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Use of di-ammonium hydrogen phosphate to attract *Bactrocera zonata* (Diptera: Tephritidae) and enhance its protein-based bait under field conditions

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Abstract

The peach fruit fly (PFF), *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is a polyphagous insect native to tropical Asia and became a serious pest in Egypt attacking a wide range of fruits. The present experiments were conducted in a mandarin (*Citrus reticulata* Blanco) orchard located in Mansoura district, Dakahlia Governorate, Egypt. This study aimed to evaluate di-ammonium hydrogen phosphate as an olfactory attractant for PFF adults and evaluate its ability as an enhancer for Buminal (The commercial product of protein-based bait for PFF). Statistically, the efficiency of di-ammonium hydrogen phosphate (As an attractant for PFF adults) increased by its concentration, increased till it reached the range between 2.5 and 3.0%; then, with the increase of di-ammonium hydrogen phosphate concentration its attractiveness decreased. As general, di-ammonium hydrogen phosphate attracted 6.25 PFF females per each male. In another hand, the elapsed time showed an adverse effect on the efficiency of all the tested concentrations of di-ammonium hydrogen phosphate as attractants for PFF except for the concentration of 5%. The obtained data showed that adding di-ammonium hydrogen phosphate to the protein-based bait (Buminal) with a concentration of 2% significantly increased Buminal’s efficiency as an attractant to PFF adults with 6.33 folds of the efficiency of Buminal alone.

Introduction

Fruit flies (Diptera: Tephritidae) are of the most economically important groups of insect pests attacking fruits and vegetables over all the world causing large losses (Allwood, 1997 and Abd El-Kareim *et al*., 2008). As a result of the infestation by these insects, quarantine restrictions are instigated in the infested areas; so that commercial fruits undergo protective and quarantine treatments before export (Vargas *et al*., 2008). The peach fruit fly (PFF), *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is a polyphagous insect native to tropical Asia, but it was spread to many regions of the world (Agarwal *et al*., 1999 and El-Minshawy *et al*., 1999). In Egypt, PFF became a serious pest attacking a wide range of fruits that differ in their ripening times and that persist almost throughout the year (White and Elson-Harris, 1992).

Nitrogen-containing food sources strongly influence physiology and behaviour of tephritid flies (Yuval *et al*., 2007). This influence is a result of the female's need for protein to produce its eggs (Epsky *et al*., 2014 and Piñero *et al*., 2015). Hence, protein baits mixed with pesticides have been used to control fruit flies worldwide (Vargas *et al*., 2001; Barry *et al*., 2003 and Ghanim, 2018). So, protein-based bait formulations must have good levels of attraction and stimulate adult flies (i.e., PFF) to ingest the lethal doses of the pesticides; then obtain effective suppression against fruit fly populations (Mangan, 2009 and 2014).

Releasing ammonia substances significantly affected the attraction of fruit flies to food sources (Epsky and Heath, 1998 and Hull and Cribb, 2001). This information led to the development of effective ammonia-containing lures (Moustafa and Ghanim, 2008 and Ragab and Youssef, 2021). According to Mazor *et al*. (2002), the attraction level of fruit flies to ammonia-based compounds is known as dose-dependent and the range of attractiveness is very narrow, while the range of repellence is much wider. So, some ammonium compounds (with accurate concentrations) were added to protein-based baits to enhance their effectiveness for fruit flies such as PFF (Moreno and Mangan, 2002 and Piñero *et al*., 2015). The addition of ammonium compounds to baits or local products led to an improvement in their effectiveness for monitoring and suppression of the populations of fruit flies (Piñero *et al*., 2015). So, the purposes of this study are:

1) Evaluating di-ammonium hydrogen phosphate as an olfactory attractant for PFF adults at different concentrations.

2) Evaluating its ability as an enhancer for Buminal (The commercial product of protein-based bait) to increase its attractiveness to PFF adults under field conditions.

Materials and methods

The present experiments were done in an area of about 5 feddans (= 21000 m^2) of mandarin (*Citrus reticulata* Blanco) orchard in the experimental farm of Faculty of Agriculture, Mansoura University (located at Mansoura district, Dakahlia governorate) by using di-ammonium hydrogen phosphate ($(NH_4)_2HPO_4$).

1. Chemicals:

Buminal was obtained from Plant Protection Research Institute, Agricultural Research Center. Di-ammonium hydrogen phosphate ($(NH_4)_2HPO_4$) was obtained from Edwic Company, Egypt.

2. Evaluation the efficiency of di-ammonium hydrogen phosphate concentrations as attractants for *Bactrocera zonata*:

Four concentrations (1, 2, 3, and 5% as w: v of di-ammonium hydrogen phosphate: water) were used in the present experiment (The concentration of 3% is the recommended one). The modified Nadel traps (Which was described by Hanafy *et al*. (2001) were used by putting 250 milliliters of each concentration inside one trap. All treatments were replicated four times. Traps were hung in a shaded place at a height of about 1.5 on the trees and distributed in a completely randomized design within the wind direction. As interval distance, 20 meters between every two successive traps were considered to avoid interference among traps.

During the period from the 13^{th} to 28^{th} of October 2022 (15 days), every 3 days traps were inspected by filtering the solutions inside traps from PFF adults and solutions were returned to the traps again. Captured females and males of PFF were counted and recorded as captured flies /trap/day (FTD values).

3. Di-ammonium hydrogen phosphate as enhancer for the efficiency of protein-based baits to attract *Bactrocera zonata*:

The mixture of buminal and $(NH_4)_2HPO_4$ was prepared first by diluting Buminal with distilled water to obtain the concentration of 5% (vol/vol) and then, used to prepare 1 and 2% of di-ammonium hydrogen phosphate. Buminal 5% without mixing with di-ammonium hydrogen was used as a positive control for comparison. Inside each modified Nadel trap, 250 ml of the mixture was put. Each treatment was replicated four times.

During the period from the 24th of November to the 9th of December 2022 (15 days), the prepared traps were distributed in a completely randomized design as previously mentioned. As an interval period, traps were inspected every three days and the captured females and males were counted and recorded as previously mentioned.

4. Statistical analysis:

To analyze the obtained data followed by the least significant difference (LSD, 0.05), One-way ANOVA was used. In addition, regression analysis was performed. The statistical analysis program CoHort Software (2004) was used to analyze all analyses.

Results and discussion

1. Evaluation of efficiency of di-ammonium hydrogen phosphate concentrations as attractants for *Bactrocera zonata*:

Data illustrated in Figure (1) showed that the highest activity of di-ammonium hydrogen phosphate after 3, 6, 9 and 12 days were recorded at the concentration of 3% (FTDs were 3.82±0.33, 1.66±0.27, 3.66±0.90 and 3.80±0.33, respectively); while the highest activity after 15 days was recorded at the concentration of 5% (FTD was 1.66). On another hand, the highest attraction of 0.5 and 3% concentrations to PFF adults was recorded after 3 and 12 days; while the highest attraction of 1, 2 and 5% was recorded after 12, 6 and 15 days, respectively.

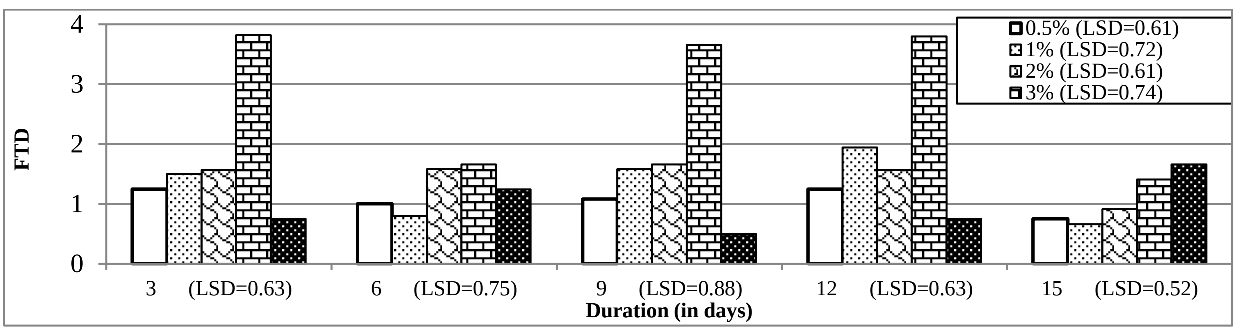

Figure (1): Attractiveness of di-ammonium hydrogen phosphate concentrations (0.5, 1, 2, 3 and 5%) to *Bactrocera zonata* adults all over 15 days under field conditions.

The general means of attracting PFF to di-ammonium hydrogen phosphate concentrations all over 15 days showed that the concentration 3% was the significantly highest effective concentration for PFF adults; while the efficiency of 0.5, 1, 2 and 5% of di-ammonium hydrogen phosphate exhibited the second rank in attracting PFF with no significant differences between them (Figure 2).

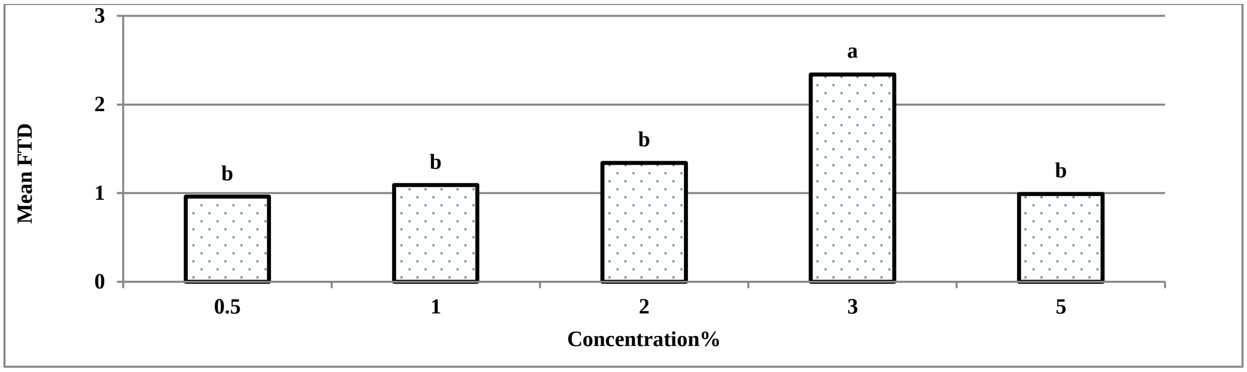

Figure (2): Mean attracted *Bactrocera zonata* adults to 0.5, 1, 2, 3 and 5% di-ammonium hydrogen phosphate all over 15 days under field conditions (Columns had the same letters did not differ at the significantly of 5%).

Figure (3) shows the relationship between the concentrations of di-ammonium hydrogen phosphate and its efficiency as an attractant to females and males PFF as well as the total of them. The efficiency of di-ammonium hydrogen phosphate increased by its concentration increased till it reached the range between 2.5 and 3.0%; then, by the increase of di-ammonium hydrogen phosphate concentration its attractancy to PFF decreased.

The statistical relationship between the concentrations of di-ammonium hydrogen phosphate and its attractancy females and males of PFF was determined as follow:

Females: $FTD = -0.14 C^2 + 0.77 C + 0.37$

Males: $FTD = -0.07 C^2 + 0.43 C – 0.18$

Total: $FTD = -0.20 C^2 + 1.19 C + 0.20$

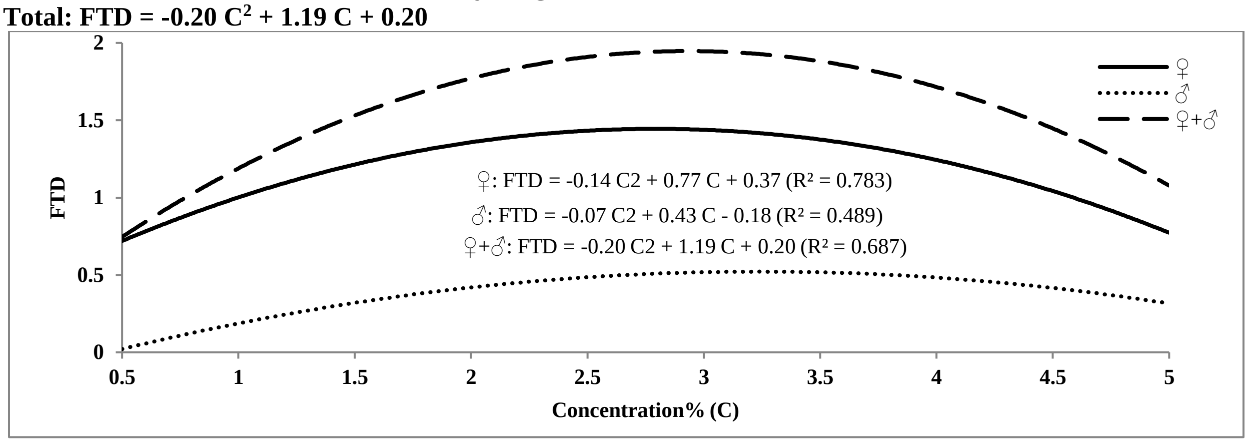

Figure (3): Relationship between the concentrations of di-ammonium hydrogen phosphate and its efficiency as attractant to females and males of *Bactrocera zonata* under field conditions.

The obtained data revealed that the highest sex ratio (As number of females per one male) was recorded when the concentration of 0.05% was used (12.71 females) followed by 2% of di-ammonium hydrogen phosphate (10.25 females/one male). The general mean number of attracted females per one male in all of the tested di-ammonium hydrogen phosphate concentrations all over the tested period was recorded as 6.25 females (Figure 4).

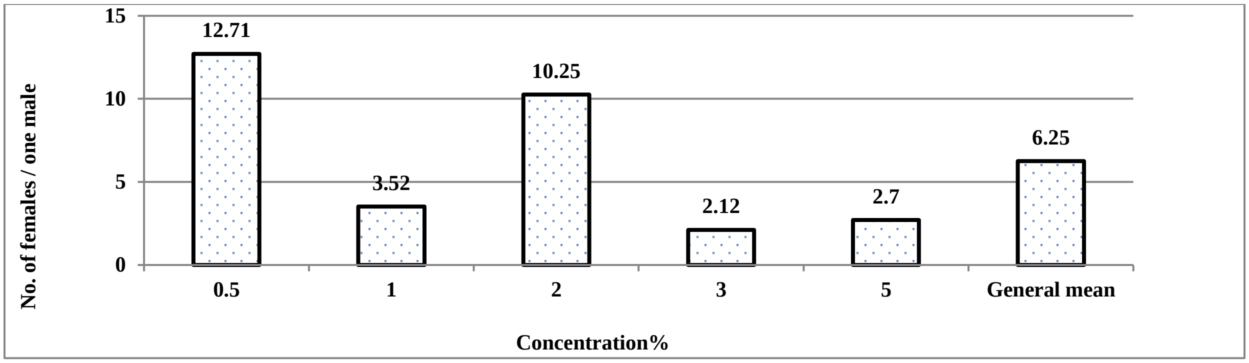

Figure (4): Number of *Bactrocera zonata* adult females per one male attracted to the concentrations of di-ammonium hydrogen phosphate (0.5, 1, 2, 3 and 5%) under field conditions.

Figure (5) shows the effect of elapsed time on the efficiency of the tested di-ammonium hydrogen phosphate concentrations as attractants for PFF adults all over the tested period. The highest concentration affected by the elapsed time was that of 2% (Where R^2 reached 0.464) followed by 0.5, 5, 3 and 1%, respectively (where R^2–values were 0.464, 0.204, 120 and 0.025, respectively). In another hand, the elapsed time showed an adverse effect on the efficiency of all the tested concentrations of di-ammonium hydrogen phosphate as attractants for PFF (Where it decreased by the elapsed time) except on the concentration of 5% (Where its efficiency increased by the elapsed time).

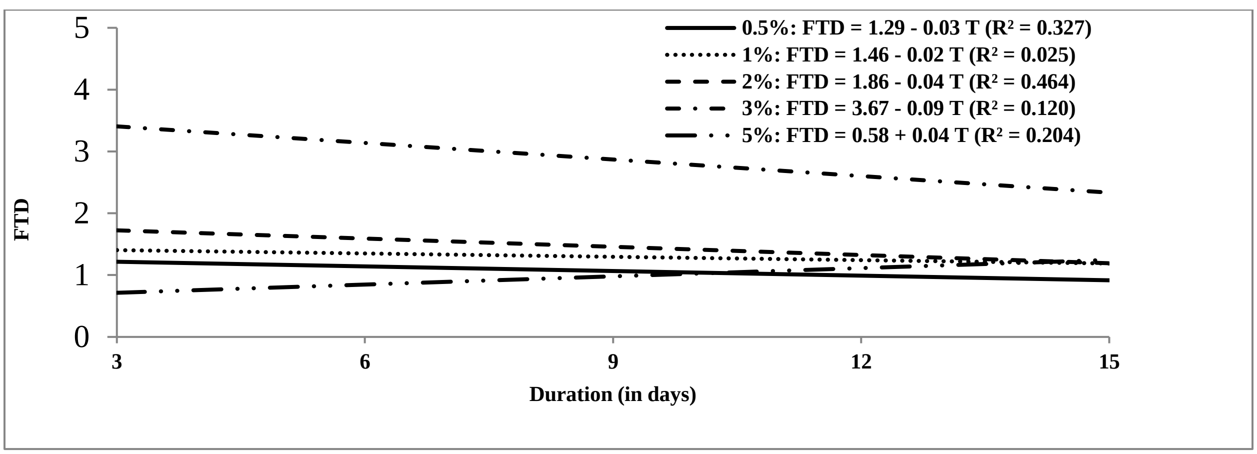

Figure (5): Effect of the elapsed time on the efficiency of di-ammonium hydrogen phosphate concentrations (0.5, 1, 2, 3 and 5%) as attractants for *Bactrocera zonata* adults under field conditions all over 15 days.

2. Di-ammonium hydrogen phosphate as enhancer for the efficiency of protein-based baits to attract *Bactrocera zonata*:

As shown in Table (1), adding di-ammonium hydrogen phosphate with a concentration of 1% to Buminal (The food attractant of PFF) with a concentration of increased its efficiency as an attractant to PFF after 3 and 15 days of the starting experiment (Mean FTDs were 0.08±0.16 and 0.58±0.19) and did not attract in the rest interval periods. While, adding di-ammonium hydrogen phosphate with a concentration of 3% to the same concentration of Buminal increased its efficiency as an attractant to PFF during all the interval periods; however, FTDs were 0.33±0.26, 0.42±0.14, 0.08±0.20, 0.25±0.5 and 0.80±0.50 after 3, 6, 9, 12 and 15 days of staring the experiment.

Table (1): Enhancement of protein-based bait (Buminal) by using 1 and 2% concentrations of di-ammonium hydrogen phosphate to attract *Bactrocera zonata* under field conditions all over 15 days.

Conc.%	FTD after (in days)					$LSD_{(P=0.05)}$
	3	6	9	12	15	
0.0	0.00±0.00	0.08±0.20	0.08±0.20	0.17±0.19	0.00±0.00	0.20
1.0	0.08±0.16	0.00±0.00	0.00±0.00	0.00±0.00	0.58±0.19	0.30
2.0	0.33±0.26	0.42±0.14	0.08±0.20	0.25±0.5	0.80±0.50	0.62
$LSD_{(P=0.05)}$	0.29	0.41	0.22	0.53	0.66	

Adding di-ammonium hydrogen phosphate to the protein-based bait (Buminal) with a concentration of 2% significantly increased Buminal's efficiency as attractant to PFF adults with 6.33 folds of the efficiency of Buminal alone all over the tested period; while, adding di-ammonium hydrogen phosphate to Buminal with a concentration of 1% significantly did not increase Buminal's efficiency as an attractant to PFF adults (Figure 6).

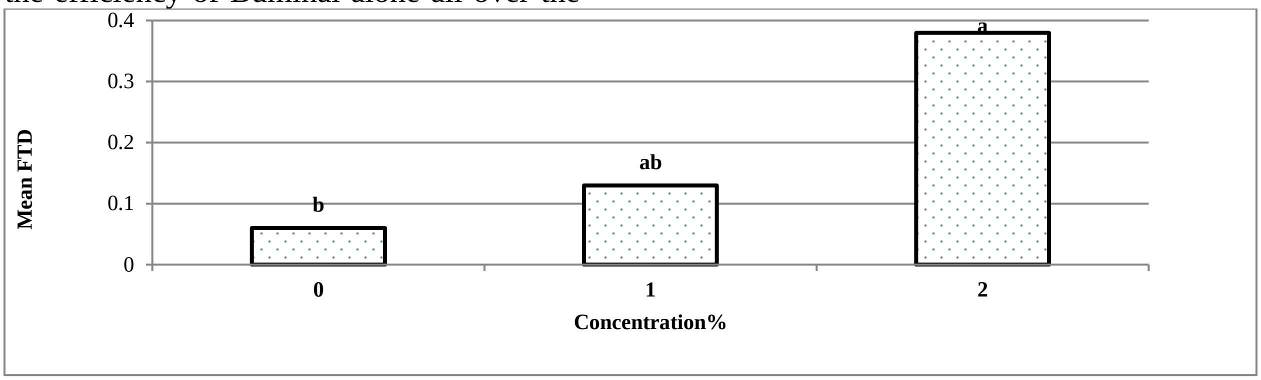

Figure (6): Mean attracted PFF adults (As FTDs) Buminal alone or enhanced with di-ammonium hydrogen phosphate 1 and 2% concentrations under field conditions all over 15 days (Columns had the same letters did not differ at the significantly of 5%).

The effect of elapsed time on the efficiency of Buminal alone (As an attractant for PFF adults) or enhanced with di-ammonium hydrogen phosphate at concentrations of 1 and 2% all over the tested period is illustrated in Figure (7). The highest treatment affected by the elapsed time was that of Buminal enhanced with 1% di-ammonium hydrogen phosphate (R^2 = 0.391) followed by enhanced with 2% di-ammonium hydrogen phosphate (R^2 = 0.206) and Buminal alone (R^2 = 0.041), respectively. In another hand, the elapsed time showed a positive effect on the efficiency of all the tested treatments as attractants for PFF (Where it increased by the elapsed time).

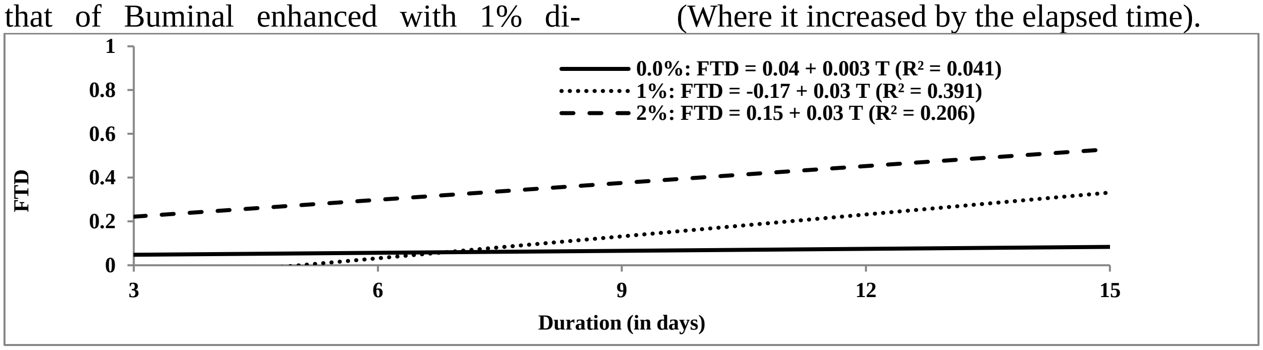

Figure (7): Effect of the elapsed time on the efficiency of the protein-based bait (Buminal) alone or enhanced with di-ammonium hydrogen phosphate concentrations (1 and 2%) as attractants for *Bactrocera zonata* adults under field conditions all over 15 days.

Increases in ammonia produced from protein bacterial degradation were correlated with increases in bait efficiency as an attractant to adults of fruit flies, whereas the high attractiveness of proteinaceous baits to the adults of fruit flies was associated with high release rates of ammonia and vice versa (Bateman and Morton, 1981 and Mazor, 2009). The obtained data showed that di-ammonium hydrogen phosphate at its concentrations ranged between 2.5 and 3.0% was a good attractant for PFF adults under field conditions. Ghanim *et al*. (2021) reported a similar conclusion, where they found that 2 and 3% of di-ammonium hydrogen phosphate are the superior of its concentrations in attracting PFF under field conditions. While Abd El-Kareim *et al*. (2008) found that di-ammonium hydrogen phosphate was superior in its attractiveness to PFF at its concentrations of 1. Also, Moustafa and Ghanim (2008) and Ragab and Youssef (2021) found that di-ammonium hydrogen phosphate at its concentration of 3% was e superior to its concentrations in attracting *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) under the field conditions.

The lower efficiency of the low concentrations (Less than 2.5%) may be attributed to their lower attractiveness to PFF adults, while the lower efficiency of high concentrations (More than 3%) may be attributed to the appearance of the relative repellency to MFF adults. These suggestions may be explained by the studies of Mazor *et al*. (2002); they mentioned that the attraction level of fruit flies to ammonia-based compounds is known as dose-dependent and the range of attractiveness is very narrow, while the range of repellence is much wider.

Dietary sources of nitrogen showed strong effects on the physiology and behavior of fruit flies (Kaspi *et al*., 2000 and Yuval *et al*., 2007). So, baits including ammonia in their formulations have a relatively high efficiency as attractants for fruit flies because of the advantage of the key role that ammonia plays in fruit fly attraction (Heath *et al*., 2004 and Leblanc *et al*., 2010). Therefore, ammonium acetate is added to the food attractant (Biolure) and so it becomes the most attractive component for *C. capitata*. Also, adding ammonium acetate or di-ammonium hydrogen phosphate to the insecticidal bait (GF-120; which considered a protein-based bait) exhibited significant positive effects of its attractiveness to *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), PFF and *C. capitata* (Piñero *et al*., 2011 and Ghanim, 2018). In the present study, the commercially protein-based bait of Buminal was enhanced as an attractant to PFF by adding di-ammonium hydrogen phosphate with a concentration of 2% to it, which was found a significant increase in attracting PFF adults. Similar findings were obtained by Hemeida *et al*. (2017); they reported that Buminal could be enhanced by adding di-ammonium hydrogen phosphate and resulting in significant increases in its efficiency as an attractant to PFF under field conditions. Also, the insecticidal bait of GF-120 can be enhanced by di-ammonium hydrogen phosphate with a concentration of 2% to increase its efficiency as an attractant to PFF and *C. capitata* (El-Metwally, 2017 and Ghanim, 2018). Hemeida *et al*. (2017) added that di-ammonium hydrogen phosphate (3%) can enhance the commercially protein-based baits of Agrinal or Amadene (5%) to increase their efficiency as attractants for PFF adults.

Females of fruit flies require a source of protein for egg maturation; so, this requirement may probably be the main cause for the strong female attraction to the protein-based baits (Epsky *et al*., 2014 and Piñero *et al*., 2015). These findings may support the present results; where, all concentrations of di-ammonium hydrogen phosphate tended to attract PFF females more than males. Similarly, Yee (2007), Moustafa and Ghanim

(2008), El-Abbassi *et al*. (2017) found that ammonium compounds attracted females of fruit flies with higher numbers than its males, whereas, adding ammonium compounds to protein baits enhanced the response of fruit fly females more than its males (Piñero *et al*., 2015).

The present study showed that the efficiencies (As attractant to PFF adults) of di-ammonium hydrogen phosphate (At concentrations 1 and 2%) were the most stable treatment over the past time of 15 days under field conditions (÷f it used lonely or for enhancing the protein-based bait, Buminal). These findings came in the same trends of Moustafa and Ghanim (2008), Ghanim *et al*. (2014) and Ghanim *et al*. (2021); whereas they reported that the efficiencies of ammonium compounds in attracting adult fruit flies were stable or tended to increase by the elapsed time under field conditions.

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