TOXICITY OF THE PHOTOACTIVE COMPOUNDS PHLOXINE B AND MENADIONE ON *BACTROCERA ZONATA* (DIPTERA: TEPHRITIDAE)

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Abstract

The photoactive compounds phloxine B and menadione are relatively safe food additives and possess efficient insecticidal properties against certain insect species. The two compounds were tested for toxicity to the peach fruit fly, *Bactrocera Zonata* (Saunders) under both dark and sun light conditions. Both compounds had no significant toxic effect in dark and concentration-dependent toxicity when insect exposed to sunlight for 2 hrs. Phloxine B was approximately 1000 time more toxic to *B. zonata* than menadione with LC_{25} in water of 0.0007 g% and 0.854 g% (w/v), respectively. Adding menadione to phloxine resulted in significant antagonistic effects on insect mortality, which could be due to menadione alteration of the pH of the feeding media and/or menadione induction of the ROS-detoxifying enzymes, particularly peroxidase. It is concluded that phloxine B and menadione are incompatible and their mixture is not likely to pose any significant addition to their control potential against *B. zonata.*

INTRODUCTION

The peach fruit fly, *B. zonata* is a serious pest of fruits over a large part of the world. In Egypt, it is is responsible for high loses in fruit crops (Saafan *et al.*, 2004). The organophosphate malathion mixed with protein bait has traditionally been used to control fruit fly populations. However, such control methodology, has a serious problem due concerns on its environmental and human safety.

Within the last decades an extensive research has been carried out on photoactive compounds called phototoxins or photosensitizers as candidate insecticides. Several photosensitizing agents have been shown to be accumulated in significant amounts in a variety of insects when they are administered in association with suitable feeding baits. The subsequent exposure of such insects to UV/visible light leads to the formation of insect-toxic reactive oxygen species (ROS) such as free radicals, hydrogen peroxide and singlet oxygen. ROS has the ability of oxidizing a variety of significant bio-molecules, including enzymes, hormones, lipids, and nucleic acids; and consequently leads to a significant drop in insect survival (Khalifa, 2012). Of such photosensitizers, phloxine B has proved to be efficient insecticides against

more than 30 insect species in both laboratory experiments and field studies, among them several species of fruit flies (El-Adely *et al.*, (2007) and Khalifa (2012). Phloxine B (2',4, 5',7'-tetrabromo-4,5,6,7-tetrachlorofluorescein; D&C Red No. 28) is a red dye found in drugs, cosmetics and foods; possesses low toxicity to mammals (Khalifa, 2012). However, in spite of growing interest in the toxicological effects of pesticide alternatives on insect pest and increasing studies on mode of action of photoactive pesticides, one of the remaining concerns in field of pest management is resistance of insect to such promising compounds. Respicio and Heitz (1983) evaluated the possible development of resistance to phloxine B in *M. domestica and* were able to establish resistance levels at 3 folds that of the unselected strain in 32 generations. Khalifa (2012) reported 2 fold resistance ratio among Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) treated with increased concentrations of phloxine B over 16 generations. Investigating the sensitivity of pests and using a mixture of chemicals is an accepted approach which has been suggested by some authorities to overcome pesticide resistance. In this respect, Liquido *et al., (*1995a&b) evaluated the joint toxicity of mixing phloxine B with fluorescein or uranine with limited synergism of the mixture. However, if resistance were to develop in the field during fruit fly control by phloxine B, addition of another photo-pesticide with the same mode of action of phloxine B such as fluorescein or uranine may not be an option (Mangan and Moreno, 2001). If resistance to phloxine B were the result of selection for decreased sensitivity to ROS or altering its production conditions, this resistance might be overcome by adding another pesticide with different mode of action.

Menadione (vitamin K_3) is a polycyclic aromatic ketone that can function as a precursor in the synthesis of vitamin K and used in poultry as a dietary supplement. According to European Food Safety Authority (EFSA) Vitamin K3 in the form of menadione nicotinamide bisulfate MNB) is authorized as feed additives and intended for use in feed all animal species without a maximum limit (EFSA, 2014). Menadione was shown to have toxic effect on different insect species such as *Dysdercus koenigii* Fabr. (Magdum and Banerjee, 2009), *Dysdercus cingulatus*-Fabricius (Singh-Gupta *et al.*, 2015) and *Drosophila melanogaster* Meigen (Lushchak, *et al.*, 2014). Although both menadione and phloxine B promote insect death by imposing massive oxidative stress upon exposure of insects to sunlight, they possess different mechanisms of exerting their toxicity. While phloxine B is an efficient singlet oxygen generator, the main mechanism of menadione seems to be through the production of hydrogen peroxide and alteration of intracellular Ca^{2+} homeostasis (Arriaga *et al.*, 1994 and McCormick *et al.*, 2000). In addition, menadione has toxic effects in the dark via

biochemical pathway as it metabolized by a variety of cellular reductive enzymes, to unstable semiquinones that can readily enter into a redox cycle when molecular oxygen is present, causing a reformation of the quinone, with the generation of ROS (Criddle *et al*., 2006).

Accordingly, this study was initiated to compare phloxine B and menadione for toxicity against the peach fruit fly, *B. Zonata* and to determine the additive, synergistic, or antagonistic effect of their combinations to overcome the expected development of resistance. Also, information on the relative toxicity of these photoactive compounds to herbivores, when ingested alone or in combination, will improve our understanding of their interaction and mode of action.

MATERIALS AND METHODS

2.1. Photosensitizers:

The photosensitizers screened in this study were: Menadione (Menadione nicotinamide bisulfate 96%, Microvit®, Adisseo, France) and phloxine-B (D&C Red #28, Oxford Laboratory, Mumbai. India)

2.2. Insects Source:

Adults of *B. zonata*, were obtained from a laboratory colony reared according to the rearing procedure described by El-Adely *et al*., (2007). All the tests were done with 3- to 7-days-old flies. The day before testing, flies were separated by aspirating and placed inside cubic (30 cm per side) screened (10 mesh per centimeter) cages. Isolated flies were fed only on water for 24 hours.

2.3. Preparation of photosensitizer sucrose media:

Agarose based sucrose media containing 10% sucrose and known concentration of the tested photosensitizes was prepared according to the method described in details by Khalifa (2012). Control agarose media contained only 10% without any photosensitizer.

2.4. Acute toxicity:

Six concentrations were prepared for each photosensitizer: 0 (control), 0.0001, 0.0005, 0.001, 0.005, and 0.01g/100 ml of sucrose media for phloxine B and 0, 0.05, 0.1, 0.5. 0.1 and 1.5 g/100 ml of sucrose media for menadione. These two ranges, was chosen following El-Adely *et al*., (2007) and Singh-Gupta *et al*., (2015). For each concentration, three cages containing a group of 40 hungry flies were prepared. Petri dishes (25-mm-diameter) containing test media were left in the presence of flies in the dark at 25±1ºC and 65-70% RH. A hungry peach fruit fly could feed in the dark (Mangan and Moreno, 2001). After 24 hours incubation at dark, number of dead flies

was recorded to determine the dark effect of the selected photosensitizer. Light-induced mortality was estimated by taking the cages outdoors from 11.00AM to 13.00 PM Cairo local time. Because sunlight fluence rates varied with time, the actual fluence rate was accounted through the average of the different intensities during the exposure time (2-hrs). The average intensity of natural sunlight in the clear day over the experimental period was measured as 400 W/m^2. After exposure, cages were returned to the laboratory and kept at 25±1ºC and 65-70% RH for 24 hrs before estimating mortality, LC_{50} and LC_{25}.

2.5. Joint toxicity of phloxine B*, and* menadione:

Joint toxicity of phloxine B*,* and menadione was determined following the procedure of Diawara *et al*., (1993) by testing combination of LC_{25s} of the two compounds for toxicity to *B. zonata.* The treatments were: control, phloxine B, menadione, and phloxine B + menadione. Bioassay procedure was the same as for the acute toxicity experiment. Expected mortality was calculated using the formula:

$E = O_a + O_b (1 - O_a/100)$ (Diawara *et al*., 1993), where E is the expected percent mortality of phloxine B and menadione *Combinations*, O_a is the expected percent mortality due to phloxine B alone, and O_b is the expected percent mortality due to menadione alone.

2.6. Chronic toxicity:

Adults of *B. zonata* were fed on agarose based media containing a concentration equal to the calculated LC_{25} from each of phloxine B, menadione and their combination for 24 hrs. The mortality of flies was monitored after 24, 48, 72 and 96-hours post exposure to sunlight for 2 hrs.

2.7. The pH dependent toxicity:

The effect of pH of the agarose based media on phloxine B toxicity against *B. zonata* was determined following the same bioassay procedure described in the acute toxicity experiment, but using concentration equal to the calculated LC_{50} of phloxine B and different feeding media pH (7.0, 6.0, 5.0, and 4.0). Feeding media with different pH were prepared using citric acid-sodium citrate buffer system instead of distilled water. Control feeding media was prepared for each pH using the same buffer system, but without addition of phloxine B. The pH of the stock buffer solutions, base feeding media, media containing phloxine B, media containing menadione, and media containing both phloxine B and menadione were measured using Corning 445 pH-meter (corning Inc, UK) at 25±2°C.

2.8. Peroxidase activity:

Three groups of flies, (each of 20 frozen flies) were homogenized in liquid nitrogen. Total soluble proteins were determined in the homogenate by the method of Bradford (1976), then peroxidase activity was determined by a direct spectrophotometric method described by Hammerschmidt and Kuc (1982). The values were expressed as Unit/min/mg protein. The assays were done at least in triplicate.

2.9. Statistical analysis.

The experiments were replicated three times. The $LC_{25\&50}$ and the slope of concentration/mortality regression were estimated by probit analysis using a software package "LD-Pline", Copyright of Ihab. M. Bakr, Plant Protection Research Institute, Egypt. Data of the other experiments were evaluated statistically using ANOVA at $P<0.05$ on the arcsin-transformed percentage mortality data. Results were recorded as mean ± standard deviation (SD).

RESULTS

3.1. Acute toxicity

Preliminary results showed that after 24 hours of feeding at dark no significant increase in mortality was observed among fly groups fed with phloxine B or menadione compared to control groups. The acute toxicity of phloxine B and menadione to *B. zonata* adults following 2 hrs exposure to sunlight is shown in Figure 1 and Figure 2, respectively. After 24 hours post- exposure to sunlight phloxine B had a significant phototoxic effect on *B. zonata* in a concentration-response relation. Figure 1 shows that feeding *B. zonata* adults on sucrose media containing a phloxine B concentration as low as 0.0005g% (w/v) resulted in a mean percent mortality of 10.0 ± 4.0 % after 24 hours following exposure to sunlight and reached 90.0 ± 5 % at a phloxine B concentration of 0.01%. Probit analysis indicated that the LC_{50} of phloxine B after 24 hours post-exposure to sunlight was 0.0031% (Table 1). On the other hand, feeding *B. zonata* adults on sucrose media containing 0.05% (w/v), menadione resulted in 7.3± 0.6% mortality after 24 hours following exposure to sunlight (Fig. 2). Mortality ratio increased slowly with increasing menadione concentration. At menadione concentration as high as 1.5% (w/v), only 35 ± 5.0% of individuals were dead after 24 hours of exposure to sunlight. Based on LC_{25} values, phloxine B was almost 1000 times more toxic to *B. zonata* adults than menadione following identical conditions of exposure with LC_{25} equal 0.0007g% compared with LC_{25} of 0.85g% for menadione (Table 1).

3.2. Joint toxicity of Phloxine B *and* menadione:

The acute toxicological response of *B. zonata* adults following exposure to a combination of phloxine B and menadione together is shown in Table 2. Flies fed on media containing a concentration equal to LC_{25} from each of the two compounds resulted in antagonistic effect on fly's mortality. Mortality ratio of the mixture was less than that of menadione alone or phloxine B alone. The difference in mortality ratio between the mixture and menadion was insignificant ($p>0.05$) while the difference in mortality ratio between the mixture and phloxine B was significant ($p<0.05$). The calculated expected combined toxicity of phloxine-menadione mixture was 43.29 while the actual observed mortality was much less (18.0± 3.0%).

3.3. Chronic toxicity:

To determine the possible delayed lethal effect of phloxine B, menadione and their combination against *B. zonata* adults, flies were fed on agarose based media containing concentration equal to the calculated LC_{25} from each of the two compounds and their combination for 24 hours. Mortality ratio was monitored after 24, 48, 72 and 96 hours post exposure to sunlight for 2 hours. Figure 3 showed that the mortality ratio gradually increased over time, reached 34.6±4.0, 30.4±6.8 and 25.0±3.8 after 96 hrs post exposure to sunlight compared with 28.6±3.5, 21.8±3.5 and 18.3±3.0 after 24 hrs post exposure to sunlight for phloxine B, menadione and their combination, respectively (Fig 3). The differences in mortality ratio between 24 hours and 96 hours post-exposure were significant for the three treatments ($P<0.05$). However, mortality ratio among flies fed on the combined LC_{25} of phloxine B and menadione was significantly lower than that of phloxine alone and comparable to that of menadione alone ($P>0.05$) even after 96 hours of exposure to sunlight.

3.4. The effect of the pH of the feeding media on phloxine B toxicity:

Table 4 indicated that the measured pH of the feeding media (Agarose + sucrose + distilled water) was 6.7. Addition of phloxine B in a concentration equal to the calculated LC_{25} did not affect greatly the neutral pH of the media. However, adding menadione alone or in combination with phloxine B to feeding media decreases dramatically the pH of the media to extremely acidic pH (4.2, and 4.1, respectively).

Figure 4 showed that the mortality ratio among *B. zonata* adults fed on agarose based media containing LC_{50} of phloxine B decreased with decreasing pH of the feeding media. Mortality ratio was 57.2 ±9.0% at pH equal 7.0, decreased to 46.3±7.2% at pH equal 6. The difference in mortality ratio between pH 7 and 6 was not significant ($P>0.05$). However, mortality ratio decreased significantly at pH 5 reached 18.0 ± 9.0% and further decreased to 6.8±1.8% at pH 4, compared with a mortality ratio of 2.2±1.1 for control media (without phloxine B, pH 6.7).

3.5. Effect of phloxine B and menadione on the Activity of peroxidase:

The activity of peroxidase in homogenate of *B. zonata* adults fed on media containing concentration equal to the calculated LC_{25} from phloxine B, menadione and their combination together after 24 hours in the dark, and after 24, 48, and 96 hours of exposure to sunlight for 2 hours is shown in table 4. Results indicated that after 24 hours in the dark menadione either alone or in combination with phloxine B cause significant increase in peroxidase activity while phloxine B did not affect peroxidase activity relative to control. After 24, 48 and 96 hrs of exposure to sunlight, all treatments resulted in a significant reduction in peroxidase activity compared with control. However, phloxine B cause significantly higher reduction in peroxidase activity than either menadione alone or menadione-phloxine B mixture.

DISCUSSION

Despite the similar photo-activity of the two compounds evaluated in this study through reactive oxygen species ROS production, there was a significant difference in their lethal effect against *B. zonata* adults. Phloxine B was significantly much more toxic to *B. zonata* than menadione following identical conditions of exposure. The apparent difference in toxicity of these two compounds is probably related to differences in their reactivity following photoactivation. Phloxine B is much more efficient in ROS production than menadione as reflected by its relatively high quantum yield of singlet oxygen species generation. The quantum yield of a singlet oxygen species generation determined in water for phloxine B was ten-fold higher than that for menadione (0.6 VS 0.06, respectively) (McCormick *et al*, 2000 and Khalifa, 2012). In this study, phloxine B was almost 1000 times more toxic to *B. zonata* adults than menadione (based on their LC_{25s}). Moreover, LC_{50} value for menadione could not be calculated because 50% mortality was not reached in any test concentration. Further increase in menadione concentration could not achieve in this study as the test concentrations above 1.5% exceeded the solubility for menadione nicotinamide bisulphate in the water (EFSA, 2014).

Adding menadione to phloxine B resulted in a significantly antagonistic effect on acute and delayed toxicity of phloxine B against *B. zonata* adults. Results of pH measurements provide an insight into the potential mechanisms of this antagonistic effect. As we observed a remarkable instant decrease in the red color intensity of phloxine B containing feeding media after addition of menadione, we suspected that menadione changed the pH of the feeding media and that effect could result in the observed de-coloration of phloxine B solution and the obtained antagonistic effect. Results of pH measurements confirmed this suspicion, while phloxine B did not affect

the pH, menadione change the pH of the feeding media containing phloxine B from weekly acidic to extremely acidic. On the other hand, our results show that toxicity of phloxine B against *B. zonata* reduced dramatically at pH of 5 and it almost loosed its toxicity at pH 4. It has been reported that some xanthene dyes, such as phloxine B, reduced from quinoid (chromophore) colored-form under week acidic conditions to leuco colorless-form under strong acidic conditions thereby drastically altering their physicochemical properties and quenching their ability to generate ROS (Fakkaew and Sopajaree 2015). The singlet oxygen formation and ROS production are positively correlated with the concentrations of phloxine B quinoid form (Keum and Li, 2003). Thus it could be concluded that phloxine B reduced very rapidly in the media containing menadione as the pH of the media changed to strong acidic pH, thereby, phloxine B loosed its ability to produce ROS and consequently its toxicity against *B. zonata* fly reduced. The mortality ratio induced by the mixture could be attributed to both dark and light toxicity of menadione alone.

A second possible mechanism would occur if the relatively small amount of hydrogen peroxide produced in dark by menadione increased ROS detoxifying enzyme activities in *B. zonata* adults, resulting in less biological effect with higher concentrations of ROS produced by the light activated phloxine B. Insect cells possess a range of enzymes, which are capable of detoxifying ROS, for example, catalase, superoxide dismutase, and particularly peroxidase for detoxify hydrogen peroxide (Jamieson,1992). Increased ROS detoxifying enzymes activity following treatment with menadione has been reported in fruit flies *Drosophila melanogaster* (Lushchak, *et al*., 2014). This explains the significant increase in peroxidase activity among *B. zonata* adults fed with menadione in dark in this study. However, with light activated phloxine B, the photoreaction is extremely rapid, resulting in the formation of thousands of reactive, singlet oxygen and hydrogen peroxide molecules per minute which overwhelms the antioxidant defensive enzymes and causes amino acid damage of the enzymes (Hirakawa *et al*. 2013). This explain the dramatic reduction in peroxidase activity among *B. zonata* flies fed on phloxine B in this study. As menadione has the ability to generate much less amount of ROS and hydrogen peroxide in dark through its cellular reduction, it is possible that the generated hydrogen peroxide caused induction of peroxidase which could result in cross-protection against subsequent lethal concentration of ROS and H_2O_2 generating by phloxine B after light exposure. Thus, we hypothesize that adding menadione to phloxine B could result in induction of the detoxifying enzymes, thereby increase their ability to break down the subsequent ROS generated by phloxine B. Menadione itself produces its light dependent lethal effect through other mechanisms such as alteration of intracellular Ca^{2+} homeostasis of insect cells (Arriaga *et al*., 1994). This

suggestion could be supported by the finding of Jamieson, (1992) that pre-treatment with menadione was protective against *Saccharomyces cerevisiae* cell killing by subsequent treatment with a lethal concentration of hydrogen peroxide. Also, Vattanaviboon *et al*., (2003) reported that pre-exposure of the luminous bacterium *Vibrio harveyi* to menadione induced elevated levels of both peroxide and superoxide detoxification enzymes and resulted in cross-protection against subsequent treatment with a lethal concentration of hydrogen peroxide. Finally, it is possible that both of the two above discussed mechanisms could involve in the antagonistic effect of menadione on phloxine B toxicity against *B. zonata*.

CONCLUSION

The obtained results suggest that while *B. zonata* adults were significantly susceptible to exposure to phloxine B, the concentration of menadione necessary to produce 25% mortality among flies was quite high and that necessary to produce a higher acceptable mortality level exceeded the water solubility limit of menadione. Also, the results shown in this study demonstrate that phloxine B and menadione are incompatible and their mixture is not likely to pose any significant addition to their control potential against B. zonata adults.

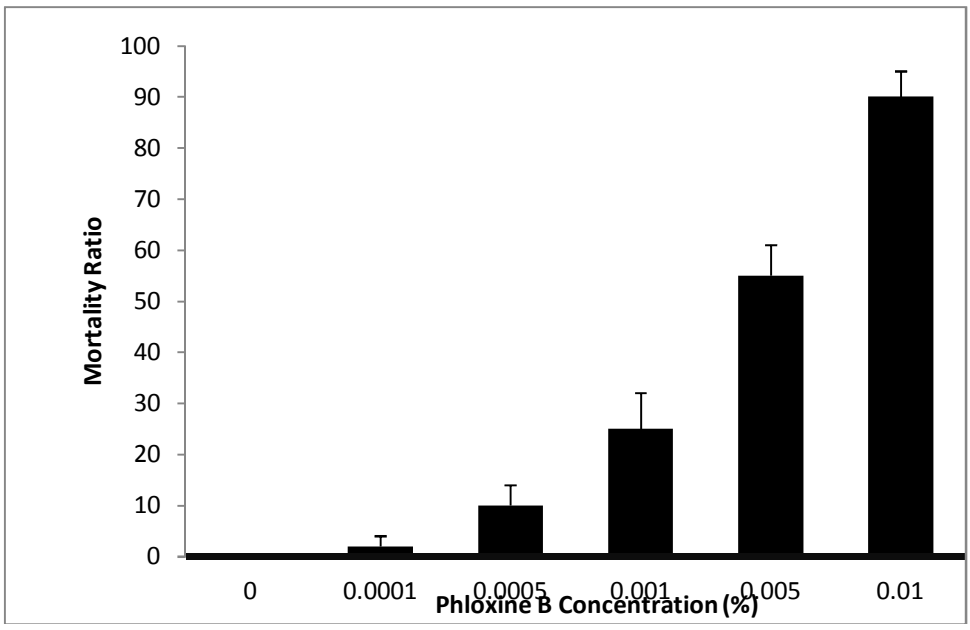

Fig. 1. Mortality ratios of *B. zonata* adults fed on media contains increasing concentrations of phloxine B after 24 hrs of exposure to sun light for 2 hrs (bars represent standard deviation).

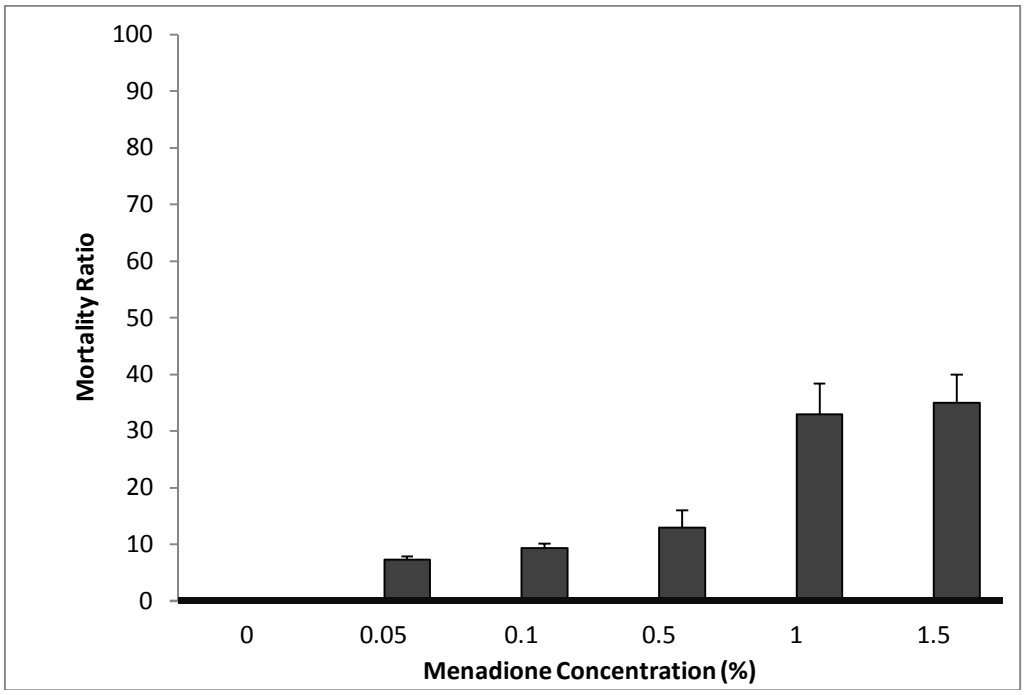

Fig. 2. Mortality ratios of *B. zonata* adults fed on media contains increasing concentrations of menadione after 24 hrs of exposure to sun light for 2 hrs (bars represent standard deviation).

Table 1. Acute toxicity of phloxine B and menadione against *B. zonata*.

Treatment	Slop	LC_{25}* (95% FL)	LC_{50}* (95% FL)
Phloxine B	1.047 ± 0.054	0.0007 (0.0002- 0.0013)	0.0031 (0.001-0.0077)
Menadione	0.666 ± 0.119	0.854 (0.485-1.153)	----------**

* Gram per 100 ml feeding media, FL = Fiducial limit

**Dashes indicate 50% mortality was not reached during test period;

Table 2. Toxicity of phloxine B and menadione and their combination against *B. zonata* when the calculated LC_{25} incorporated into the feeding media.

	Expected Mortality (%)	Observed Mortality (%)	Effect interpretation
Phloxine B	25	28.6±3.5[a]	
Menadione	25	21.8±3.5a[b]	
Phloxine B+ Menadione	43.29	18.0± 3.0[b]	Antagonism

Means within a column followed by different letters are significantly different ($P < 0.05$) by LSD test

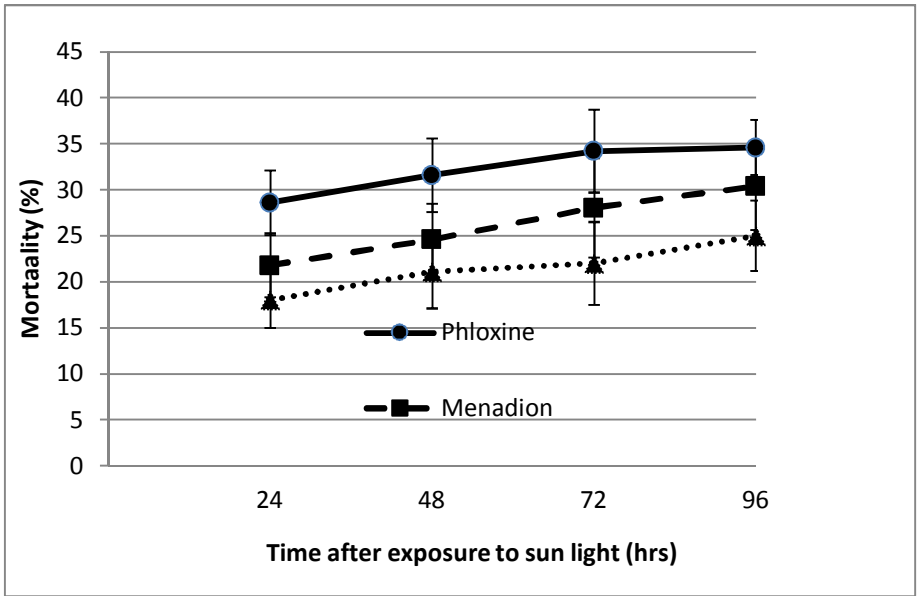

Figure 3. Influence of time after exposure to sunlight on mortality ratio among *B. zonata* adults fed on media contains LC_{25} of phloxine B, menadione and their combination (bars represent standard deviation).

Table 3. The measured pH of various feeding media preparations.

Media composition	Measured pH
Base feeding Media	6.7
Base feeding media + LC_{25} of Phloxine B	6.6
Base feeding media + LC_{25} of Menadione	4.2
Base feeding media + LC_{25} of Phloxine B and Menadione	4.1

Base feeding media (agarose + sucrose + dist. Water)

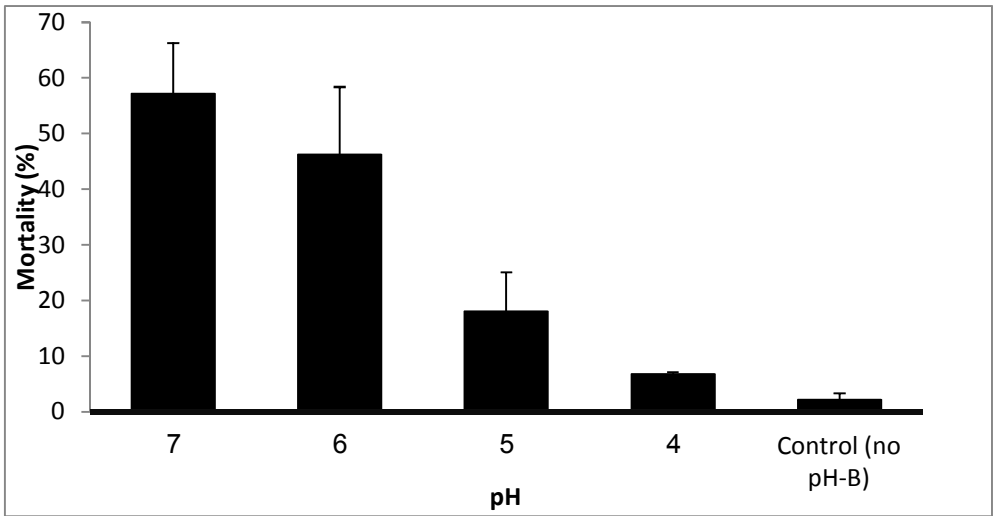

Figure 4. Influence of pH of feeding media on mortality ratio among *B. zonata* adults fed on media containing LC_{50} of phloxine B (bars represent standard deviation).

Table 4. The effect of LC_{25} from phloxine B, menadione and their combination on peroxidase activity in *B. zonata* adults

Treatments	Peroxidase activity (Unit/min/mg Protein)			
	Dark	24 hrs	48 hrs	96 hrs
Control	7.58± 0.55[a]	7.14± 0.25[a]	7.45± 0.82[a]	7.76± 0.59[a]
Menadione	9.23± 1.03[b]	5.08± 1.40[b]	5.71±0.60[b]	5.61± 0.48[b]
Phloxine B	7.28± 0.16[a]	3.65± 0.48[c]	3.80± 0.75[c]	3.69± 0.25[c]
Menadione + Phloxine B	8.31±0.96[b]	4.03± 0.93b[c]	4.25± 0.34b[c]	4.56± 0.27b[c]

Means within a column followed by different letters are significantly different ($P < 0.05$) by LSD test.

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سمية المركبين النشطين ضوئيا –الفولاكسين بى والمناديون على ذبابة فاكهة الخوخ *Bactrocera zonata* (Diptera: Tephritidae)

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يعتبر المركبان النشطان ضوئيا: الفولاكسين بى والمناديون من الإضافات الغذائية الآمنة نسبيا وفى نفس الوقت لهما خصائص المبيدات الحشرية الفعالة ضد عدد من أنواع الحشرات. وقد تم اختبار سمية هذين المركبين على ذبابة فاكهة الخوخ تحت كل من ظرفي الظلام وضوء الشمس . كلا المركبين لم يكن لهما أى تأثير سام معنوي في الظلام، وظهرت لهما سمية معتمدة على التركيز عند تعريض الحشرات لضوء الشمس لمدة ساعتين. وقد كان الفولاكسين بى أكثر سمية بألف ضعف تقريبا عن المناديون حيث كان التركيز ربع المميت ٠,٠٠٠٧ جرام من الفولاكسين بى لكل ١٠٠ مللى ماء مقارنة ب٠,٨٥٤ جرام من المناديون لكل ١٠٠ مللى ماء. وقد تسببت إضافة المناديون إلى الفولاكسين بى إلى تأثير تثبيطى معنوي على سمية الفولاكسن بى والذي قد يكون سببه التغير الذي يسببه المناديون في درجة حمضية الوسط الذي تتغذى عليه الحشرات بالإضافة إلى احتمال تسبب المناديون في زيادة فاعلية ونشاط الإنزيمات التي تزيل سمية نواتج الأكسجين المتفاعلة وخاصة إنزيم البيروكسيديز. وقد خلص البحث إلى أن خلط الفلوكسين بى على المناديون لا يتوافق، وان خلطهما سويا قد لا يكون له اى إضافة محسوسة على فاعليتهما في مكافحة ذبابة فاكهة الخوخ.