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Influence of mixing ammonium acetate and di-ammonium phosphate on their attraction to the peach fruit fly *Bactrocera zonata* (Diptera: Tephritidae) under field conditions

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Abstract:

Peach fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is one of the most destructive key pests infesting fruit orchards. Ammonium acetate and di-ammonium phosphate were evaluated as attractants for the peach fruit fly *B. zonata* in a mandarin orchard (Using concentrations of 1, 2 and 3% of each ammonium compound lonely or mixed between them interchangeably). The highest attractive treatments to *B. zonata* were ammonium acetate 3% mixed with di-ammonium phosphate 3 or 1%, while; the lowest attractive treatments to this pest were ammonium acetate 3% and di-ammonium phosphate 1% (With no mixing). On the other hand, all of the tested treatments attracted females of *B. zonata* obviously more than males. The concentration of ammonium acetate showed a positive effect on the attraction of *B. zonata*. Females and males of *B. zonata* exhibited positive responses to the increase of the pH-level of the tested treatments. As conclusion, the present results may be useful in applying integrated pest management control programs by using mixtures of ammonium acetate and di-ammonium phosphate (Ammonium acetate 3% mixed with di- ammonium phosphate 3 or 1%) because of its good attraction for *B. zonata* adults.

Introduction

Peach fruit fly *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is one of the most dominant and destructive key pests in of fruit orchards in different agro-ecosystem in Egypt (Ghanim, 2009 and Dras *et al*., 2016). It is a polyphagous species attacking a wide range of fruits that differ in their ripening time stage all over the year (White and Elson-Harris, 1992 and Ghanim, 2013). According to Kaspi *et al*. (2000), Yuval *et al*. (2007), Hemeida *et al*. (2017), El-Metwally (2017) and Ghanim and El-Metwally (2019) a strong influence on the physiology and behavior of tephritid flies are correlated with food sources which are rich in nitrogen. Epsky *et al*. (2014) and Pinero *et al*. (2015) mentioned that the effectiveness of protein bait is behaviorally relying on the fact that immature females need a protein meal to reach sexual maturity and for the development of eggs to maturity. So, food sources which are rich in nitrogen act as food attractants. Ghanim *et al*. (2014), Bayoumy and El-Metwally (2017), Hemeida *et al*. (2017) and Ghanim and El-Metwally (2019) added that ammonia is associated with protein-rich foods and has long between known to attract fruit flies.

Ammonium compounds are considered to be from the most important fruit fly attractants. However, Moore (1969), M oustafa and Ghanim (2008), Abd El-Kareim *et al*. (2008), Ghanim *et al*. (2014) and El-Abbassi *et al*. (2017) used various formulations of synthetic ammonium compounds as baits for fruit flies. These compounds, including ammonium acetate di-ammonium phosphate, tri-ammonium phosphate, ammonium carbonate and ammonium chloride. Mazor *et al.* (1987) used dilutions of a pure ammonia solution to obtain a direct correlation between the capture of *Ceratitis capitata* (Wiedemann) females and concentration of ammonia. Also, the relationship between ammonia release rate and captured *C. capitata* and *Anastrepha suspensa* (Loew) females were reported by Heath *et al.* (1994) and Epsky *et al.* (1993).

According to Abd El-Kareim *et al*. (2008), Ghanim *et al*. (2014), Ghanim (2018) and El-Metwally (2017); attractants of fruit flies are used for two objectives: the first one to detect and monitor populations of fruit flies; while, the second one is for control of fruit flies. So, the present study aimed to mix the solutions of ammonium acetate and di-ammonium phosphate for detecting a new treatment which may be more attractive to *B. zonata* under field conditions.

Materials and methods

The commercial products of ammonium acetate (Aa) and di-ammonium phosphate (Da) were obtained from El-Naser for Drugs and Chemicals Company. These two ammonium compounds were evaluated as olfactory attractants for the peach fruit fly, *B. zonata* under field conditions. The concentrations of 1, 2 and 3% (As w: v of ammonium compound: water) of each ammonium compound were investigated lonely and mixed with the concentrations of the other ammonium compound (As interchangeably mixing the concentrations of ammonium acetate and di- ammonium phosphate).

An experiment was carried out in a mandarin orchard on the Experimental Farm of the Faculty of Agriculture, Mansoura University at Mansoura district, Dakahlia Governorate. The total area of the selected orchard was about six feddans (One feddan equal 4200 m^2). The experiment was carried out during the period from the 9^{th} of till the 25^{th} of September 2020.

By using the modified Nadel traps (Described by Hanafy *et al.*, 2001), 300 ml of each treatment was put in a trap. Each treatment was replicated four times. All prepared traps were distributed in a completely randomized design in the orchard. The distance between every two successive hanged traps was not less than 20 meters to avoid interference among traps loaded with different treatments. The traps were hanged at about 1.5 meters in a shady site of the trees.

Along the period of study, the traps were inspected every 4 days (as interval period) with no renewal of solutions. Captured females and males of *B. zonata* were counted and recorded as FTD (Number of flies per trap per day). Also, relative attraction (RA) was calculated as a contribution percentage of each treatment from the total attracted flies.

Data were analyzed by using one-way ANOVA followed by least significant difference (LSD) at probability level of 0.05. Regression analysis was also performed. All analyses were performed using CoHort Software (2004).

Results and discussion

Data in Table (1) showed that peach fruit fly, *B. zonata* adults exhibited variance preferences to the tested ammonium compounds.

However, the highest attractive treatment to *B. zonata* after 4 days was that of ammonium acetate 3% mixed with di- ammonium phosphate 2% (FTD= 0.81). After 8 days, ammonium acetate 3% mixed with di-ammonium phosphate 3% was the most effective treatment in attracting *B. zonata*. The mixture of ammonium acetate and di-ammonium phosphate as 3% of each exhibited the highest attraction to this pest after 12 days. While, ammonium acetate 3% mixed with di-ammonium phosphate 2% was the highest attractant to *B. zonata* after 16 days. On the other hand, all of the tested treatments attracted females of *B. zonata* obviously more than males.

Table (1): Attracted *Bactrocera zonata* adults to different preparations of ammonium acetate (Aa) and di-ammonium phosphate (Da) under field conditions.

Treatment	FTD after											
	4 days			8 days			12 days			16 days		
	♀	♂	♀+♂	♀	♂	♀+♂	♀	♂	♀+♂	♀	♂	♀+♂
Aa1	0.19	0.00	0.19	0.69	0.00	0.69	0.44	0.00	0.44	0.44	0.00	0.44
Aa2	**0.25**	**0.00**	**0.25**	**0.44**	**0.00**	**0.44**	**0.25**	**0.06**	**0.31**	**0.88**	**0.06**	**0.94**
Aa3	**0.19**	**0.00**	**0.19**	**0.19**	**0.00**	**0.19**	**0.06**	**0.00**	**0.06**	**0.44**	**0.00**	**0.44**
Da1	**0.125**	**0.00**	**0.125**	**0.44**	**0.00**	**0.44**	**0.19**	**0.00**	**0.19**	**0.31**	**0.00**	**0.31**
Da2	**0.31**	**0.00**	**0.31**	**0.50**	**0.00**	**0.50**	**0.56**	**0.00**	**0.56**	**0.44**	**0.125**	**0.56**
Da3	**0.25**	**0.00**	**0.25**	**0.31**	**0.00**	**0.31**	**0.38**	**0.00**	**0.38**	**0.44**	**0.06**	**0.50**
Aa1+Da1	**0.25**	**0.00**	**0.25**	**0.44**	**0.00**	**0.44**	**0.69**	**0.00**	**0.69**	**0.69**	**0.06**	**0.75**
Aa1+Da2	**0.25**	**0.00**	**0.25**	**0.31**	**0.00**	**0.31**	**0.44**	**0.19**	**0.63**	**0.56**	**0.00**	**0.56**
Aa3+Da3	**0.19**	**0.00**	**0.19**	**0.125**	**0.00**	**0.125**	**0.56**	**0.00**	**0.56**	**0.56**	**0.19**	**0.75**
Aa2+Da1	**0.25**	**0.00**	**0.25**	**0.50**	**0.00**	**0.50**	**0.44**	**0.00**	**0.44**	**1.25**	**0.25**	**1.50**
Aa2+Da2	**0.56**	**0.00**	**0.56**	**0.56**	**0.00**	**0.56**	**0.88**	**0.00**	**0.88**	**0.69**	**0.31**	**1.00**
Aa2+Da3	**0.125**	**0.00**	**0.125**	**0.44**	**0.06**	**0.50**	**0.44**	**0.00**	**0.44**	**0.50**	**0.00**	**0.44**
Aa3+Da1	**0.75**	**0.00**	**0.75**	**0.50**	**0.00**	**0.50**	**0.50**	**0.00**	**0.50**	**1.31**	**0.38**	**1.69**
Aa3+Da2	**0.81**	**0.00**	**0.81**	**1.00**	**0.00**	**1.00**	**0.44**	**0.06**	**0.50**	**0.50**	**0.06**	**0.56**
Aa3+Da3	**0.63**	**0.00**	**0.63**	**0.94**	**0.00**	**0.94**	**1.38**	**0.00**	**1.38**	**1.00**	**0.19**	**1.19**
LSD	**0.333**	**0.00**	**0.333**	**0.351**	**0.045**	**0.348**	**0.355**	**0.005**	**0.356**	**0.328**	**0.207**	**0.304**

Note: 1,2,3 in all treatments mean concentrations of 1%, 2%, 3% respectively

Data illustrated in Figure (1) showed that the concentration of 2% was the most attractive treatment of ammonium acetate alone to *B. zonata* (Mean FTD was 0.61). While, the concentration of 2% was the highest of di-ammonium phosphate alone (Mean FDT was 0.44). When the concentrations of ammonium acetate and di-ammonium phosphate were mixed interchangeably, ammonium acetate 3% mixed with di-ammonium phosphate 3% exhibited the highest attractive treatment to *B. zonata* (Mean FTD was 1.03) followed by the mixture of ammonium acetate 3% and di-ammonium phosphate 1% (Mean FTD was 0.86).

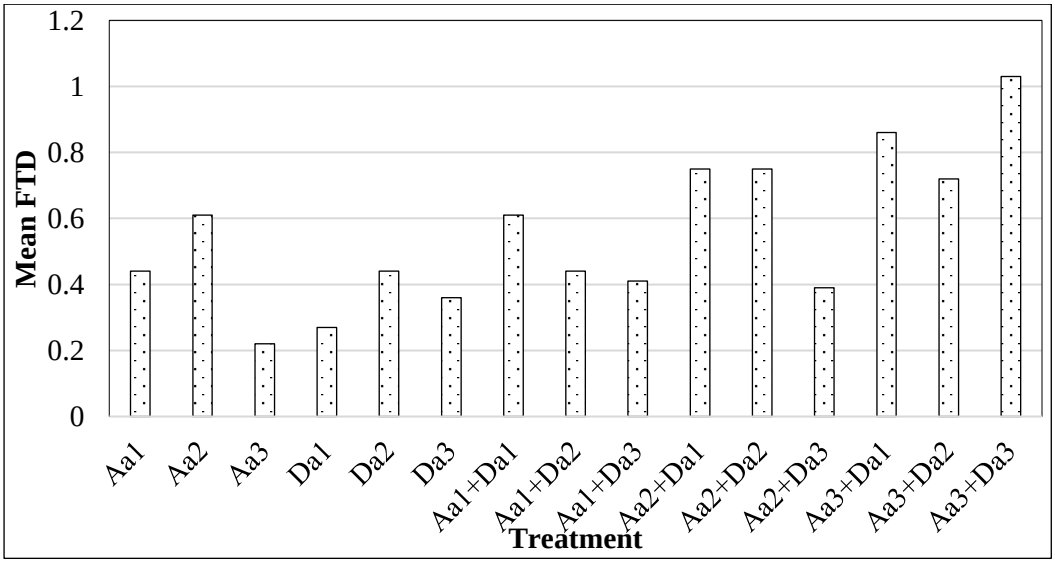

Figure (1): Mean numbers of attracted *Bactrocera zonata* (As females + males) to different concentrations of ammonium acetate (Aa) and di-ammonium phosphate (Da) allover 16 days under field conditions (Note: 1,2,3 in all treatments mean concentrations of 1%, 2%, 3% respectively).

To evaluate the effect of concentration of ammonium compounds on the attractiveness of the tested compounds (As lures for *B. zonata* adults), regression analysis has been done between the relative attraction and concentrations of each ammonium compound (Figures 2 and 3).

As shown as in Figure (2), the concentrations of ammonium acetate showed positive effects on attracting *B. zonata* when it was alone or mixed with di-ammonium phosphate 1, 2 or 3%. However, each increase of ammonium acetate concentration by 1%; the attracted *B. zonata* (As relative attraction) increased by 0.003, 0.015, 0.017 and 0.038% all over 16 days when ammonium acetate was alone or mixed with di-ammonium phosphate 1, 2 and 3% respectively. On another hand, the highest effect of ammonium acetate concentration was recorded when it was mixed with di-ammonium phosphate 1%; where, the calculated R^2-value was 0.922. On the contrary, the lowest effect of ammonium acetate concentration was recorded when it was not mixed; where calculated R^2-value was 0.030 (Figure 2).

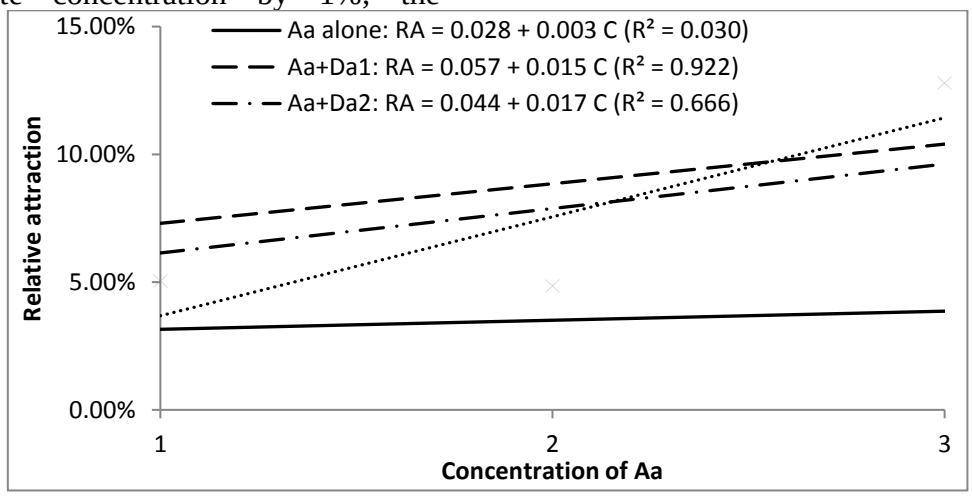

Figure (2): The relationship between concentration (C) of ammonium acetate (Alone or mixed with di-ammonium phosphate) and its relative attraction (RA) to *Bactrocera zonata* under field conditions (Note: 1,2,3 in all treatments mean concentrations of 1%, 2%, 3% respectively).

Data illustrated in Figure (3) showed that the concentration of di-ammonium phosphate showed positive effects on attracting *B. zonata* when it was alone or mixed with ammonium acetate 3%. However, each increased of di-ammonium phosphate concentration by 1%; the relative attraction increased by 0.005 and 0.010%, respectively all over the 16 days. In contrary, di-ammonium phosphate showed a negative effect when it mixed with ammonium acetate 1 and 2% (b-regression = -0.012 and -0.017). As it was previously recorded in the case of ammonium acetate, the highest effect of di-ammonium phosphate concentration was recorded when it was mixed with ammonium acetate 1% (R^2 = 0.862); while, the lowest was recorded when it was not mixed (R^2 = 0.298) (Figure 3).

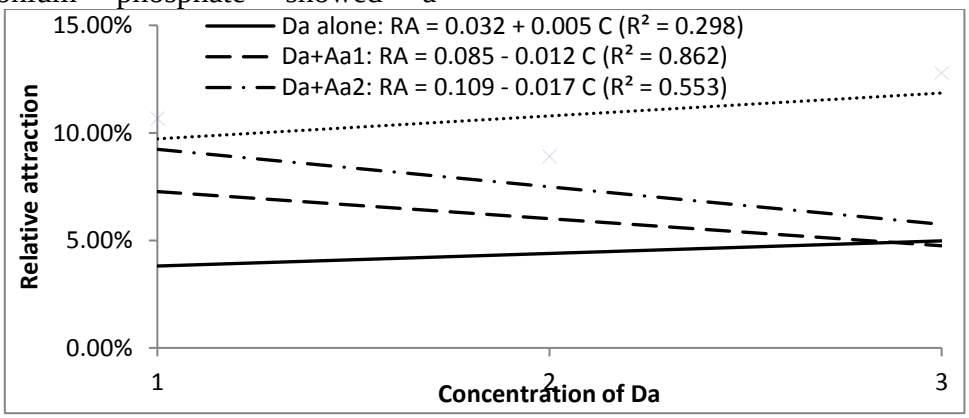

Figure (3): The relationship between concentration (C) of di-ammonium phosphate (Aone or mixed with ammonium acetate) and its relative attraction (RA) to *Bactrocera zonata* under field conditions (Note: 1,2,3 in all treatments mean concentrations of 1%, 2%, 3% respectively).

As shown in Table (2), the elapsed time (T) showed its highest effect on the mixture of ammonium acetate 3% + di-ammonium phosphate 2%; where, the calculated R^2-value was 0.952 (RA = 0.199 - 0.010 T) followed by the mixture of ammonium acetate 3% + di-ammonium phosphate 1% (R^2 = 0.773 and RA = 0.157 - 0.008 T), di-ammonium phosphate 2% alone (R^2 = 0.680 and RA = 0.069 - 0.001 T) and the mixture of ammonium acetate 2% + di-ammonium phosphate 1% (R^2 = 0.667 and RA = 0.017 + 0.006 T). In contrast, the elapsed time exhibited its lowest effect on the mixture of ammonium acetate 3% + di-ammonium phosphate 3%, ammonium acetate alone 3 and 1%; where, the calculated R^2- values were 0.002, 0.015,0.024 , respectively (Table 2).

Table (2): Effect of passed time (T) on the relative attraction (RA) of ammonium acetate, di-ammonium phosphate and the mixtures between them towards *Bactrocera zonata* under field conditions.

Treatment	Relationship	R^2
Aa1%	RA = 0.062 + 0.000 T	0.024
Aa2%	RA = 0.039 + 0.001 T	0.299
Aa3%	RA = 0.030 - 0.0003 T	0.015
Da1%	RA = 0.039 + 0.000 T	0.041
Da2%	RA = 0.069 - 0.001 T	0.680
Da3%	RA = 0.048 - 0.0002 T	0.083
Aa1% + Da1%	RA = 0.065 + 0.001 T	0.052
Aa2% + Da1%	RA = 0.017 + 0.006 T	0.667
Aa3% + Da1%	RA = 0.157 - 0.008 T	0.773
Aa1% + Da2%	RA = 0.045 + 0.001 T	0.080
Aa2% + Da2%	RA = 0.103 - 0.0008 T	0.043
Aa3% + Da2%	RA = 0.199 - 0.010 T	0.952
Aa1% + Da3%	RA = 0.012 + 0.004 T	0.486
Aa2% + Da3%	RA = 0.035 + 0.001 T	0.124
Aa3% + Da3%	RA = 0.135 - 0.0003 T	0.002

Note: 1,2,3 in all treatments mean concentrations of 1%, 2%, 3% respectively

Data represented in Figure (4) showed that females and males of *B. zonata* exhibited positive responses to the increase of the pH- level of the tested treatments. However, the relative attraction (RA) of females, males and total of them increased by 0.017, 0.030 and 0.030%, respectively by increasing one degree of pH- level.

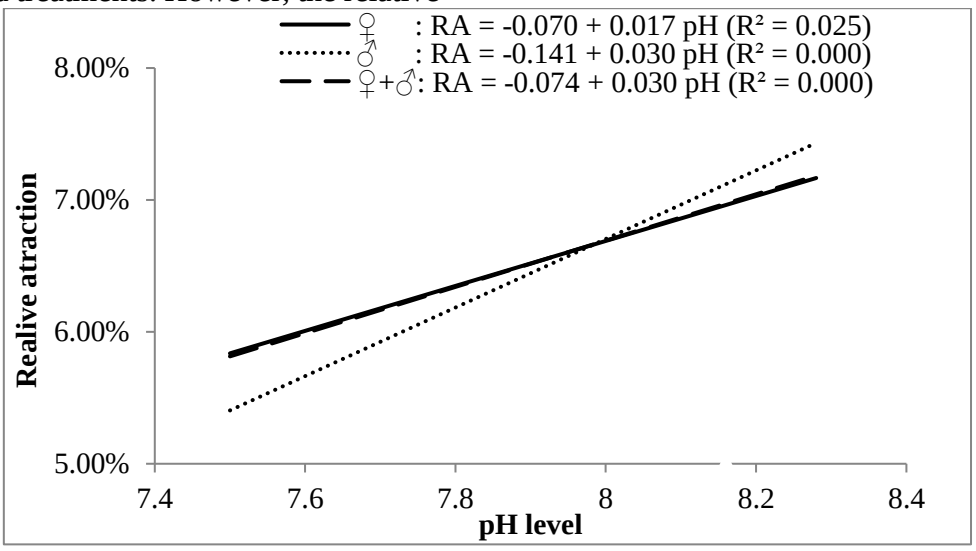

Figure (4): Effect of pH-level of the tested compounds on the attracted *Bactrocera zonata* (Females, males and total adults) under field conditions (Note: 1,2,3 in all treatments mean concentrations of 1%, 2%, 3%, respectively).

Peach fruit fly *B. zonata* is one of the most destructive key pests infesting fruit orchards in different agro-ecosystems. Like other tephritid flies, it is physiologically and behaviorally influenced by food sources which are rich in nitrogen. Ammonia is associated with protein-rich foods and has long between known to attract fruit flies. According to the obtained data, *B. zonata* adults exhibited different preferability to the tested ammonium compounds. Where, the highest attractive treatment to *B. zonata* all over 16 days was that of ammonium acetate 3% mixed with di- ammonium phosphate 3%, followed by ammonium acetate 3% mixed with di- ammonium phosphate 1%. On another hand, the lowest attractive treatments to this pest were ammonium acetate 3% and di-ammonium phosphate 1% (which were not mixed with other treatments). The present results are in agreement with that of El-Abbassi *et al*. (2017); they mentioned that mixing of ammonium acetate and di-ammonium phosphate was more attractive to the tephritid, *C. capitata* adults than the use of each compound lonely. Also, Abd El-Kareim *et al*. (2008), Moustafa and Ghanim (2008), Ghanim *et al*. (2014), Hemeida *et al*. (2017) and Ghanim *et al*. (2018) stated that ammonium compounds could be used in monitoring populations of fruit flies or in mass trapping as a part of integrated control of fruit flies.

The present study explained that the mixture of ammonium acetate and di- ammonium phosphate as 3% of each exhibited the highest attraction to this pest after 12 days; while, most of the rest tested treatments exhibited its highest attraction to *B. zonata* after 16 days. Approximately the same results were obtained by Moustafa and Ghanim (2008), Abd El-Kareim *et al*. (2008) and Ghanim *et al*. (2014) recorded that the highest attraction of ammonium acetate was recorded after 12-15 days; while, the highest attraction of di-ammonium phosphate was recorded after 3–6 days of hanging traps. According to El-Abbassi *et al*. (2017), mixtures of ammonium acetate and di-

ammonium phosphate exhibited its attraction to *C. capitata* until 8 weeks; while, when these compounds were used lonely, the highest attraction was recorded during the first 4 weeks. The differences between the present study and others may be attributed to the variation of the tested form of treatments, fruit fly species, weather factors and/ or host plants.

On the other hand, the present results showed that the efficiency of all treatments containing ammonium acetate 1% increased in attracting *B. zonata* adults by the elapsed time. With respect to the other treatments, it showed differences in their attractiveness toward *B. zonata* with the elapsed time. Also, the results of Abd El-Kareim *et al*. (2008) and Moustafa and Ghanim (2008) came in the line of the present one. However, they reported that the efficiency of ammonium acetate 1% in attracting *B. zonata* and *C. capitata* increased by the elapsed time. In contrary, Ghanim *et al*. (2014) reported that the efficiency of all concentration of ammonium acetate as lures for the tephritid, *Carpomya incompleta* in Saudi Arabia decreased by the elapsed time. The variation between these results and the present may be attributed to the variation of fruit fly species and/or climatic factors.

Concentrations of ammonium acetate and di-ammonium phosphate (lonely or mixed together) significantly attracted females more than males of *B. zonata*. Similar results were obtained by Delrio and Orto (1989), Hanafy *et al.* (2001), Saafan (2005), Abd El-Kareim *et al*. (2008), Moustafa and Ghanim (2008) and Hemeida *et al*. (2017); they mentioned that ammonium compounds attracted females of *B. zonata* and *C. capitata* than males. Also, Ghanim *et al*. (2014) found that ammonium compounds significantly attracted more females of *Carpomya incomplete* (Beeker) than males. Because of the great need for protein by females for egg development; it would be reflected in increased numbers of antennal receptor neurons sensitive to volatile by products of protein degradation, and consequently, in an increased physiological response to ammonia (Arn *et al.*, 1975; Mayer *et al.*, 1987 and Landolt and Davis-Hernandez, 1993).

The concentration of ammonium acetate showed a positive effect on the attraction of *B. zonata* when it was used alone or mixed with di-ammonium phosphate 1, 2 and 3%. These findings are in agreement with the studies of Abd El-Kareim *et al*. (2008), Moustafa and Ghanim (2008), Ghanim *et al*. (2014) and Ghanim and El-Metwally (2019). They reported that attraction of fruit flies showed positive response to the increase of ammonium acetate concentration when it was used lonely or mixed with other attractants. Also, the present results revealed that concentration of di-ammonium phosphate (alone or mixed with ammonium acetate 3%) showed a positive effect on the attraction of *B. zonata*; on contrary, di-ammonium phosphate mixed with ammonium acetate 1 or 2% exhibited adverse effect on the attraction of this pest. These findings are in the same trend of Moustafa and Ghanim (2008) and El-Abbassi *et al*. (2017); they reported that *C. capitata* adults exhibited a negative response to the increase of di-ammonium phosphate concentration.

The obtained data revealed that there was a positive response of attracting *B. zonata* flies (Females and males) to the increase of the pH-level. However, the highest number of attracted flies was recorded pH-level of 8.14. These results came in the same line with those of El-Metwally (2017), Ghanim (2018) and Ghanim and El-Metwally (2019); they reported that attraction of *B. zonata* and *C. capitata* adults is positively correlated with pH-

level of the attraction solutions. The same authors added that the highest attraction of *B. zonata* and *C. capitata* were occurring between 6.32 and 8.29 of pH- level.

From the previously mentioned of view, it could be concluded that the present results may be useful in applying integrated pest management control programs by using mixtures of ammonium acetate and di- ammonium phosphate (Ammonium acetate 3% mixed with di- ammonium phosphate 3 or 1%) because of its good attraction for *B. zonata* adults.

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