarW 90% + ME 10%	PW 90% + ME 10%
SRF-2	LW 80% + ME 20%	CanW 80% + ME 20%	BW 80% + ME 20%	CarW 80% + ME 20%	PW 80% + ME 20%
SRF-3	LW 70% + ME 30%	CanW 70% + ME 30%	BW 70% + ME 30%	CarW 70% + ME 30%	PW 70% + ME 30%
SRF-4	LW 60% + ME 40%	CanW 60% + ME 40%	BW 60% + ME 40%	CarW 60% + ME 40%	PW 60% + ME 40%
SRF-5	LW 50% + ME 50%	CanW 50% + ME 50%	BW 50% + ME 50%	CarW 50% + ME 50%	PW 50% + ME 50%
SRF-6	LW 40% + ME 60%	CanW 40% + ME 60%	BW 40% + ME 60%	CarW 40% + ME 60%	PW 40% + ME 60%
SRF-7	LW 30% + ME 70%	CanW 30% + ME 70%	BW 30% + ME 70%	CarW 30% + ME 70%	PW 30% + ME 70%
SRF-8	LW 20% + ME 80%	CanW 20% + ME 80%	BW 20% + ME 80%	CarW 20% + ME 80%	PW 20% + ME 80%
SRF-9	LW 10% + ME 90%	CanW 10% + ME 90%	BW 10% + ME 90%	CarW 10% + ME 90%	PW 10% + ME 90%

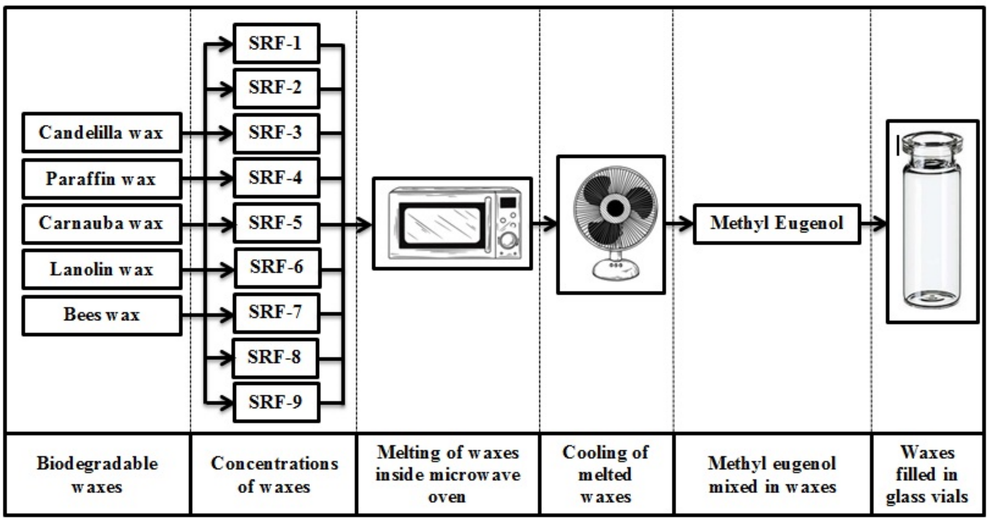

Figure 1. Preparation of slow-release formulations of biodegradable waxes.

2.2. Experiment-I: Evaluation of Trapping Efficiency of Slow-Release Formulations Prepared in Different Types of Biodegradable Waxes

Fifty-four biodegradable waxes were prepared along with a Standard trap (ST) containing only Conventional ME and were installed in the center of the tree canopy of each plant by following the methodology of Nisar et al. [14]. The plants in each orchard (mango, citrus, and guava) were selected based on their availability in the area. Flowering/fruiting seasons were not considered as a selection criterion, except for mango, and plants were chosen randomly within the layout plan.

The traps (made up of plastic bottles) were installed at the height of about 450 cm on the horizontal branch of the central stem inside the tree canopy where *B. zonata* activity is typically highest, while avoiding interference with ground-level activity [14]. Each trap was fixed by knotting one end of a string (30 cm long) with the trap's hook and its other end with the horizontal branch of stem (Figure 2). The trapping efficiency of each formulation was counted daily till no *B. zonata* was trapped in each trap. The trapped fruit flies were collected in polythene bags separately for each trap, brought to the laboratory, identified and separated into males and females of *B. zonata* based on morphological characteristics, as described by White and Elson-Harris [55]. This involved careful examination of the insects' physical traits such as size, coloration, and the presence or absence of specific features.

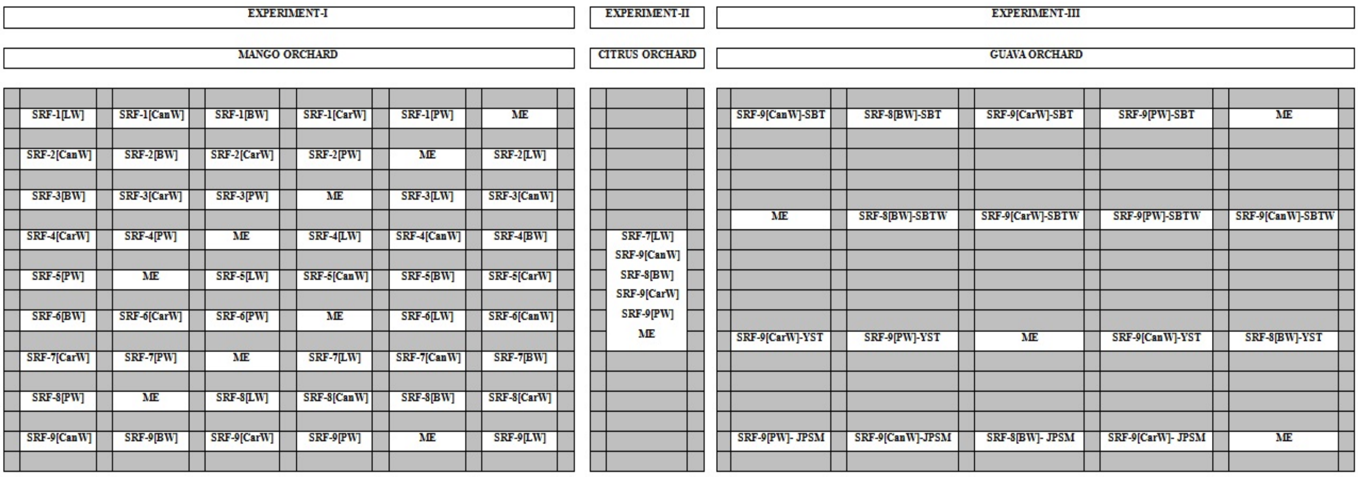

Figure 2. Layout of the experiments (Experiment-I. Experiment-II and Experiment-III).

2.3. Experiment-II: Evaluation of Comparative Trapping Efficiency of a Highly Attractive Combination of Slow-Release Formulations Prepared in Different Types of Biodegradable Waxes

Highly attractive formulations were selected from Experiment-I based on their trapping efficiency. The six highly attractive formulations along with ST (Conventional ME) were installed in the center of the tree canopy of one plant to maximize exposure to *B. zonata*, as this is the area where their activity is concentrated during feeding and mating by following the methodology of Nisar et al. [14], similar to Experiment-I (Figure 2).

The trapping efficiency of highly attractive formulations was counted daily till no *B. zonata* was trapped in each trap. The trapped fruit flies were collected in polythene bags separately for each trap, brought to the laboratory, identified and separated into males and females of *B. zonata* based on morphological characteristics, as described by White and Elson-Harris [55].

2.4. Experiment-III: Evaluation of Trapping Efficiency of the Highly Attractive Slow-Release Formulation by Different Implementation Techniques

The highly attractive and slow-release formulations determined from Experiment-II were assessed by following implementation techniques (Figure 2):

2.4.1. Simple Bottle Trap

In this method, traps were made as previously used in Experiment-I. The formulation was prepared and filled in glass vials, installed in plastic bottles and these bottles were used for trapping in the field.

2.4.2. Simple Bottle Trap with Water

This method was the same as "Method-I" but in this method, the bottom of the plastic bottles (1.5 L) was filled with simple water and then installed in field for trapping.

2.4.3. Yellow Sticky Trap

In this method, a triangular hut (5″ width × 7″ length) was made of yellow color charts. In the bottom of the hut a sliding tray was adjusted. Sticky material (APSPRAT, Hubei Fengde Weiye New Material Co. Ltd., Xianning, China) and the concerned formulation were admixed and applied to the sliding tray which was then put in triangular hut. The triangular hut was then installed in the field for trapping of *B. zonata*.

2.4.4. Jute Piece with Sticky Material

In this method, a jute piece measuring 10 × 5 inches was used. At first, sticky material was added on piece then the formulation was admixed on it, this piece was hanged in the field for the attraction of *B. zonata*.

These four methods were kept in observation and the *B. zonata* trapped in each method were counted daily till no *B. zonata* was trapped. Conventional ME trapping technique was used for comparison ST. The trapped fruit flies were collected in polythene bags separately for each trap, brought to the laboratory, identified and separated into males and females of *B. zonata* based on morphological characteristics, as described by White and Elson-Harris [55].

2.5. Data Analyses

All the treatments were applied in a Randomized Complete Block Design (RCBD) in the fields and replicated thrice. The collected data was transformed into percentage attracted and data were analyzed using ANOVA technique and means were compared by Tukey's HSD test. The data were also transformed into attractancy rating using the following formula described by Beroza and Green [56].

$$AI = \frac{IA_{TR} - IA_S}{IA_T} \times 100$$

where: AI = Attractancy index; IA_{TR} = Insects attracted in treatment; IA_S = Insects attracted in Standard trap; IA_T = Total insects attracted

The treatments were then classified into different classes based on attractancy indices as describes by Beroza and Green [56] (Table 2).

Table 2. Different classes of Attractancy index described by Beroza and Green [56].

Class	Male *B. zonata*
I	>11
II	11–50
III	<50

I = Non or little attractive; II = Moderately attractive; III = Strongly attractive

3. Results

3.1. Trapping Efficiency of Slow-Release Formulations Prepared in Different Types of Biodegradable Waxes

3.1.1. Lanolin Wax

The results indicated that SRF-7 captured maximum *B. zonata* (42.1 male per trap per day), which was found approximately 3.6 times higher than ST (11.7 male per trap per day) and statistically different from all other SRFs. The performance of all formulations of LW was found in order of SRF-7 > SRF-8 > SRF-9 > SRF-6 > SRF-3 > SRF-5 > SRF-4 > SRF-2 > SRF-1 > ST (Figure 3).

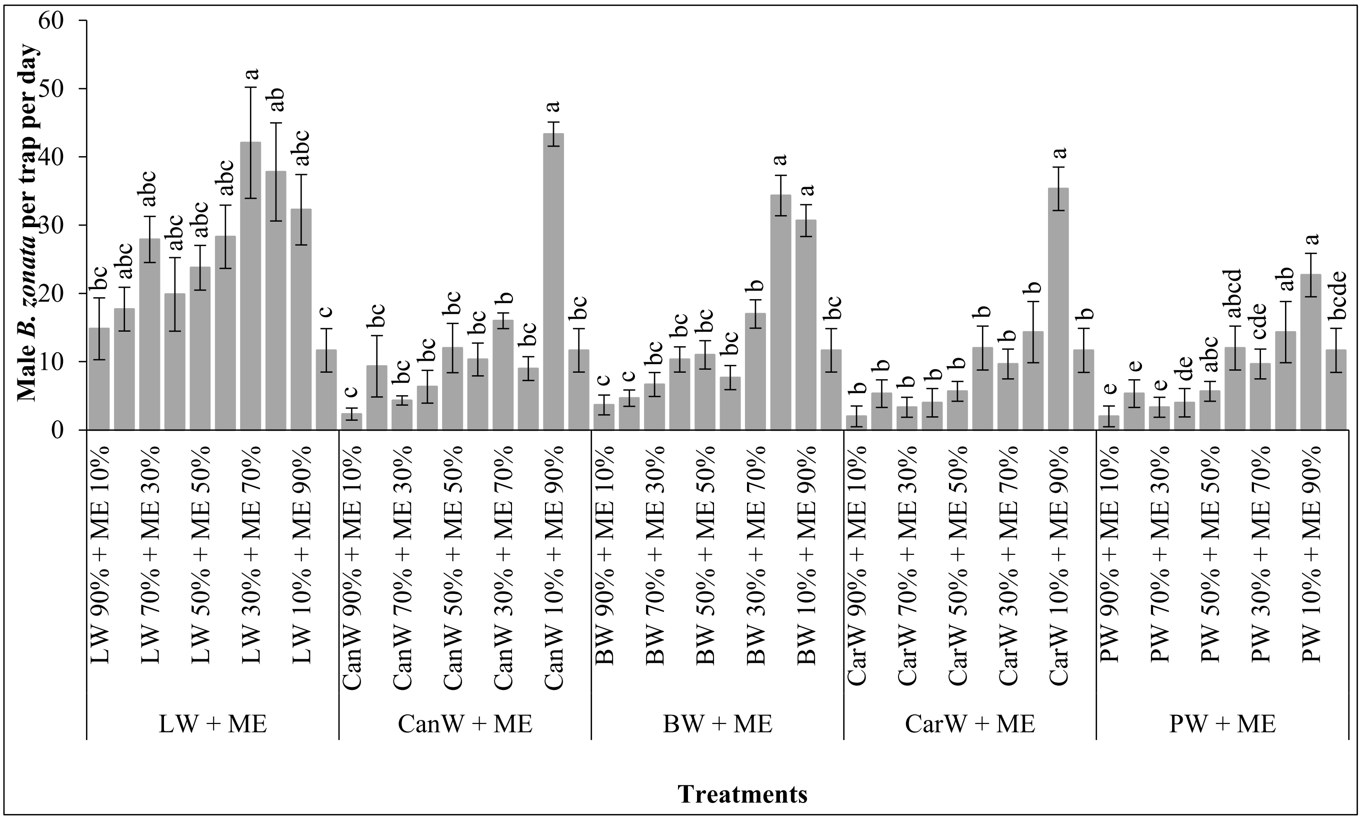

Figure 3. Population of *Bactrocera zonata* captured per trap per day (Means ± SE) in different slow-release formulations containing various concentrations of Lanolin wax, Candelilla wax, Bees wax, Carnauba wax and Paraffin wax with methyl eugenol. Means sharing similar style letters don't differ significantly at a probability level of 5%.

The results of AI under field conditions revealed that only SRF-7 exhibited 51.71% AI proved strongly attractive SRF to *B. zonata* and was categorized as Class-III SRF (AI > 50%) (Table 3). The SRF-2, SRF-3, SRF-4, SRF-5, SRF-6, SRF-8 and SRF-9 exhibited 20.54%, 41.02%, 26.00%, 34.15%, 43.50%, 49.86% and 46.07% AI, respectively, proved moderately attractive SRFs and were categorized as Class-II SRF (AI = 11–50%) (Table 3). Only SRF-1 exhibited 0.71% AI proved little or non-attractive SRF and was categorized as Class-I SRF (AI < 11%) (Table 3).

Table 3. Attractive index regarding the attraction of *B. zonata* to the different slow-release formulations of various concentrations of waxes with methyl eugenol.

Treatments	LW		CanW		BW		CarW		PW	
	Attractive Index	Class	Attractive Index	Class	Attractive Index	Class	Attractive Index	Class	Attractive Index	Class
SRF-1	0.71	I	−66.67	I	−52.17	I	−70.73	I	−66.67	I
SRF-2	20.54	II	−11.11	I	−42.86	I	−37.26	I	−70.73	I
SRF-3	41.03	II	−55.56	I	−27.27	I	−55.56	I	−62.79	I
SRF-4	26.00	II	−29.63	I	−6.06	I	−48.94	I	−55.56	I
SRF-5	34.15	II	1.41	I	−2.94	I	−34.62	I	11.39	II
SRF-6	43.50	II	0.00	I	−20.69	I	1.41	I	6.67	I
SRF-7	49.71	III	15.66	II	18.60	II	−9.38	I	−40.00	I
SRF-8	51.86	II	−12.90	I	49.27	II	10.25	I	22.22	II
SRF-9	46.07	II	57.57	III	44.88	II	50.35	III	32.04	II

3.1.2. Candelilla Wax

The results of means indicated that SRF-9 captured maximum *B. zonata* (43.3 male per trap per day), which was found approximately 3.7 times higher than ST (11.7 male per trap per day) and statistically different from all other SRFs. The performance of all formulations of CanW was found in order of SRF-9 > SRF-7 > SRF-5 > ST > SRF-6 > SRF-2 > SRF-8 > SRF-4 > SRF-3 > SRF-1 (Figure 3).

The results of AI under field conditions revealed that only SRF-9 exhibited 57.57% AI proved strongly attractive SRF to *B. zonata* and was categorized as Class-III SRF (AI > 50%) (Table 3). Only SRF-7 exhibited 15.55% AI proved moderately attractive SRF and was categorized as Class-II SRF (AI = 11–50%) (Table 3). The SRF-1, SRF-2, SRF-3, SRF-4, SRF-5, SRF-6 and SRF-8 exhibited −66.66%, −11.11%, −55.55%, −29.63%, 1.40%, −0.001% and −12.90% AI, respectively, proved little or non-attractive SRF and were categorized as Class-I SRF (AI < 11%) (Table 3).

3.1.3. Bees Wax

The results of means indicated that SRF-8 captured maximum *B. zonata* (34.3 male per trap per day), which was found approximately 3 times higher than ST (11.7 male per trap per day) and statistically different from all other SRFs. The performance of all formulations of BW was found in order of SRF-8 > SRF-9 > SRF-7 > ST > SRF-5 > SRF-4 > SRF-6 > SRF-3 > SRF-2 > SRF-1 (Figure 3).

The results of AI under field conditions revealed that only SRF-7, SRF-8 and SRF-9 exhibited 18.60%, 49.27% and 44.88% AI, respectively, proved moderately attractive SRF and was categorized as Class-II SRF (AI = 11–50%) (Table 3). The SRF-1, SRF-2, SRF-3, SRF-4, SRF-5 and SRF-6 exhibited −52.17%, −42.85%, −27.27%, −6.06%, −2.94% and −20.69% AI, respectively, proved little or non-attractive SRF and was categorized as Class-I SRF (AI < 11%) (Table 3).

3.1.4. Carnauba Wax

The results of means indicated that SRF-9 captured maximum *B. zonata* (35.3 male per trap per day), which was found approximately 3 times higher than ST (11.7 male per trap per day) and statistically different from all other SRFs. The performance of all formulations of CarW was found in order of SRF-9 > SRF-8 > SRF-6 > ST > SRF-7 > SRF-5 > SRF-2 > SRF-4 > SRF-3 > SRF-1 (Figure 3).

The results of AI under field conditions revealed that only SRF-9 exhibited 50.35% AI proved strongly attractive SRF to *B. zonata* and was categorized as Class-III SRF (AI > 50%) (Table 3). The SRF-1, SRF-2, SRF-3, SRF-4, SRF-5, SRF-6, SRF-7 and SRF-8 exhibited −70.73%, −37.25%, −55.55%, −48.93%, −34.61%, 1.40%, −9.37% and 10.25% AI, respectively, proved little or non-attractive SRF and were categorized as Class-I SRF (AI < 11%) (Table 3).

3.1.5. Paraffin Wax

The results of means indicated that SRF-9 captured maximum *B. zonata* (22.7 male per trap per day), which was found approximately 1.9 times higher than ST (11.7 male per trap per day) and statistically different from all other SRFs. The performance of all formulations of PW was found in order of SRF-9 > SRF-8 > SRF-5 > SRF-6 > ST > SRF-7 > SRF-4 > SRF-3 > SRF-1 > SRF-2 (Figure 3).

The results of AI under field conditions revealed that only SRF-5, SRF-8 and SRF-9 exhibited 11.39%, 22.22% and 32.03% AI, respectively, proved moderately attractive SRF to *B. zonata* and was categorized as Class-II SRF (AI = 11–50%) (Table 3). The SRF-1, SRF-2, SRF-3, SRF-4, SRF-6 and SRF-7 exhibited −66.66%, −70.73%, −62.79%, −55.55%, 6.66% and −40.00% AI, respectively, proved little or non-attractive SRF and was categorized as Class-I SRF (AI < 11%) (Table 3).

3.2. Comparative Trapping Efficiency of a Highly Attractive Combination of Slow-Release Formulations Prepared in Different Types of Biodegradable Waxes

The results of means indicated that CanW 10% + ME 90% captured maximum *B. zonata* (13.77 male per trap per day), which was found approximately 2.6 times higher than ST (5.29 male per trap per day) and statistically different from all other SRFs. The PW 10% + ME 90% demonstrated a capture of 11.00 male per trap per day, which was approximately two times higher than ST. Similarly, BW 20% + ME 80% and CarW 10% + ME 90% exhibited a capture of 8.15 and 7.23 male per trap per day, which were approximately 1.5 and 1.4 times higher than ST respectively. Unlikely, LW 30% + ME 70% exhibited a capture of 1.81 male per trap per day and explained approximately three times less capture than ST (Figure 4).

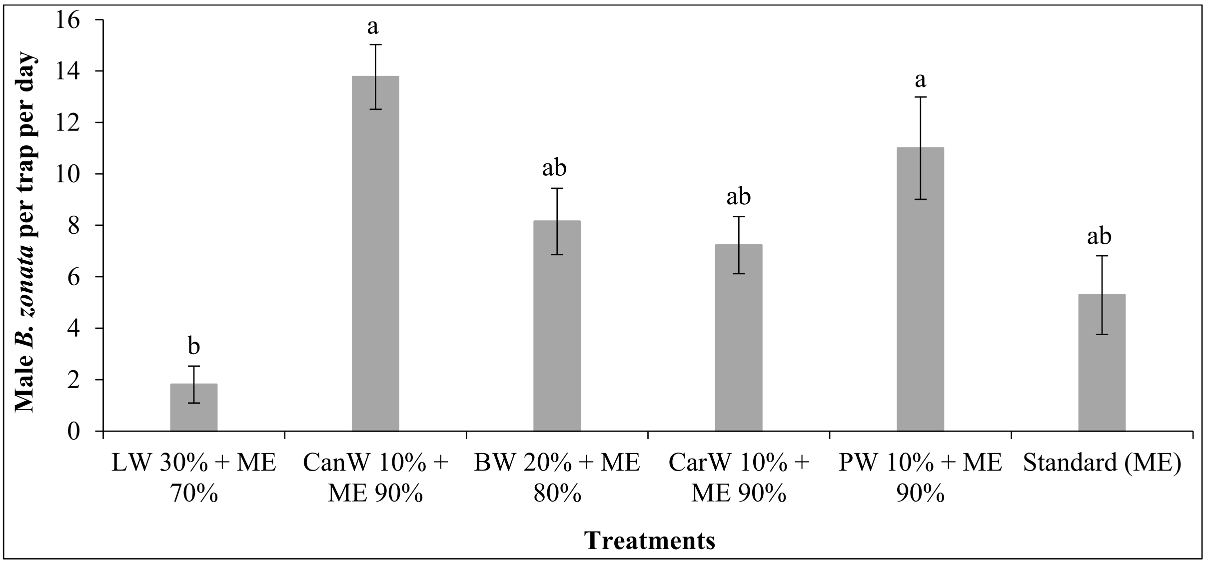

Figure 4. Population of *Bactrocera zonata* captured per trap per day (Means ± SE) in different highly attracted slow-release formulations. Means sharing similar style letters don't differ significantly at a probability level of 5%.

The results of AI under field conditions revealed that CanW 10% + ME 90%, PW 10% + ME 90%, BW 20% + ME 80% and CarW 10% + ME 90% exhibited 41.42%, 32.05%, 20.98% and 12.87% AI, respectively, proved moderately attractive SRF to *B. zonata* and was categorized as Class-II SRF (AI = 11–50%) (Table 4). The LW 30% + ME 70% exhibited −61.86% AI proved little or non-attractive SRF and was categorized as Class-I SRF (AI < 11%) (Table 4).

Table 4. Attractive index regarding the attraction of *B. zonata* to different highly attracted slow-release formulations.

Treatments	Formulation	Attractive Index	Class
SRF-1	LW 30% + ME 70%	−61.868	I
SRF-2	CanW 10% + ME 90%	41.428	II
SRF-3	BW 20% + ME 80%	20.986	II
SRF-4	CarW 10% + ME 90%	12.878	II
SRF-5	PW 10% + ME 90%	32.052	II

3.3. Trapping Efficiency of the Highly Attractive Slow-Release Formulation by Different Implementation Techniques

3.3.1. Candelilla Wax 10% + Methyl Eugenol 90%

The results of means indicated that CanW 10% + ME 90% captured maximum *B. zonata* (21.49 male per trap per day) when evaluated by YST method which was found approximately 1.9 times higher than ST (11.00 male per trap per day) and statistically different from all other SRFs. When CanW 10% + ME 90% was assessed by simple bottle trap (SBT) technique, 19.31 male per trap per day were captured which was found approximately 1.8 times higher than ST and statistically like YST technique. Application of CanW 10% + ME 90% by SBTW and jute piece with sticky material (JPSM) demonstrated 7.74 and 8.94 male per trap per day, respectively, which were found approximately 1.4 and 1.2 times less than ST and statistically different from all other SRFs. Overall, CanW 10% + ME 90% proved more attractive with YST method followed by SBT method, JPSM and SBTW (Figure 5).

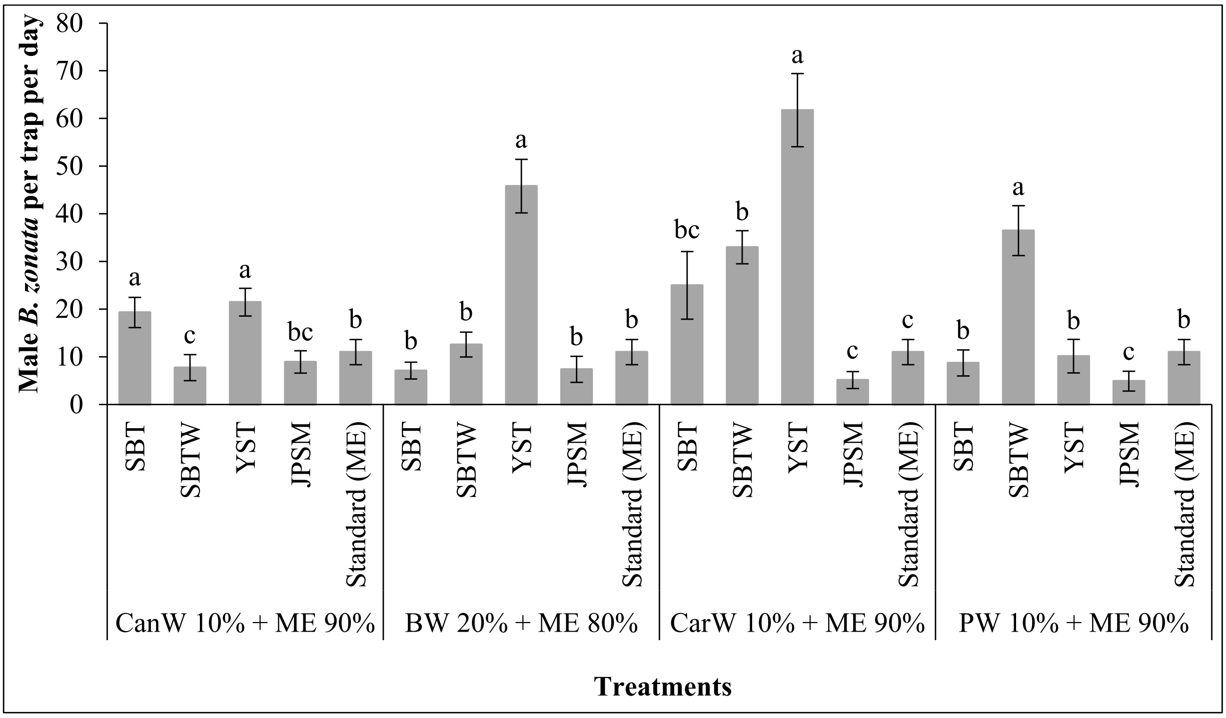

Figure 5. Population of *Bactrocera zonata* captured per trap per day (Means ± SE) in different application techniques of highly attracted slow-release formulations. Means sharing similar style letters don't differ significantly at a probability level of 5%.

The results of AI under field conditions revealed that none of the application techniques proved strongly attractive application technique to *B. zonata* and was categorized as Class-III SRF (AI > 50%). CanW 10% + ME 90% with YST and SBT exhibited 32.28% and 27.41% AI, respectively, proved moderately attractive application technique and was categorized as Class-II SRF (AI = 11–50%). CanW 10% + ME 90% with SBTW and JPSM exhibited −17.39% and −10.33% AI proved little or non-attractive application technique and was categorized as Class-I SRF (AI < 11%). These results of AI also explained that CanW 10% + ME 90% with YST and SBT exhibited higher attractancy over the ST. However, CanW 10% + ME 90% with SBTW and JPSM demonstrated less attractancy than the ST (Table 5).

Table 5. Attractive Index regarding the attraction of *B. zonata* to different application techniques at various concentrations of waxes with methyl eugenol.

Application Technique	CanW 10% + ME 90%		BW 20% + ME 80%		CarW 10% + ME 90%		PW 10% + ME 90%	
	Attractive Index	Class	Attractive Index	Class	Attractive Index	Class	Attractive Index	Class
SBT	27.4166	II	−21.4798	I	38.8889	II	−11.562	I
SBTW	−17.3959	I	6.700594	I	50.11	III	53.6549	III
YST	32.2868	II	61.27442	III	69.7553	III	−4.0681	I
JPSM	−10.331	I	−19.6953	I	−36.392	I	−38.278	I

3.3.2. Bees Wax 20% + Methyl Eugenol 80%

The results of means indicated that BW 20% + ME 80% captured maximum *B. zonata* (45.81 male per trap per day) when evaluated by YST method which was found approximately 4.1 times higher than ST (11.00 male per trap per day) and statistically different from all other SRFs. When BW 20% + ME 80% was assessed by SBTW technique, 12.58 male per trap per day were captured which was found approximately 1.2 times higher than ST. Application of BW 20% + ME 80% by SBT and JPSM demonstrated 7.11 and 7.38 male per trap per day, respectively, which were found approximately 1.5 times less than ST and statistically different from all other SRFs. Overall, BW 20% + ME 80% proved more attractive with YST method followed by SBTW method, JPSM and SBT (Figure 5).

The results of AI under field conditions revealed that BW 20% + ME 80% with YST technique exhibited 61.27% AI proved strongly attractive application technique to *B. zonata* and was categorized as Class-III SRF (AI > 50%). None of the application techniques with BW 20% + ME 80% proved moderately attractive and was categorized as Class-II SRF (AI = 11–50%). BW 20% + ME 80% with SBTW, SBT and JPSM exhibited 6.70%, −21.49% and −19.69% AI proved little or non-attractive application technique and was categorized as Class-I SRF (AI < 11%). These results of AI also explained that BW 20% + ME 80% with YST and SBTW were higher attractancy over the ST. However, BW 20% + ME 80% with SBT and JPSM demonstrated less attractancy than the ST (Table 5).

3.3.3. Carnauba Wax 10% + Methyl Eugenol 90%

The results of means indicated that CarW 10% + ME 90% captured maximum *B. zonata* (61.74 male per trap per day) when evaluated by YST method which was found approximately 5.6 times higher than ST (11.00 male per trap per day) and statistically different from all other SRFs. When CarW 10% + ME 90% was assessed by SBTW technique, 33.00 male per trap per day were captured which was found approximately 3 times higher than ST. Application of CarW 10% + ME 90% by SBT demonstrated 25.00 male per trap per day were captured which was found approximately 2.3 times higher than ST. Unlikely, the application of CarW 10% + ME 90% by JPSM exhibited 5.13 male per trap per day, which was found approximately 2.1 times less than ST and statistically different from all other SRFs. Overall, CarW 10% + ME 90% proved more attractive with YST method followed by SBTW, SBT and JPSM (Figure 5).

The results of AI under field conditions revealed that CarW 10% + ME 90% with YST and SBTW technique exhibited 69.75% and 50.11% AI, respectively, proved strongly attractive application techniques to *B. zonata* and were categorized as Class-III SRF (AI > 50%). CarW 10% + ME 90% with SBT exhibited 38.88% AI proved moderately attractive application technique and was categorized as Class-II SRF (AI = 11–50%). CarW 10% + ME 90%

with JPSM exhibited −36.39% AI proved little or non-attractive application technique and was categorized as Class-I SRF (AI < 11%). These results of AI also explained that CarW 10% + ME 90% with YST, SBTW and SBT were higher attractancy over the ST. However, CarW 10% + ME 90% with JPSM demonstrated less attractancy than the ST (Table 5).

3.3.4. Paraffin Wax 10% + Methyl Eugenol 90%

The results of means indicated that PW 10% + ME 90% captured maximum *B. zonata* (36.47 male per trap per day) when evaluated by SBTW which was found approximately 3.3 times higher than ST (11.00 male per trap per day) and statistically different from all other SRFs. When PW 10% + ME 90% was assessed by YST technique, 10.14 male per trap per day were captured that was found approximately similar ST. Application of PW 10% + ME 90% by SBT and JPSM demonstrated 8.72 and 4.91 male per trap per day, respectively, which were found approximately 1.2 and 2.2 times less than ST and statistically different from all other SRFs. Overall, PW 10% + ME 90% proved more attractive with SBTW followed by YST method, SBT method and JPSM (Figure 5).

The results of AI under field conditions revealed that PW 10% + ME 90% with SBTW exhibited 53.65% AI proved strongly attractive application technique to *B. zonata* and was categorized as Class-III SRF (AI > 50%). None of the application techniques proved moderately attractive application technique and was categorized as Class-II SRF (AI = 11–50%). PW 10% + ME 90% with SBT, YST and JPSM exhibited −11.56%, −4.06% and −38.27% AI proved little or non-attractive application technique and were categorized as Class-I SRF (AI < 11%). These results of AI also explained that PW 10% + ME 90% with SBTW was higher attractancy over the ST. However, PW 10% + ME 90% with SBT, YST and JPSM demonstrated less attractancy than the ST (Table 5).

Regardless of the application techniques, results revealed that the CarW 10% + ME 90% attracted 27.5 male *B. zonata* per trap per day, which was 2.5 times higher than ST. CarW 10% + ME 90% proved to be highly attractive in in-season and in off-season environments. After CarW 10% + ME 90%, BW 20% + ME 80% was important in the attraction of *B. zonata*. CarW 10% + ME 90% captured 17.0 male per trap per day that was 1.5 times higher than ST. Application of CanW 10% + ME 90% and PW 10% + ME 90% demonstrated a capture of 14.0 and 15.0 male per trap per day, respectively, which were found approximately 1.2 and 1.3 times higher respectively than ST. Overall, CarW 10% + ME 90% proved most attractive followed by BW 20% + ME 80%, PW 10% + ME 90% and CanW 10% + ME 90% (Figure 6).

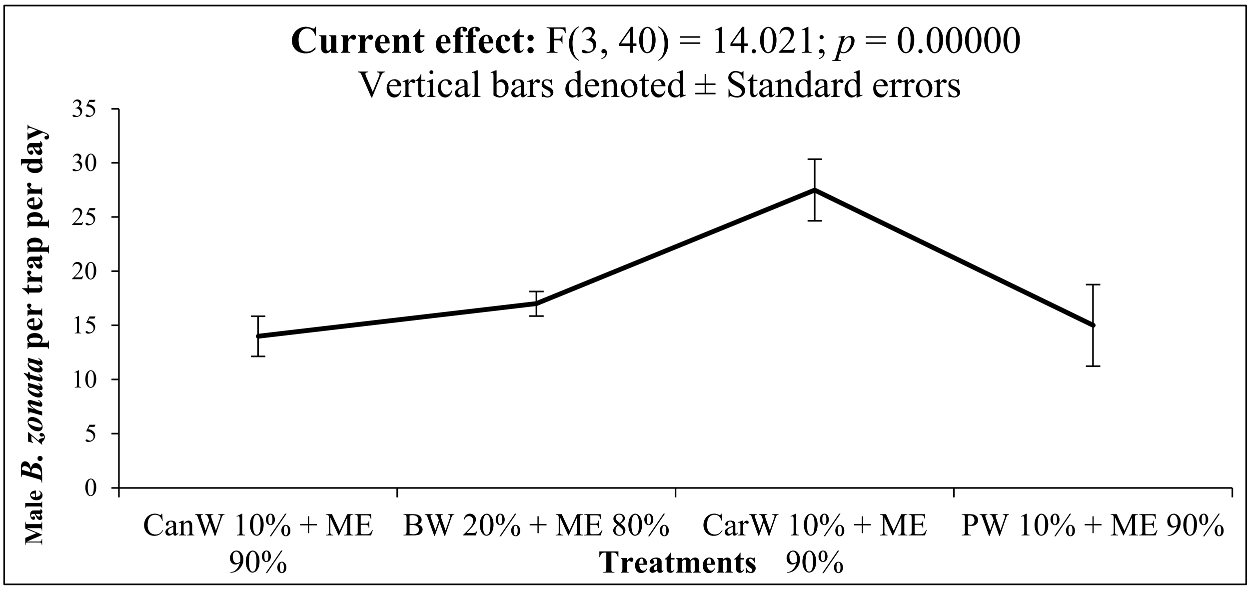

Figure 6. Population of *Bactrocera zonata* captured per trap per day (Means ± SE) in highly attracted slow-release formulations.

Regardless of the SRFs, results revealed that the YST was more attractive in the attraction of male *B. zonata* and it was more effective to be used with SRFs that capture 35.0 male per trap per day which was 3 times higher attractancy than ST. SBTW was also showed maximum attractancy after the YST that capture 23.0 male per trap per day and this method was 2 times higher than ST. After this, SBT exhibited a capture of 15.0 male per trap per day which was found approximately 1.3 times higher attractancy than ST. Unlikely, the JPSM exhibited a capture of 6.1 male per trap per day was found approximately 1.8 times less attractancy than ST. Overall, YST was the most attractive application technique in the attraction of male followed by SBTW, SBT and then JPSM (Figure 7).

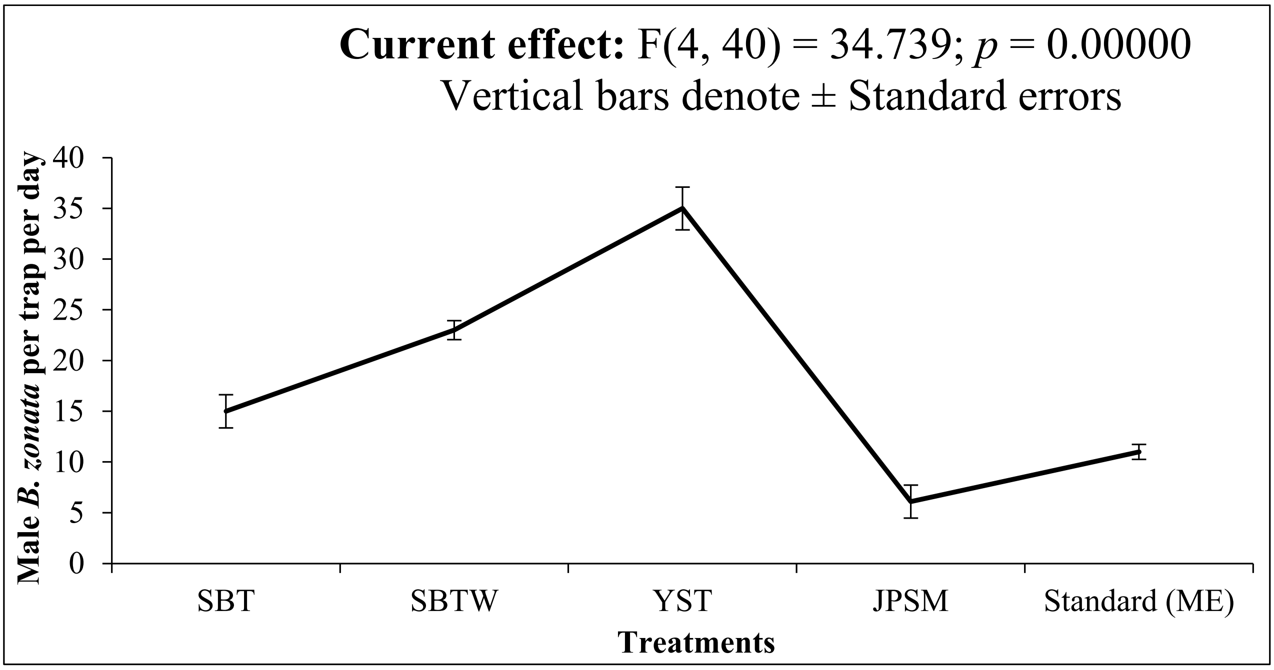

Figure 7. Population of *Bactrocera zonata* captured per trap per day (Means ± SE) in different application techniques.

The results of these experiments exhibited that CarW 10% + ME 90% and BW 20% + ME 80% attracted the maximum number of flies that was 61.74 and 45.81 male *B. zonata* per trap per day, when assessed by YST. However, CanW 10% + ME 90% demonstrated a capture of 21.49 and 19.31 male per trap per day when assessed by YST and SBT technique, respectively. While, PW 10% + ME 90% demonstrated a capture of 36.47 male per trap per day when assessed by SBTW technique. Overall, CarW 10% + ME 90% proved more attractive with YST method followed by SBTW, SBT and JPSM. BW 20% + ME 80% proved more attractive with YST method followed by SBTW method, JPSM and SBT. PW 10% + ME 90% proved more attractive with SBTW followed by YST method, SBT method and JPSM and CanW 10% + ME 90% proved more attractive with YST method followed by SBT method, JPSM and SBTW (Figure 8).

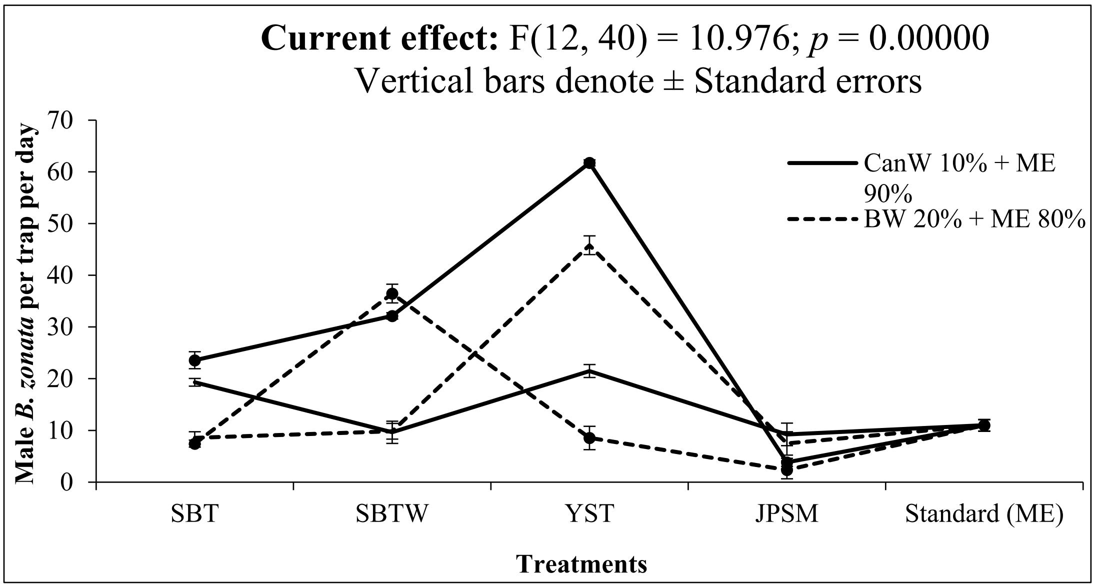

Figure 8. Population of *Bactrocera zonata* captured per trap per day (Means ± SE) in different application techniques of different highly attracted slow-release formulations.

4. Discussion

Insect pheromones have high vitality but exhibit photo-degradability and instability due to their chemical structure. These pheromones reduce the biological activity (attractiveness) of the insects quickly due to photo-oxidation, auto-oxidation, isomerization and volatility [57–59]. Complex chemical reactions, which are influenced by factors like exposure to UV light and oxygen, affect the photo-oxidation, auto-oxidation and isomerization of insect pheromones. Photo-oxidation is a reaction where pheromones react to oxygen and UV radiation, resulting in the formation of reactive oxygen species that cause their molecules to be degraded. Auto-oxidation is when pheromone reactions with oxygen in the atmosphere take place over time. Isomerization is the process by which chemical structure of pheromone molecules changes due to a number of factors. Protection formulations, e.g., pheromones in the wax-based slow release system or additives to an antioxidant formulation, can be used for both prevention and control of these processes. These strategies can contribute to protecting pheromones from the effects of UV light and oxidative reactions [60–62].

This necessitates developing an approach that protect them from photo-degradation by UV light and oxidation, ensures their slow releases, prolong their bio-life and biological activity and enhance their efficiency in pheromone-based mating disruption technique [63]. Finding adequate material for encapsulation, managing release and maintaining the stability of pheromone compounds is one of the most important challenges in protecting pheromones from photo-degradation and oxidation. Exposure to temperature variations and humidity, which also may have an effect on the stability of pheromones and release, can be another potential degradability mechanism [64–66]. Mating disruption is a pest management technique based on the release of large amounts of synthetic sex pheromones in the field, to destroy the sexual communication between insects [58,59].

The mating behavior of tephritid fruit flies, including *B. zonata*, is predominated by "Lekking" comportment which is characterized by aggregation of males on host and/or non-host plantation, attraction of females to lekking-sites and selection of potential counterpart for courtship followed by multiple-mating/polyandry [67–69]. Availability of wild male fruit flies in the orchard and field-crop ecosystem enhances lekking and promotes multiple-mating/polyandry in tephritid fruit flies that ultimately stimulates oviposition in fruits and fruit damage by post-mated females [69,70]. Trapping male population not only inhibits/lessens chances of mating of wild female flies, fertilization of eggs, oviposition and future progeny development but also indirectly minimizes the fruit losses and enhances marketable yield as well as economic benefits [69].

Male tephritid flies themselves do not cause fruit damages directly, however, their indirect role in damage stimulation in post-mated female tephritid flies is too momentous and crucial [69]. If this indirect vital role of male flies is checked by mass male-trapping, through Male-Annihilation-Technique (MAT), post-mating effects (oviposition, fruit damages, remating/multiple-mating behavior) can be controlled or minimized and ultimately, yield and economic benefits can be enhanced [69].

Developing wax-based slow-release-formulations of sex pheromones is an effective approach that enhances the efficiency of a pheromone-based mating disruption system [71]. Their chemical properties have a bearing on the choice of waxes for slow-release formulations. The potential for forming a coherent matrix with pheromones, controlling the diffusion rate of pheromone molecules and protecting them from environmental influences are among the specific characteristics of waxes which increase their retention and release. For these formulations it is often preferable to use wax with appropriate melting points, viscosity and solubility in the carrier solution [72,73].

The lekking behavior of *B. zonata* is mostly observed around the fruits and underside the leaves of the host crop [69,74]. So, mass trapping of male population of *B. zonata* through slow-release wax formulation of para-pheromone (methyl eugenol) in host crop can impact lekking and post-mating effects (oviposition, fruit damages, remating/multiple-mating behavior) negatively [69].

Para-pheromones are highly volatile in nature. When these para-pheromones are installed inside the traps, these are quickly volatilized, photodegraded and lose their bio-efficacy (attractancy) within a short period of time (less than a week) due to their exposure to high temperature in field conditions. So, sole application of para-pheromones in the field in traps proves least effective. The attract-and-kill potential of para-pheromones can be enhanced and prolonged by developing their slow-release formulations in biodegradable natural waxes. Such wax based slow-release formulation can protect para-pheromones from weathering impacts, prolong their bio-life, and enhance their bio-efficacy. A prolonged mass trapping of male tephritid flies by wax based slow-release formulation of para-pheromones in host crop can reduce population of male flies significantly, impact lekking behavior, and influence post-mating effects (remating/multiple-mating behavior, oviposition, fruit damages, yield losses) negatively [69].

In the present study, different bio-waxes-based ME formulations were prepared and assessed for their trapping efficacy against *B. zonata*. The first experiment was designed to indicate the most attractive ratios of ME that attract the flies over a long period with different waxes. Zada et al. [72] used waxes for the slow release of pheromones and proved that waxes sustain the pheromone for over 28 days. Gomez and Coen [75] reported that biodegradable waxes were effective for the continuous discharge of ME for a long period from 4 to 12 weeks. They assessed different biodegradable waxes and results exhibited that 10–45% by weight of these waxes gave long persistency. These results are in agreement with the results of present study which demonstrate that LW 30%, CanW 10%, BW 20%, CarW 10% and PW at 10% gave maximum attractancy and attracted flies for over 30 days. Slow-release formulations (SRFs) based on LW, CanW, CarW demonstrated 3–3.7 times higher attraction than standard (only ME) and were categorized as class-III formulation (AI > 50%). While SRFs based on BW, and PW demonstrated 1–2 times higher attraction than standard and were categorized as class-II formulation (AI = 11–50%). The results regarding the efficacy of PW emulsion of present study conforms with the results of Atterholt et al. [76] who documented that a paraffin emulsion exhibited a longer (more than 100 days) and constant release rate of pheromone at 27°C in the laboratory.

However, the results regarding the attractancy of other wax-based SRFs cannot be compared and contrasted as very little information are available in the literature reviewed on these lines of study involving these waxes. The degradation and release of the pheromone in the wax mixture depend on the types of wax used in emulsion-matrix and vary with the type of wax [77,78]. Yoon et al. [78] reported that the maximum release rate of pheromone was found in an emulsion containing PW. These reports of Yoon et al. [77] also confirm our results regarding the variation in the attractancy of various wax-based SRFs in the field where these SRFs are exposed to various physical factors especially temperature that affect the release rate of pheromone from different waxes. The variation in the temperature-based release rate of pheromone from waxes and ultimately the attractancy of these biodegradable waxes based SRFs may be attributed to the difference in physic-chemical properties of these waxes including thermal properties, chemical composition, hardness/brittleness, crystallinity, contraction, emulsification, oil-binding capacity, solubility in solvent/chemicals, viscosity, and retention-capacity. But these properties of waxes were not studies in the present research and need to be investigated for a better conclusion on the causes of variation in the efficiency of waxes-based slow-release formulations.

The second experiment was designed to compare the attractancy of five selected slow-release formulations from experiment-1 (SRF based on CanW, BW, CarW, PW and LW). CanW based SRF (10% CanW + 90% ME) proved highly attractive (2.6 times higher than standard) followed by Bees wax (20% BW + 80% ME) and PW (10% PW + 90% ME) based SRF (1.5 times higher than standard). The better efficiency of these waxes based SRF over the standard may be attributed to the fact that these waxes improve the chemical retention and surface tension properties as well as coating plasticity of the emulsion prepared by admixing ME and these waxes by forming a protective barrier around pheromone molecules. This barrier prevents the pheromones from being dispersed or evaporating quickly, which allows their release to be controlled and sustained. Waxes can also improve surface tension of the product by facilitating its spread and application to surfaces such as those targeted for insect pests [79]. Similar reasons have also been explained by various researchers [58,63,71]. These results are highly inconsistent with the results of Zisopoulou et al. [59] who reported that different waxes and mineral oil-based emulsion improve surface tension properties and coating plasticity. Overall, the results of Zisopoulou et al. [59] are highly in agreement with the results of present study which demonstrate that maximum attractancy was found in different waxes and SRFs based on these four waxes were found moderately attractive when checked in comparison. The more attractancy of CanW may be attributed to its more solubility towards pheromone and sustained release at the higher rate of pheromone from its emulsion followed by that of BW, CarW and PW in their respective formulations/emulsions. Similar reasons have been explained by Yoon et al. [77] behind the differential sustained release of pheromone from different emulsions prepared from different waxes. According to Gomez and Coen [75], ME, cue-lure and biodegradable wax by weight of the total emulsion-formulation remain effective in providing a continuous release of ME and cue-lure from the carrier over an extended period of at least four weeks. The results of second experiment also demonstrate that three SRFs having 10% CanW + 90% ME, 20% BW + 80% ME, 10% PW + 90% ME and 10% CarW + 90% ME proved more attractive than standard against *B. zonata* in a comparative study. The partial inconsistency in the composition by weight of biodegradable wax is due to variation in components of the emulsion as they have admixed two pheromones (ME and cue-lure) while we have admixed only one pheromone (ME) in an emulsion. The wax-based ME-emulsions prove more effective for a longer period (30 days) than standard ME against *B. zonata*. These results of the present study are highly in conformity with the results of Gomez and Coen [75] who documented that a formulation containing ME, cue-lure and a biodegradable wax carrier proved significantly attractive while the same formulation admixed with toxicant proved highly effective in MAT (attract-and-kill system) for *B. dorsalis* and *B. cucurbitae*.

The efficiency of the pheromones and wax-based pheromone emulsions/formulations depend on the properties of the pheromone, physic-chemical properties of the waxes and other carriers used in the matrix of emulsion and techniques used for their dissemination in the field where so many abiotic factors like temperature, relative humidity and rainfall affect their efficiency [58]. The fruit damage can be significantly reduced when an efficient application technique or traps is used [80].

The third experiment was conducted to assess different implementation techniques of four waxes-based ME-emulsions which demonstrated maximum attractancy over a long period in Experiment-II. The efficacy of each wax base SRF varies with the application technique. This variation in the performance of application techniques for different aforementioned four SRFs may be attributed to the variation in the ability of each technique to protect the SRFs from exposure to heat, UV light, rain etc. During data collection, it was observed that YST technique exhibited more protection to the SRFs from these adverse factors as compared to other application techniques. The CanW-ME SRF (10% CanW + 90% ME), BW-ME SRF (20% BW + 80% ME) and CarW-ME SRF (10% CarW + 90% ME) proved more effective when applied by YST; while PW-ME SRF (10% PW + 90% ME) exhibited more effectiveness when applied using SBTW technique. Depending on their ability to protect the slow-release formulation against adverse external factors, protective measures offered by application techniques like YST, SBT, JPSM and SBTW may differ. Moreover, owing to its physical design, YST can offer better protection than SBTW and it may be able to provide different safety mechanisms. Such techniques may differ, affecting the efficacy of slow-release formulations due to factors such as temperature, UV exposure and humidity. The overall result of experiment shows that the orders of efficacy for application techniques for CanW-ME SRF (10% CanW + 90% ME), BW-ME SRF (20% BW + 80% ME), CarW-ME SRF (10% CarW + 90% ME) and PW-ME SRF (10% PW + 90% ME) were: YST > SBT > JPSM > SBTW; YST > SBTW > JPSM > SBT; YST > SBT W> JPSM > SBT; and SBTW > YST > SBT > JPSM. Khosravi et al. [6] evaluated different methods like Jute sacks, bucket method and spray method for application of pheromone base formulation (7% ME + 7% technical malathion) in a management program of *B. zonata* for successive two years and their results revealed that soaking 8–10 layers of jute sacks, bucket trap and spray method proved more effective in controlling *B. zonata*. These results contradict the findings of the present study. These variations in results may be attributed to the difference in formulation. They have used insecticides-based ME formulation while wax-based SRFs of ME were used in present study. However, the results of third experiment cannot be compared and contradicted because information exactly on these lines of study is not available in the literature reviewed. There is a need to assess the performance of these SRFs in different ecological zones with varying climatic conditions as well as with more application techniques in order to provide sounder and more efficient recommendations against tephritid pests in various fruit orchards and vegetable crops.

5. Conclusions

A promising result has been obtained through field trials evaluating the slow-release wax formulations to control *B. zonata* in agriculture, with important implications for sustainable agricultural pest management. SRF-9[CanW] and SRF-9[CarW] have been found to be among the most efficient tools of attracting and controlling *B. zonata* flies in a number of different wax formulation tests. In particular, the CarW 10% + ME 90% was consistently characterized by high attractiveness throughout the course of the year making it a good option for controlling pests during the whole year. The most efficient application method was identified to be YST, which have been shown to outperform the methods used in earlier studies. The findings offer a breakthrough in the fight against environmentally unfriendly pests that can reduce reliance on chemical pesticides, as well as promote healthy farming practices. In order to increase effectiveness and adoption in agriculture, further research should explore the long-term impact of these formulations on pest management, crop yields and ecological health as well as consider scaling up and integrating them into Integrated Pest Management program.

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