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Di-ammonium hydrogen phosphate as olfactory attractant and enhancer for the efficiency of protein-based baits to attract *Ceratitis capitata* (Diptera: Tephritidae) under field conditions

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Abstract

The Mediterranean fruit fly (MFF) *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is considered the most economically important pest attacking many fruit hosts. The present study was conducted under field conditions of a mandarin (*Citrus reticulata* Blanco) orchard in Dakahlia Governorate, Egypt aiming to evaluate di-ammonium hydrogen phosphate as an olfactory attractant for adults of MFF and evaluate its efficiency as an enhancer for the commercial product of the protein-based bait (Buminal). The obtained results showed that the efficiency of di-ammonium hydrogen phosphate as an attractant to MFF adults increased as its concentration increased till it reached the range between 3.0 and 3.5%, and then its efficiency decreased with the increase in concentration. The statistical relationship between the concentrations of di-ammonium hydrogen phosphate and its efficiency as an attractant to MFF was evaluated. Concentrations of di-ammonium hydrogen phosphate attracted females obviously more than males. On another hand, adding di-ammonium hydrogen phosphate with concentrations of 1 and 2% to Buminal increased its efficiency as an attractant to MFF adults with 1.46 and 2.86 folds of the efficiency of Buminal alone.

Introduction

Citrus fruits host many pests worldwide and fruit flies of the family Tephritidae are of these pests (Onah *et al*., 2015). Tephritid fruit flies are recognized as major insect pests in horticultural and vegetable orchards causing large losses of fruits (Allwood, 1997). The Mediterranean fruit fly (MFF), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is considered one of the most economically important pests attacking many fruit hosts (Liquido *et al*., 1991 and Ghanim and Moustafa, 2009). It is one of the quarantine important pest species because of its extensive damage to a wide range of cultivated and wild fruit and vegetable crops (FAO and IAEA, 2013). The larvae of MFF devour into the fruits' pulp causing great damage and making fruits unfavorable for exportation or marketing (White and Elson-Harris, 1992 and Borge and Basedow, 1997). According to Sayed *et al*. (1970), Pena *et al*. (1998), Ghanim and

Moustafa (2009) and Papadopoulos *et al*. (2015) reported that MFF causes considerable damage in many fruit species.

As a result of the lack of an effective female attractant; several studies had been done to examine some odors as lures for attracting female of fruit flies such as ammonium compounds. According to Moustafa and Ghanim (2008), Ghanim *et al*. (2014), Ghanim (2018), Ghanim and El-Metwally (2019) and Ghanim *et al*. (2021); ammonium compounds play significant roles in attracting tephritid fruit flies.

Where Piñero *et al*. (2011) mentioned that ammonia-releasing substances could be developed as effective synthetic food-based lures for fruit flies; however, females of tephritid flies require protein sources for egg production and ammonia plays a role in locating protein-food sources (Piñero *et al*., 2015 and 2017). Also, ammonium compounds could be used for enhancing some protein-based baits for attracting adults of MFF and *Bactrocera zonata* (Saunders) (Hemeida *et al*., 2017; El-Metwally, 2017 and Ghanim and El-Metwally, 2019). While Ragab and Youssef (2021) and Ghanim *et al*. (2021) used ammonium compounds (*i.e.* Ammonium acetate and di-ammonium hydrogen phosphate) to activate each other (As attractants for MFF and *B. zonata*) by mixing them together at different ratios of each.

The present study is aimed to evaluate di-ammonium hydrogen phosphate as an olfactory attractant for adults of MFF at different concentrations in addition to evaluating it as an enhancer for the commercial product of the protein-based bait (Buminal) to increase its attractiveness to adults of MFF under field conditions.

Materials and methods

1. Chemicals:

Di-ammonium hydrogen phosphate ($(NH_4)_2HPO_4$) was obtained from Edwic Company, Egypt. Buminal (39.78%) was obtained from Horticultural Insect Pests Research Department, Plant Protection Research Institute.

2. Di-ammonium hydrogen phosphate as olfactory attractant for the Mediterranean fruit fly:

Di-ammonium hydrogen phosphate was tested as olfactory attractants for MFF, *C. capitata* under field conditions of mandarin (*Citrus reticulata* Blanco) orchard (about 5 feddans = 21000 m^2) located at Mansoura district, Dakahlia governorate, Egypt (the experimental farm of Faculty of Agriculture, Mansoura University). The four concentrations 1, 2, 3 (which is the recommended concentration) and 5% as w: v of di-ammonium hydrogen phosphate: water was used inside the modified Nadel traps that were described by Hanafy *et al*. (2001) during the period from the 13th till 28th of October 2022.

Each concentration of di-ammonium hydrogen phosphate (250 ml) was put in one trap and repeated four times. Traps were distributed in a completely randomized design in the previously mentioned orchard and hung in a shaded place of the trees at a height of about 1.5 meters from the ground on the trees within the wind direction. To prevent interference among the traps, there had to be at least 20 meters between each pair of hanging traps.

Every 3 days (As an interval period), traps were inspected over a period of 15 days by filtering the solutions from MFF adults and returned to the traps again without renewing the solution. Captured MFF females and males were counted and recorded as calculated FTD values (Captured flies /trap/day).

3. Enhancement of protein-based bait by di-ammonium hydrogen phosphate to attract Mediterranean fruit fly:

Buminal (The commercial product of the protein-based bait as hydrolyzed protein 39.78%) was prepared at the concentration of 5% (v/v) by diluting with distilled water. The mixture of di-ammonium hydrogen phosphate and Buminal 5% was prepared. Each one liter of Buminal 5% was mixed with 10 g of di-ammonium hydrogen phosphate to obtain 1% (w/v) or mixed with 20 g to obtain 2% concentration. The prepared mixtures were compared with Buminal 5% alone as a positive control. Each mixture (250 ml) was put inside the modified Nadel traps and repeated four times.

In the previously mentioned mandarin orchard, experiments were carried out during the period from the 24th of November to 9th of December 2022. The prepared traps were distributed in the orchard as previously mentioned. After hanging the traps, they were checked every three days (as an interval period) for a total of 15 days. As indicated before, both male and female caught files were counted and recovered.

4. Statistical analysis:

One-way ANOVA was used to analyze the obtained data followed by the least significant difference (L.S.D) at a probability level of 5%. In addition, regression analysis was performed. CoHort Software (2004) was used to perform all analyses.

Results and discussion

1. Di-ammonium hydrogen phosphate as olfactory attractant for Mediterranean fruit fly:

Data represented in Table (1) showed that the highest activity of 0.5, 3 and 5% concentrations of di-ammonium hydrogen phosphate was recorded after 12 days (FTDs were 1.08±0.42, 5.99±0.94 and 1.83±0.33, respectively); while the highest activity of 1 and 2% concentrations of di-ammonium hydrogen phosphate was recorded after 9 and 15 days (FTDs were 1.33±0.47 and 0.94±0.19). On another hand, the highest activity of di-ammonium hydrogen phosphate after 9, 12 and 15 days were recorded at the concentration of 3%.

Table (1): Efficiency of di-ammonium hydrogen phosphate concentrations (Conc.) as attractants for Mediterranean fruit fly adults during 15 days in mandarin orchard.

Conc.	FTD after (In days)					$LSD_{(P=5\%)}$
	3	6	9	12	15	
0.5%	0.25±0.16	0.41±0.31	0.90±0.31	1.08±0.42	0.66±0.47	0.53
1%	0.00±0.00	0.00±0.00	1.33±0.47	1.25±0.31	0.25±0.16	0.39
2%	0.17±0.09	0.17±0.09	0.17±0.09	0.33±0.00	0.94±0.19	0.25
3%	0.17±0.09	0.17±0.09	5.66±0.81	5.99±0.94	2.75±0.50	0.92
5%	0.17±0.09	0.25±0.16	0.41±0.20	1.83±0.33	1.41±0.41	0.48
$LSD_{(P=5\%)}$	0.24	0.30	0.73	0.76	0.56	0.80

Figure (1) shows the general means of attracting MFF to di-ammonium hydrogen phosphate concentrations all over 15 days. This figure showed that the concentration of 3% was the significantly highest effective concentration as an attractant for MFF adults, while the significantly lowest concentration was of 2%. The concentrations of 0.5, 1 and 5% showed moderate ranks as attractants for MFF adults.

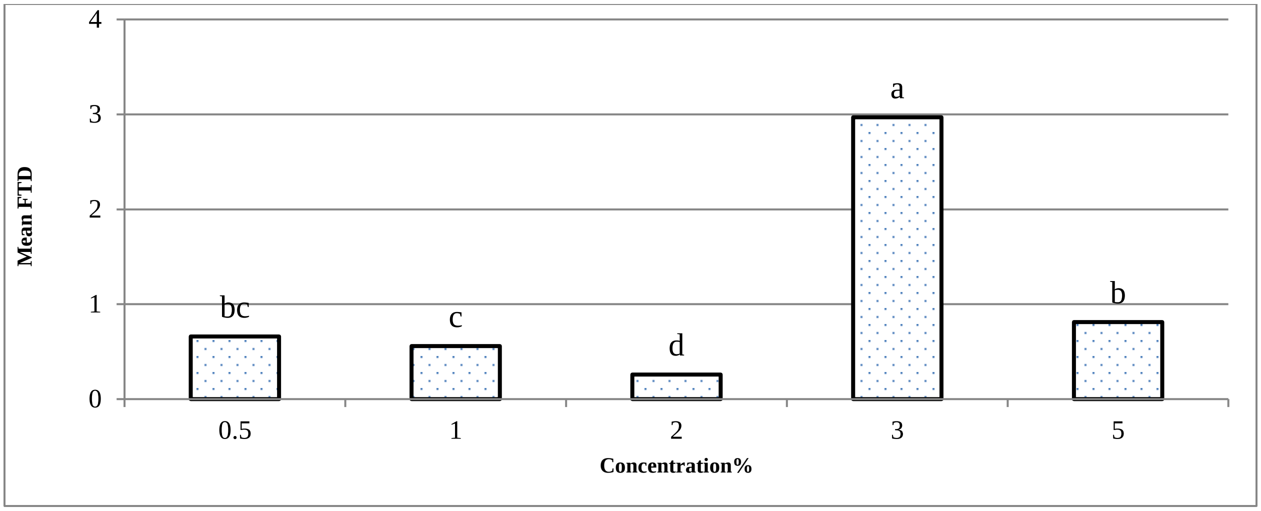


Figure (1): General mean of attracted MFF adults (as FTDs) to di-ammonium hydrogen phosphate concentrations during 15 days in mandarin orchard (Columns had the same letters did not differ at the significantly of 5%).

The relationship between the concentrations of di-ammonium hydrogen phosphate and its efficiency as an attractant to MFF was illustrated in Figure (2). As shown in this figure, it can be noticed that the efficiency of di-ammonium hydrogen phosphate increased as its concentration increased till it reached the range between 3.0 and 3.5%. After that the efficiency of di-ammonium hydrogen phosphate as an attractant to MFF decreased with the increase of its concentration.

The statistical relationship between the concentrations of di-ammonium hydrogen phosphate and its efficiency as attractant to MFF was evaluated as follow:

$FTD = -0.23\ C^2 + 1.42\ C - 0.42$

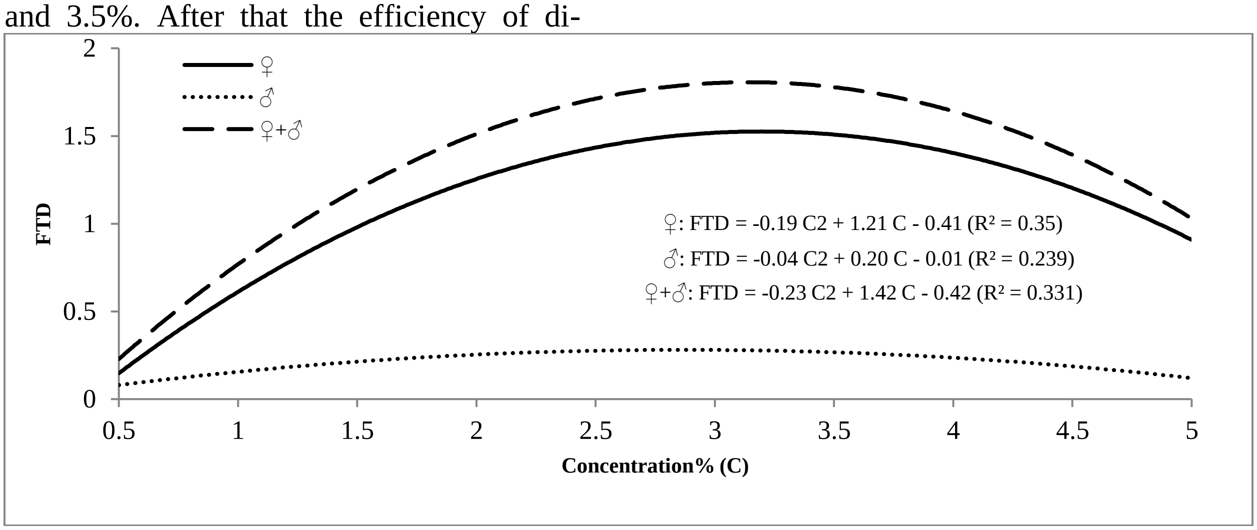


Figure (2): The relationship between the concentrations of di-ammonium hydrogen phosphate and its efficiency as attractant to Mediterranean fruit fly in mandarin orchard.

Sex ratios of the attracted MFF adults to the tested concentrations of di-ammonium hydrogen phosphate were evaluated and illustrated in Figure (3). The highest number of females per one male was recorded with a concentration 5% (9.12 females/one male) followed by the di-ammonium hydrogen phosphate concentration of 1% (8.00 females). The general mean sex ratio in all of the tested di-ammonium hydrogen phosphate concentrations reached 5.57 females per one male.

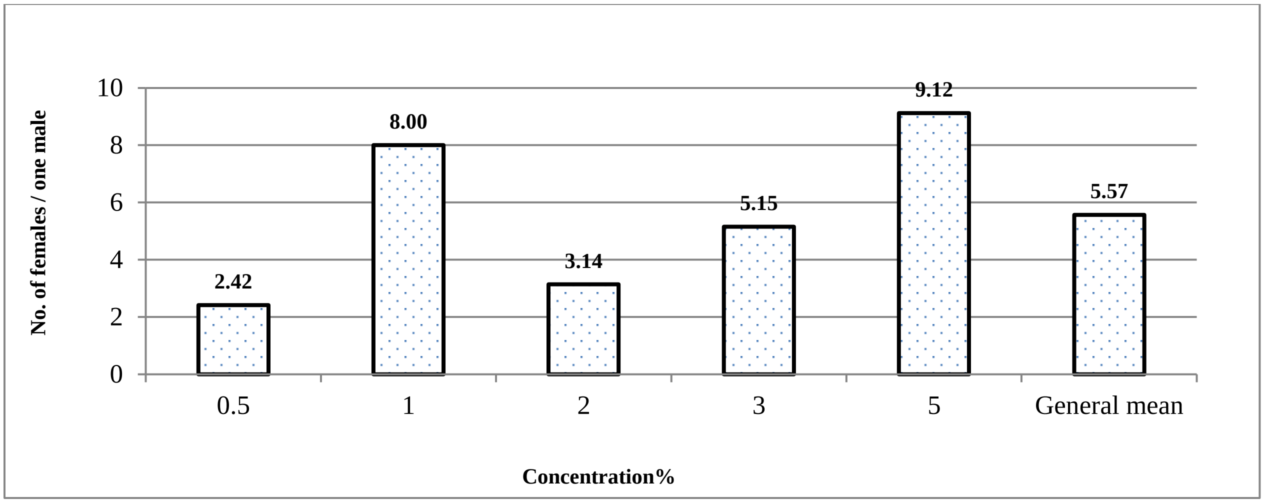


Figure (3): Sex ratios of the attracted Mediterranean fruit fly adults to the tested concentrations of di-ammonium hydrogen phosphate in mandarin orchard.

As shown in Figure (4), the efficiency of all the tested di-ammonium hydrogen phosphate concentrations increased by the passed time; however, each passed day increased the attracted MFF adults (As FTDs) by 0.05, 0.06, 0.06, 0.37 and 0.14 individuals when used 0.5, 1, 2, 3 and 5% concentrations of di-ammonium hydrogen phosphate, respectively. So, the relative stability showed that a 3% concentration of di-ammonium hydrogen phosphate was the most effective concentration to attract MFF adults.

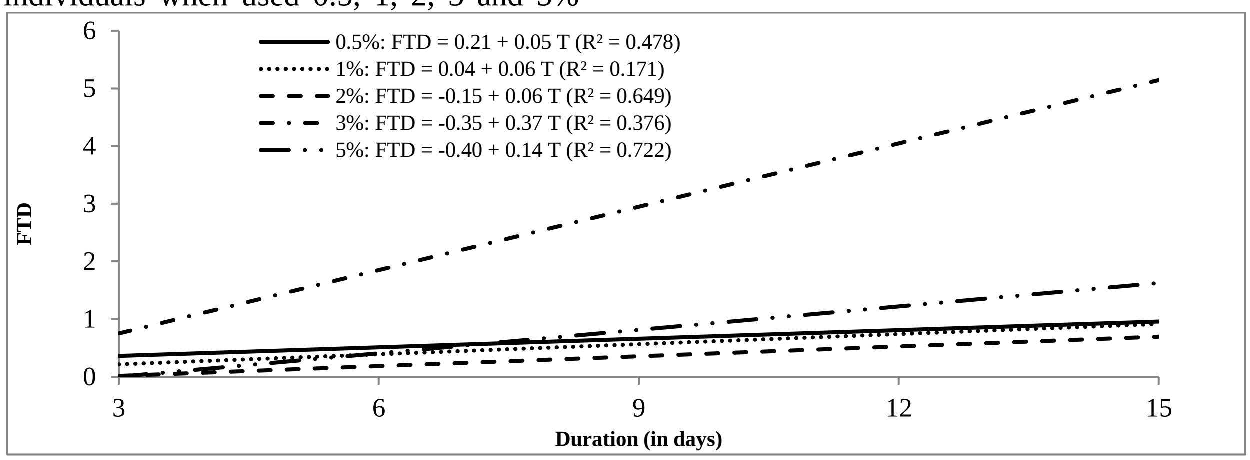


Figure (4): Stability of the tested concentrations of di-ammonium hydrogen phosphate as attractants for Mediterranean fruit fly adults against time all over 15 days in mandarin orchard.

2. Enhancement of protein-based bait by di-ammonium hydrogen phosphate to attract Mediterranean fruit fly:

Data represented in Figure (5) shows that adding di-ammonium hydrogen phosphate 1% to the food attractant (Buminal 5%) increased its efficiency as an attractant to MFF by 5.76 times more than Buminal alone after three days of hanging the traps. At all of the other inspections (6, 9, 12 and 15 days) there were no significant differences between adding di-ammonium hydrogen phosphate 1% to Buminal and Buminal alone. When adding di-ammonium hydrogen phosphate 2% to Buminal, it increased Buminal's efficiency as an attractant to MFF by 8.74, 5.55, 1.78, 2.55 and 1.61 times in comparison with Buminal alone after 3, 6, 9, 12 and 15 days of hanging traps, respectively.

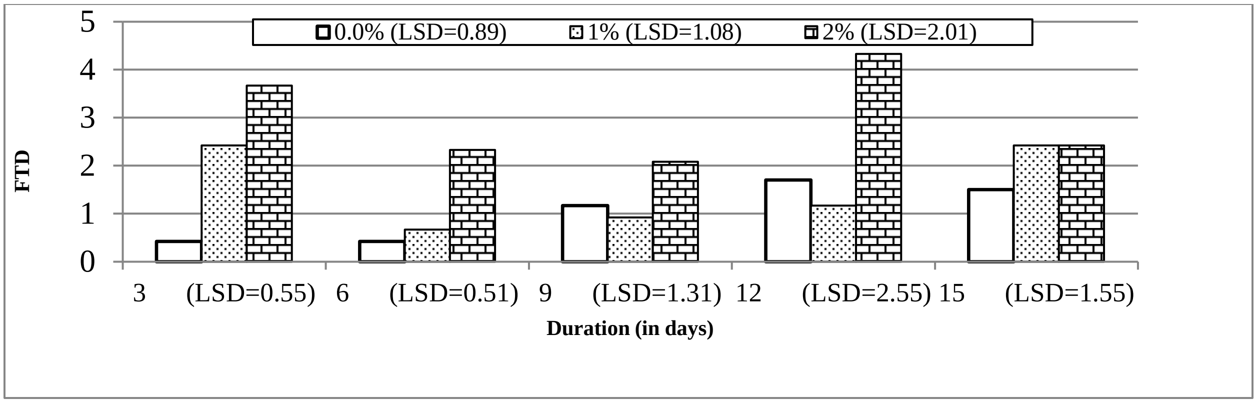
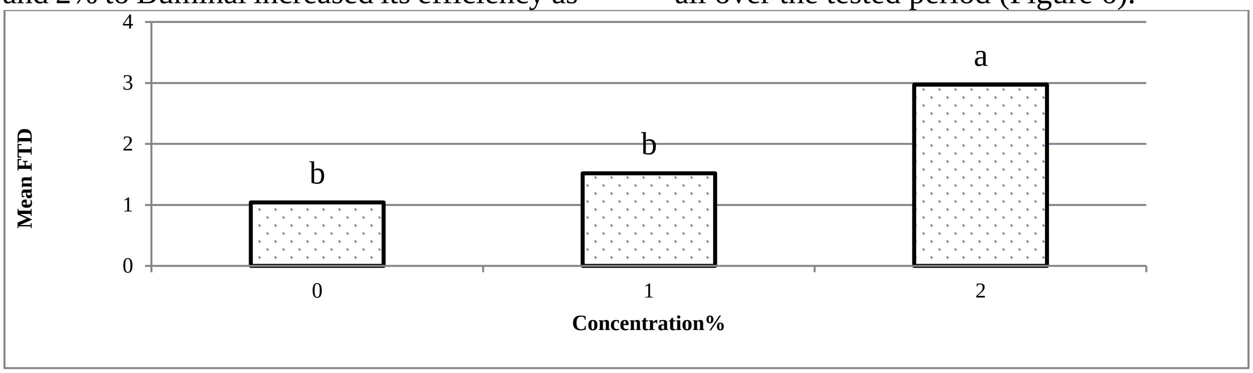


Figure (5): Enhancement of protein-based bait (Buminal) by using 1 and 2% concentrations of di-ammonium hydrogen phosphate to attract Mediterranean fruit fly all over 15 days in mandarin orchard.

As general, adding di-ammonium hydrogen phosphate with concentrations of 1 and 2% to Buminal increased its efficiency as an attractant to MFF adults with 1.46 and 2.86 folds of the efficiency of Buminal alone all over the tested period (Figure 6).



Figure(6): Mean FTDs of attracted Mediterranean fruit fly to protein-based bait (Buminal) alone or enhanced with 1 and 2% concentrations of di-ammonium hydrogen phosphate all over the tested period (15 days) in mandarin orchard (Columns had the same letters did not differ at the significantly of 5%).

Figure (7) shows that Buminal alone or enhanced with di-ammonium hydrogen phosphate 1% were more stable against the passed time after hanging traps; however, each passed day increased their efficiency as attractants to MFF by 0.11 and 0.02 adults per trap per day. With respect to Buminal which was enhanced with di-ammonium hydrogen phosphate 2%, it was relatively less stable against the passed time; however, each passed day decreased the recorded FTDs of MFF by 0.02 adults.

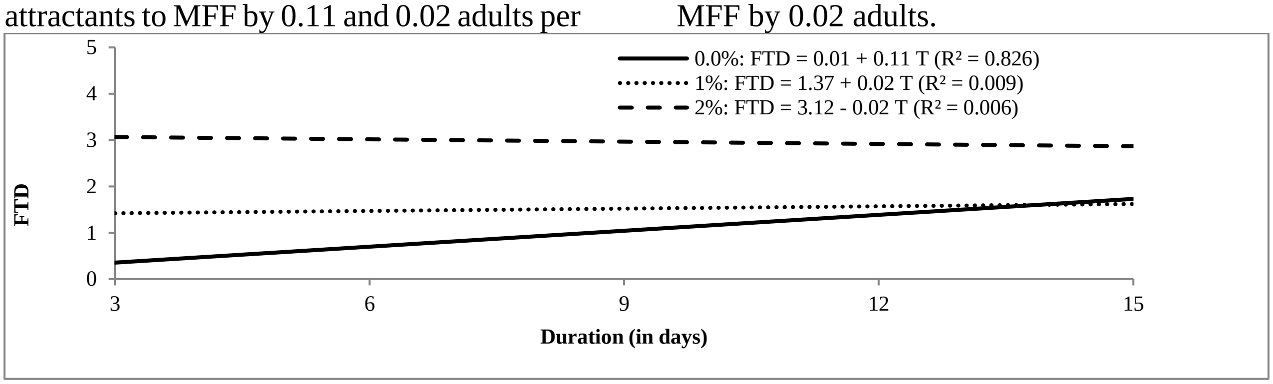


Figure (7): Stability of Buminal alone or enhanced with di-ammonium hydrogen phosphate 1 and 2% as attractants for MFF adults against time all over 15 days in mandarin orchard.

Nitrogen sources dietary has a very strong influence on the behavior and physiology of fruit flies (Hemeida *et al*., 2017 and Ghanim and El-Metwally, 2019). According to the role of ammonia in fruit fly attraction, we can increase the efficiency of fruit fly attractants by changing the formulations of variety of baits including ammonia such as ammonium acetate has been shown to be the most attractive component of food attractant (Biolure) for MFF (Leblanc *et al*., 2010). Also, Ghanim (2018) reported significant effects of adding ammonium acetate and di-ammonium hydrogen phosphate to GF-120; however, there were significant positive relationships obtained between relative amounts of ammonium compounds in the bait and the numbers of attracted *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), *B. zonata* and MFF. The production of ammonia because of protein bacterial degradation was correlated with increases in bait efficiency as an attractant to females of fruit flies (Bateman and Morton, 1981). As well as low attractiveness of proteinaceous baits to MFF was associated with low release rates of ammonia and vice versa (Mazor, 2009). The range of attractiveness of fruit flies to ammonia-based compounds is very narrow, while the range of repellence is much wider (Bateman and Morton, 1981 and Mazor *et al*., 2002). The present results showed that di-ammonium hydrogen phosphate exhibited a good efficiency in attracting MFF at concentrations ranging between 3.0 and 3.5%. The lower efficiency of lower concentrations is probably due to their lower attractiveness to MFF adults; while, the lower efficiency of higher concentrations may be due to their repellent action to MFF adults.

Similar results were obtained by Ragab and Youssef (2021) who reported that di-ammonium hydrogen phosphate at its concentration of 3% was superior to its concentrations in attracting MFF in guava and mandarin orchards. While Abd El-Kareim *et al*. (2008) and Ghanim *et al*. (2021) found that di-ammonium hydrogen phosphate at its concentrations of 1 and 2% were superior of its concentrations in attracting *B. zonata* under field conditions. In another hand, adding di-ammonium hydrogen phosphate with a concentration of 2% to the commercially protein-based bait of Buminal significantly increased Buminal's efficiency as an attractant for MFF adults.

Similar results were reported by Ghanim and El-Metwally (2019) that adding di-ammonium hydrogen phosphate at its concentrations of 2 and 3% to Buminal significantly increased its efficiency as an attractant to MFF under field conditions. Also, El-Metwally (2017) and Ghanim (2018) found that the insecticidal bait (GF-120; which considered a protein-based bait) can be enhanced by di-ammonium hydrogen phosphate with a concentration of 2% to increase its efficiency as an attractant to *B. zonata* and MFF. Hemeida *et al.* (2017) reported that when added di-ammonium hydrogen phosphate (3%) to the commercially protein-based baits of Buminal (5%) with a ratio of 1:1 significantly increased its efficiency as attractants for *B. zonata* adults from 3.4 to 6.8 times.

The attractants affected the sexes similarly in terms of relative responses (Yee, 2007). Epsky *et al*. (2014) and *Piñero et al*. (2015) explained the importance of proteins for fruit flies females to complete egg maturation; so, this indicated the main cause for the strong attraction of females to the protein-based baits. These may support the present results which showed that all concentrations of di-ammonium hydrogen phosphate tended to catch MFF females more than males. A similar conclusion was obtained by Yee (2007), Moustafa and Ghanim (2008), El-Abbassi *et al*. (2017), Ghanim (2018) and Ghanim *et al*. (2021); they reported that various ammonium compounds attracted higher numbers of females of fruit flies than males. *Piñero et al*. (2015) explained that adding ammonium compounds to a variety of protein baits and materials enhanced a response of MFF females more than males.

On the contrary, the present results revealed that the efficiency of all concentrations of di-ammonium hydrogen phosphate increased as attractants for MFF adults by the passed time over 15 days of the experiment. Also, the results of Abd El-Kareim *et al*. (2008), Moustafa and Ghanim (2008), Ghanim *et al*. (2014) and Ghanim *et al*. (2021) came in the trend of the present one. However, they noted that the efficiency of ammonium compounds in attracting fruit flies increased as time passed. On the other hand, Ghanim *et al*. (2014) reported that the efficiency of ammonium acetate was less effective for the tephritid, *Carpomya incompleta* (Becker) (Diptera: Tephritidae) decreased by the passed time in Saudi Arabia. The difference between these results and the present may be attributed to the variation of fruit fly species and/or climatic factors.

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