# Toxicity of malathion and spinosad to *Bactrocera zonata* and *Ceratitis capitata* (Diptera: Tephritidae)

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**Abstract**

Recently, an outbreak of the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), in the metropolitan area of Tel Aviv in central Israel was reported. The default action taken in response was the intensive use of the male attractant methyl eugenol applied together with the organophosphate insecticide malathion, which is toxic to a wide range of insects. In agricultural groves, the spinosad bait formulation GF-120™ is routinely used to control the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). In this study, we evaluated the toxicity of malathion and spinosad to *B. zonata* and *C. capitata* in Israel following both contact exposure (tactile) and feeding (insecticides mixed with bait). Whereas doses of 1,000 and 2,000 ppm of malathion were highly toxic to *C. capitata* both upon contact and when eaten with bait, a dose of 10,000 ppm (1%) caused only 10 to 35% mortality of *B. zonata*. This insensitivity to the toxicant cannot be explained by feeding avoidance. On the other hand, the toxicity of spinosad to *B. zonata* was high with $LC_{80}$, $LC_{90}$, and $LC_{99}$ values of 12.28, 17.67, and 33.62 ppm, respectively. This result suggests that the spinosad-based control measures routinely taken against *C. capitata* in Israel could be effective against *B. zonata*.

Key Words: Mediterranean fruit fly; peach fruit fly; bait spray; phagostimulation

**Resumen**

Recientemente se detectó un brote de mosca del durazno, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), en la zona metropolitana de la ciudad de Tel Aviv en el centro de Israel. El programa de acción aplicado inmediatamente a partir de la detección fue la aplicación masiva del atrayente masculino, metileno de eugenol, mezclado con el insecticida organofosforado malation, que se le conoce por ser toxico para una gran cantidad de insectos. En hortalizas de Israel el compuesto GF-120™ es usado rutinariamente para controlar a la mosca de las frutas del Mediterraneo, *Ceratits capitata* (Wiedemann) (Diptera: Tephritidae). El presente estudio examina el efecto toxico de malathion y espinosad en poblaciones de *B. zonata* y *C. capiata* de Israel. El estudio investigo el efecto de la exposición de contacto (táctil) así como el efecto de la mezcla ingerida (insecticida más cebo). Aunque las dosis de 1,000 y 2,000 partes por millón (ppm) de malathion fueron altamente toxicas para *C. capitata* tanto al contacto como al ser ingeridas con cebo, dosis de 10,000 ppm (1%) solo generaron una mortalidad de entre 10 to 35% en *B. zonata*. Este resultado no puede ser justificado en base a un rechazo de las moscas a la ingestión de la mezcla. Por otro lado, la toxicidad de espinosad en *B. zonata* fue significativa, con valores respectivos de $LC_{80}$, $LC_{90}$ y $LC_{99}$ de 12.28, 17.67, y 33.62 ppm, respectivamente. Estos resultados sugieren que los tratamientos rutinarios en hortalizas realizados en contra de *C. capitata* en Israel puedenserefectivos contra de *C. capitata* en Israel deben de ser también efectivos contra *B. zonata*.

Palabras Clave: mosca de las frutas del Mediterráneo; mosca de durazno; aspersión de cebos tóxicos; fagoestimulación

One of the key strategies for the control of tephritid fruit flies is the use of the correct combination of attractive bait and effective insecticide. Malathion is an organophosphate insecticide with contact toxicity to a wide range of insects. When combined with a food-based bait, malathion provides selective control of fruit flies (Steiner 1952). Until 2009, malathion was commonly used in Israel to control the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), in citrus orchards, and was applied mainly by aerial, ultra-low volume spraying (Rössler 1989). Buminal® bait (containing hydrolyzed proteins and malathion; (Bayer, Leverkusen, Germany) lured the fly into contact with the insecticide, which killed it within minutes. Since 2009, the use of malathion for control of *C. capitata* in Israeli citrus orchards has been completely prohibited and growers are now using the spinosad bait formulation GF-120™ Fruit Fly Bait (Dow AgroSciences, Indianapolis, Indiana) instead.

Recently, there was an outbreak of the peach fruit fly, *Bactrocera zonata* (Saunders), in the metropolitan area of Tel Aviv (EPPO 2014). The default response was the intensive use of the male annihilation technique as the most suitable method available for the eradication or control of *B. zonata* (EPPO 2010). The male annihilation technique is based on the *B. zonata* male attractant and the para-pheromone methyl eugenol (4-allyl-1, 2-dimethoxybenzene-carboxylate) (Hardy 1979) combined with malathion and applied as spot treatments and lure-and-kill stations (EPPO 2010). In such programs, the toxicity of malathion is essential for successfully suppressing the *B. zonata* male population.

Over the past decade, several studies have examined the toxicity of malathion and spinosad to *B. zonata*. Some of these studies have reported that *B. zonata* is affected by both insecticides (El-Aw et al. 2008; Ahmad et al. 2010; Ghanim et al. 2010). However, in recent reports, there is evidence of *B. zonata* resistance to malathion. In Egypt, field-collected *B. zonata* were highly resistant to malathion (resistance ratio >30) when compared with laboratory susceptible insects as a result of qualitative effects on the acetylcholinesterase enzyme (Radwan 2012). In Pakistan, field populations of *B. zonata* ranged from susceptible to

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malathion to moderately resistant (20-fold; Haider et al. 2011; Nadeem et al. 2014).

The objective of this study was to compare the toxicity of malathion and spinosad to *C. capitata* and *B. zonata* in Israel. We evaluated both the contact (tactile) toxicity of the insecticides, and feeding toxicity when mixed with bait.

## Materials and Methods

### INSECTS

*Ceratitis capitata* was obtained from the 'Sade' laboratory colony (Rössler 1975; Gazit et al. 2013), which is maintained in Bet Dagan, Israel, at the Israel Cohen Institute for Biological Control, Plant Production and Marketing Board, Citrus Division. This colony is replenished with new field-collected males every 2 yr, in order to maintain genetic diversity and a range of genotypes that is as similar as possible to that found in field populations.

*Bactrocera zonata* was obtained from the laboratory colonies that were kept under quarantine restrictions in the Plant Protection and Inspection Services (PPIS) in Bet Dagan, Israel. These colonies were established independently in 2012 and in 2014. Each colony was initiated from flies that emerged from infested fruits collected in backyards in Tel Aviv. The age of each colony used in this study was <2 yr old. The flies were kept in ventilated acrylic cages (30 × 30 × 40 cm), with approximately 3,000 adult flies in each cage. Beginning at 24 d after adult emergence, twice a week, we placed 2 to 4 oviposition devices in each cage for 24 to 72 h. Each oviposition device consisted of a 100 mL plastic flask (Actimel, Danone, Trowbridge, Wiltshire, UK) perforated by a needle from 2 cm above the bottom to the rim, with 5 to 10 mL of water at the bottom of the flask. Each flask was plugged with a 50 mL conical centrifuge tube (Miniplast, Ein Shemer, Israel), which had 4 holes (10 mm diameter) drilled in its center and contained about 5 mL of orange juice. The females landed on the flask, sensed the odor from the orange, inserted their ovipositor through the perforation and laid their eggs in the space between the flask wall and the centrifuge tube. To recover the eggs from the ovipositing device, the water containing the eggs was collected from the bottom of the flask and the flask was flushed with additional water from a squirt bottle to thoroughly wash the eggs off the inner walls. After the eggs had sunk in the water, they were pipetted and seeded on medium containing a larval diet, similar to the diet used for *C. capitata* (Rössler 1975), consisted of 26.8% wheat bran, 8.1% brewers yeast,12.1% sucrose, 1.6% technical-grade HCl, 51% water, and 0.4% fungicide sodium benzoate instead of methylparaben (nipagin), which was found to harm *B. zonata* larvae.

### MAINTAINING ADULT FLIES

Adult flies (*B. zonata* and *C. capitata*) were supplied with ad libitum water soaked in cotton wool and with a dry mixture of sucrose and enzymatic hydrolyzed yeast (BMP Biomedicals, Solon, Ohio) 4:1 (sucrose:yeast; w/w). The flies were kept at 24 ± 1 °C, 60 to 80% relative humidity, with a 14:10 h L:D photoperiod. Unless indicated otherwise, all tested flies were protein-deprived. That is, they were supplied from emergence with ad libitum water and dry sucrose only.

### INSECTICIDES

We tested 3 formulations of malathion. Commercial malathion 1,040 (1,040 g/L) and malathion 50 (500 g/L) were obtained from Tarsis Ltd. (Petah Tikva, Israel). In addition, and a custom-made emulsifiable formulation of Fyfanon® (96–97% malathion, Cheminova, Lemvig, Denmark) at 250 g/L was obtained from Luxembourg Industries Ltd. (Tel Aviv, Israel). Two formulations of spinosad, Tracer® Ultra (consisting of 11.05% spinosad) and GF-120™ (consisting of 0.02% spinosad in the bait), were obtained from Dow Agrosciences (Indianapolis, Indiana).

### BAITS

The 3 baits used in this study were: (a) "hydrolyzed yeast," which consisted of 89 mL water, 1 g enzymatic hydrolyzed yeast (BMP Biomedicals, Solon, Ohio), and 10 g dry sucrose; (b) "Buminal", which consisted of 83.75 mL water, 6.25 mL Buminal® (Bayer, Leverkusen, Germany), and 10 g dry sucrose; and (c) "Success", which consisted of 89 mL water, 1 mL GF-120™ and 10 g dry sucrose.

When we added food coloring to the bait, we used Royal Blue (E-133 and E-153, Magic Colours™, Kibbutz Ma'ale Ha'Chamisha, Israel) at a rate of 1:100 v/v. The coloring improved the visibility of the drops and served as a marker for feeding as the fly abdomen turned blue.

### BIOASSAYS

#### Contact Toxicity Assay

All tests were carried out indoors under quarantine conditions at the PPIS, Bet-Dagan, Israel. Relatively small, <8-cm-long citrus leaves were individually inserted by their petiole into microcentrifuge tubes containing agarose gel (2% agar agar), which provided moisture and support to the leaf. After malathion 1,040 was suspended in water to make 1,000 and 2,000 ppm suspensions, we immersed each leaf in 1 of the suspensions for a few seconds and then placed it on a paper towel to dry. After 1 h, we placed each leaf in a small, ventilated 390 mL plastic container (9.5 cm diameter, 5.5 cm height), which contained wet cotton wool and dry sucrose (water and food ad libitum), and then used an aspirator to insert 10 *B. zonata* (5 males and 5 females) into the container. Mortality was recorded after 24 h. Then, in order to assure the toxicity of the malathion, we added 10 *C. capitata* (5 males and 5 females) to the same cages in addition to the *B. zonata*. Mortality of both fly species was recorded after 24 h. It should be noted that, in this assay, the flies could stay on the plastic walls of the container and avoid contact with the leaf.

#### Feeding Toxicity Assay

All tests were carried out indoors under quarantine conditions at the PPIS, Bet-Dagan, Israel. We diluted the insecticides with the baits to obtain a gradient of doses in a constant amount of bait. To eliminate any possible effects of the leaf surface, we used the microscope slide method (plain glass, 2.5 × 7.6 cm) as described by Gazit et al. (2013). For each tested compound, we pipetted fifteen 3 µL drops onto microscope slides. We then left the drops to dry overnight. We carried out the feeding-toxicity assays in perforated plastic containers (as mentioned above) that were placed upside down to form a bottomless ventilated arena. Wet cotton wool (water ad libitum) was provided in each arena. Ten *C. capitata* or *B. zonata* (5 males and 5 females) were inserted into each arena. Then, we carefully lifted the container a little and slid a slide with drops inside. After 2 h, we used a spatula wrapped in sticky tape to remove the slides and inserted 2.5 mL plastic caps with dry sucrose into the arena. Mortality was determined 24 h after exposure. Fly consumption of the insecticide was evaluated by observation, by subtracting the number of remaining drops and drop fragments from the initial 15 drops. At least 5 replicates were performed for each dose. The mortality rate was calculated relative to the control (untreated flies) with Abbott formula (Abbott 1925). When we used

Success bait, which contained 2 ppm of spinosad, we calculated the mortality relative to that of untreated flies that were supplied with 10% sucrose. It is important to note that, in this assay, the 390 mL plastic arena eliminated the role of long-range attraction.

### STATISTICAL ANALYSES

Differences in fly consumption of bait containing different doses of insecticide were determined using analysis of variance (ANOVA). Data that yielded significant overall *P* values by ANOVA were subsequently subjected to Tukey honest significant difference (HSD) tests ($P < 0.05$). Probit analysis of the mortality was carried out with JMP 5.0 (SAS 2002). The doses (ppm) generating 80, 90 and 99% mortality ($LC_{80}$, $LC_{90}$, and $LC_{99}$ values, respectively), with 95% fiducial intervals, were calculated by an inverse prediction, following the fitting of the model of mortality as a function of dose. Differences in consumption rates were determined by analysis of covariance (ANCOVA).

## Results

*Bactrocera zonata* and *C. capitata* responded differently when they were exposed to the 2 doses of malathion (1,000 and 2,000 ppm). Although *B. zonata* flies made contact with the leaves with no repellent behavior, they remained alive. On the other hand, all of the *C. capitata* that were introduced on the next day into the same arena with the living *B. zonata* died within 24 h.

To further examine this difference in malathion toxicity between the 2 species of flies, we tested their responses in a feeding-toxicity assay that included 3 different formulations. The first step toward that was to find appropriate baits that would generate maximal consumption. In a preliminary study, we found that *B. zonata* flies were not stimulated to feed by the commercial bait Buminal when it was diluted with water. However, the addition of sucrose transformed this bait into a phagostimulant. Therefore, we mixed each of our tested baits (Buminal, GF-120, and hydrolyzed yeast) with a 10% sucrose solution, which was found to generate maximal intake. The phagostimulation as a function of bait concentration was similar in the 3 baits (Fig. 1) and the ANOVA revealed significant differences in consumption among concentrations: $F = 98.26$, df = 5, 18, $P < 0.001$; $F = 18.58$, df = 5, 18, $P < 0.001$; and $F = 8.62$, df = 4, 14, $P < 0.001$, for Buminal, GF-120, and hydrolyzed yeast, respectively. Maximal consumption occurred at the lowest bait concentrations (6.25% Buminal, 1% GF-120, and 1% hydrolyzed yeast) and was similar to that observed for the no bait control (10% sucrose only). Increasing the dose of each of the baits resulted in significant decreases in consumption.

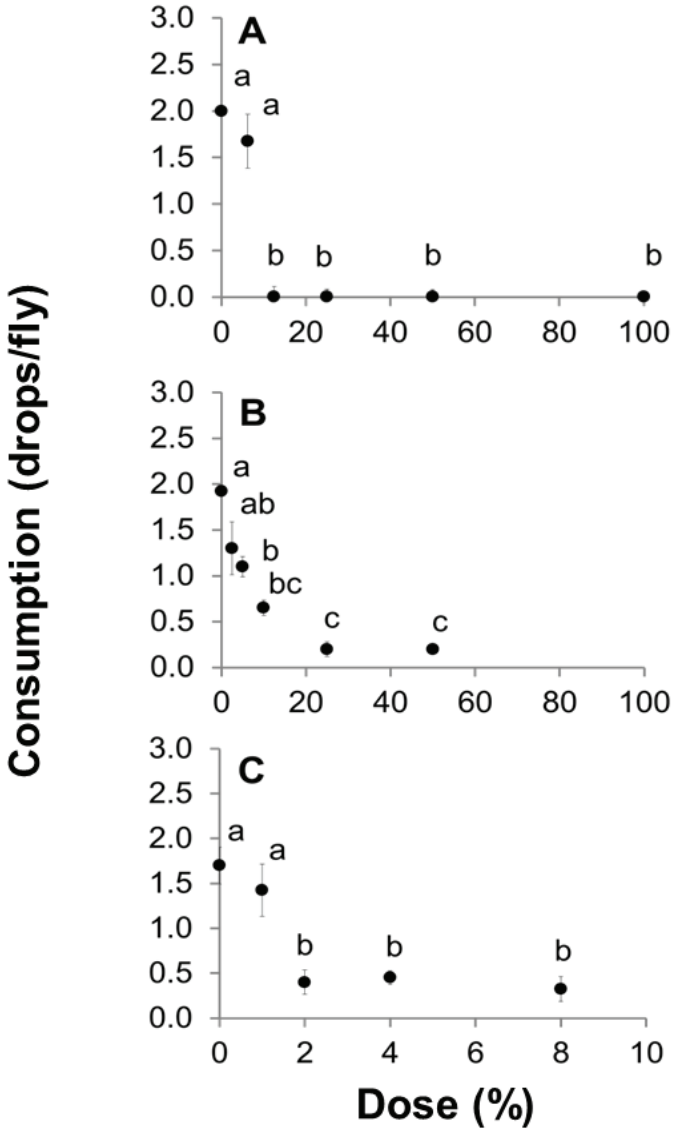


**Fig. 1.** Mean (± SE) consumption rate (drops per fly) of *Bactrocera zonata* exposed to drops of 10% sucrose with Buminal (A); GF-120 (B); and hydrolyzed yeast (C). The flies had access to the drops for 2 h and consumption was evaluated by observation. Statistical analysis was performed separately for each bait. Means labeled with different letters are significantly different from each another (Tukey HSD test, $P = 0.05$).

When *B. zonata* flies from a colony established in 2012 were exposed to drops of Buminal-baited malathion 1,040 and *B. zonata* flies from a colony established in 2014 were introduced to the hydrolyzed yeast bait + malathion 50, the maximal insecticide dose of 10,000 ppm (1%) generated mortality rates of 35.0 ± 9.6% and 10.0 ± 3.5% among the flies of the 2 colonies, respectively (Fig. 2A & B). Although the $R^2$ levels of the logarithmic slopes indicated a relationship between dose and mortality, the values were too low for further calculations of dose–mortality models. When a different formulation of emulsifiable Fyfanon® was tested with the Buminal bait, the maximal tested dose of 5,000 ppm resulted in a slight mortality rate of 10%, with no significant mortality observed for the lower doses.

On the other hand, for *C. capitata*, the Buminal bait + malathion 1,040 treatment and the hydrolyzed yeast bait + 1,250 ppm malathion 50 treatment yielded 100% mortality (Figs. 2 and 3). For malathion 1,040, the predicted $LC_{80}$, $LC_{90}$, and $LC_{99}$ values (with 95% fiducial intervals) were 391.65 (341.36–438.39), 576.94 (532.48–651.77), and 1,124.84 (977.05–1,367.66) ppm, respectively ($\chi^2 = 328.5$, df =1, $P < 0.001$). For the hydrolyzed yeast bait + malathion 50, the predicted $LC_{80}$, $LC_{90}$, and $LC_{99}$ values (with 95% fiducial intervals) were somewhat lower: 131.80 (110.44–151.58), 245.12 (221.18–276.39), and 580.22 (508.17–685.93) ppm, respectively ($\chi^2 = 148.2$, df = 1, $P < 0.001$).

The food coloring added to the bait enabled the visual observation of the consumption of the malathion 50 + hydrolyzed yeast bait within the 2 h exposure period (Fig. 3). Consumption (drops per fly) was significantly lower when greater doses of malathion were used. To further compare the consumption of the 2 species, we performed an ANCOVA, which revealed a significant interaction between fly and dose ($F = 7.95$, df = 1, $P = 0.009$). Separate evaluations of the effects of the different doses of malathion 50 (i.e., 312.5, 625.0, and 1,250.0 ppm) on the 2 species revealed that *B. zonata* consumed significantly more drops than *C. capitata* ($t = 2.447$, $P = 0.001$; $t = 2.447$, $P = 0.003$; and $t$

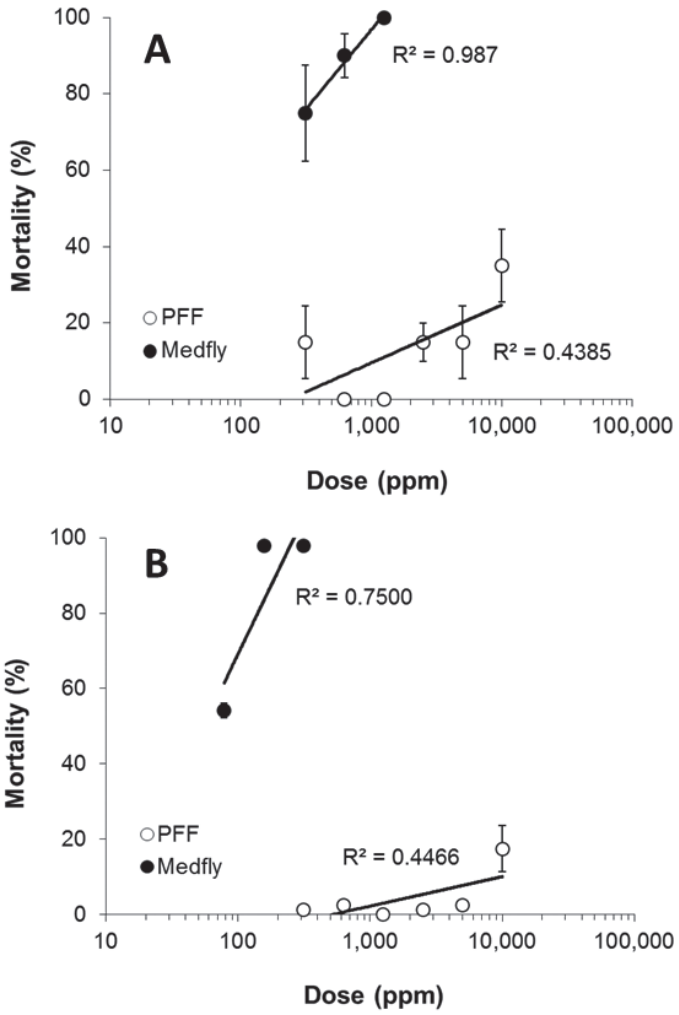


**Fig. 2.** Mortality (± SE) of *Ceratitis capitata* (Medfly) and *Bactrocera zonata* (PFF) following 2 h of exposure to glass slides with 3 µL drops of Buminal bait containing various doses (ppm) of malathion 1,040 (A) and of hydrolyzed yeast bait containing various doses of malathion 50 (B).

= 2.447, $P$ = 0.039, respectively). The dosage of 2,500 ppm, a dose that killed 100% of *C. capitata*, produced only 1.25% mortality in *B. zonata*, and the differences between the consumption rates of the 2 species were not significant ($t$ = 2.571, $P$ = 0.332).

Because *B. zonata* was found to be almost completely unaffected by malathion, we decided to evaluate its response to spinosad, which is the principal insecticide used to control *C. capitata* in citrus in Israel. The toxicity data showed that the *B. zonata* was affected by spinosad (Fig. 4) and the predicted $LC_{80}$, $LC_{90}$, and $LC_{99}$ values (with 95% fiducial intervals) were 12.28 (11.03–13.86), 17.67 (15.81–20.17), and 33.62 (29.64–39.15) ppm, respectively ($\chi^2$ = 511.4, df = 1, $P$ < 0.001). Consumption was significantly affected by dose ($R^2$ = 0.501, $F$ = 22.096, df = 1, 22, $P$ < 0.001), yet even at doses of 600 and 1,200 ppm, which generated 100% mortality, consumption was more than 1 drop per fly.

## Discussion

This toxicity study was based on our ability to generate substantial intake of the insecticides by both the *B. zonata* and *C. capitata*. This was done by (a) depriving the tested flies of protein and (b) by finding good phagostimulants for the 2 fly species that not only contained 10% sucrose, but also contained Buminal® and GF-120™, which are used in the field to attract fruit flies. Such phagostimulation by low concentrations of attractants was previously reported for *C. capitata* and for the lesser pumpkin fly, *Dacus ciliatus* (Loew) (Diptera: Tephritidae) (Nestel et al. 2004). Among fruit flies, attraction to food-based baits is not necessarily correlated to actual phagostimulation. Therefore, for ingested insecticides, phagostimulation of the targeted fly by the bait should be carefully studied.

The organophosphate malathion is considered toxic to a wide range of insects, including *C. capitata*. However, this study demonstrated that *B. zonata* in Israel were not affected by malathion when contacting treated leaves or upon feeding on malathion mixed with phagostimulant baits. Unlike *C. capitata*, which was killed upon contact and feeding, *B. zonata* was insensitive even to doses of 10,000 ppm (1%). This dose is 10-fold higher than the dose generating >99% mortality of *C. capitata*. This insensitivity in *B. zonata* was demonstrated with 3 different formulations of malathion (i.e., malathion 50, malathion 1,040 and emulsifiable Fyfanon®), using 2 different colonies of *B. zonata* that

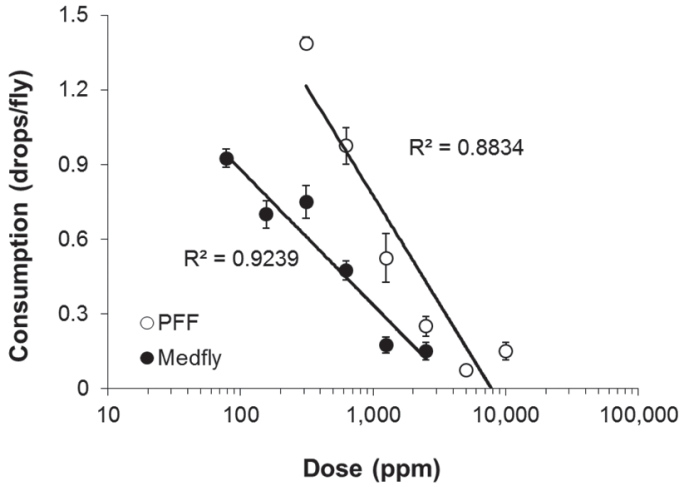


**Fig. 3.** Consumption (drops per fly; ± SE) of drops of hydrolyzed yeast bait containing various doses of malathion 50 on glass slides by *Ceratitis capitata* (Medfly) and *Bactrocera zonata* (PFF) following 2 h of exposure.

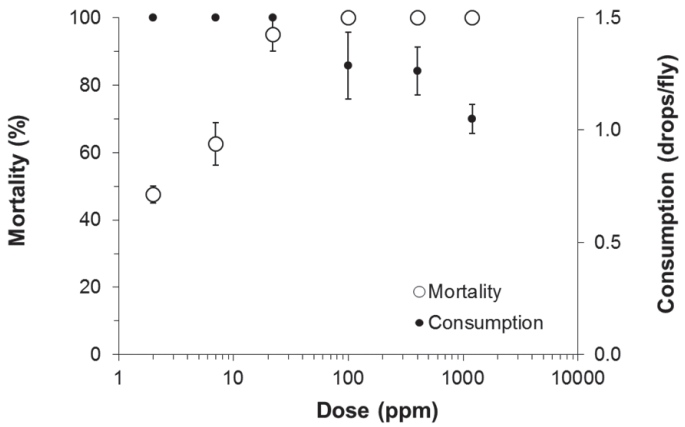


**Fig. 4.** Mortality (± SE) and consumption (drops per fly; ± SE) of 3 µL drops of Success bait (1% GF-120 with 10% sucrose) containing various doses of spinosad on glass slides among *Bactrocera zonata* following 2 h of exposure.

were established from infested fruits collected on 2 occasions from 2 different backyards in Tel Aviv. This lack of toxicity cannot be explained by feeding avoidance because *B. zonata* consumed significantly more malathion-baited drops than *C. capitata*.

A similar insensitivity to malathion also was reported for *D. ciliatus* by Maklakov et al. (2001). Because other studies have found malathion to be effective against *B. zonata*, it is possible that the resistance demonstrated in our study is localized to the population of *B. zonata* found in the metropolitan area of Tel Aviv, possibly due to the intensive use of malathion-based male annihilation technique in this area. Consequently, when malathion is the key insecticide in campaigns to control *B. zonata* that are based on both male annihilation techniques using methyl eugenol with malathion and malathion-bait sprays, this insensitivity of the fly to malathion may cause those control efforts to fail.

On the other hand, the toxicity of spinosad to *B. zonata* suggests that the current control measures based on this insecticide routinely used against *C. capitata* could also be effective against *B. zonata.* We point out that in Israel, the recommended GF-120™ formulation for ground sprays is 1:10 (v/v) in water, which contains 20 ppm spinosad. The $LC_{99}$ of spinosad to *B. zonata* observed in this study (i.e., 33.62 ppm) suggests that this formulation may be too weak, and inadequate for *B. zonata* control. The attractiveness and phagostimulatory effect of spinosad baits on *B. zonata* in the field merit further investigation.

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