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Effect of blending ammonium acetate and di-ammonium phosphate solutions on their attractance to mediterranean fruit fly, *Ceratitis capitata* in mandarin orchids under field conditions

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Abstract
The present investigation aims to evaluate the efficacy of blending ammonium acetate and di- ammonium phosphate solutions on their attractance to Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in mandarin orchids under field conditions. Trap tests indicated that *C. capitata* adults exhibited different preferability to the tested ammonium compounds. Data showed that, the highest attractive treatment to *c. capitata* after 4 days was that of ammonium acetate 3% mixed with di-ammonium phosphate 2% (FTD= 1.31). After The mixture of ammonium acetate and di- ammonium phosphate as 3% of each exhibited the highest attraction to this pest. While, ammonium acetate 2% mixed with di- ammonium phosphate 1% was the highest attractant to *C. capitata* after 16 days. On the other hand all of the tested treatments attracted females of *C. capitata* obviously more than males. To evaluate the potency of the tested compounds, the concentrations of ammonium acetate showed adverse effect on the attractancy of *C. capitata* when it was alone or mixed with di- ammonium phosphate 1%. When the concentration of di- ammonium phosphate showed positive effect on the attractancy of *C. capitata* when it was alone or mixed with ammonium acetate 3%. The elapsed time showed its highest effect on the mixture of ammonium acetate 3% + di- ammonium phosphate followed by di- ammonium phosphate1% alone.

Keywords: Blending, ammonium acetate, di- ammonium phosphate, attractance, *Ceratitis capitata* and mandarin orchids

Introduction
Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is a one of the most important pests destructing fruits of over 350 species of fruits, nuts and vegetables round the world (Liquido *et al*., 1991; White and Elson-Harris, 1992; Papadopoulos, 2014) [20, 29, 25]. It is a species of Afrotropical origin which has adapted to the climatic conditions of the Mediterranean basin (Franco *et al.*, 2006) [8]. Tephritid fruit flies (including *C. capitata*) use chemical stimuli in the form of nutrients (Joachim-Bravo *et al*., 2001) [16]. Food sources which are rich in nitrogen have a strong influence on the physiology and behavior of tephritid flies (Kaspi *et al*., 2000; Yuval *et al*., 2007; Hemeida *et al*., 2017; El-Metwally, 2018 and Ghanim and El-Metwally, 2019) [6, 30, 14, 4, 11]. So, protein bait acts as food attractant and its effectiveness behaviorally relies on the fact that immature females need a protein meal to reach sexual maturity and for development of eggs to maturity (Epsky *et al*., 2014 and Pinero *et al*., 2015) [7, 5]. According to Ghanim *et al* (2014) [9], Bayoumy and el- Metwally (2017), Hemeida et al (2017) [14] and Ghanim and El-Metwally (2019) [11] ammonia are associated with protein-rich foods and has long between known to attract fruit flies.
Ammonia and acetic acid are considered to be from the most important fruit fly attractants. Various formulations of synthetic ammonia have been used as baits for fruit flies, including ammonium acetate di- ammoniu phosphate tri-ammonium phosphate, ammonium carbonate, ammonium chloride, (Moore, 1969, Mostafa and Ghanim, 2008, Abd El-Kateim et., al 2008, Ghanim., *et al.*, 2014 and El-Abbassi *et al.* 2017) [1, 23, 9]. Documentation of the role of ammonia was provided by Mazor *et al.* (1987), who used dilutions of a pure ammonia solution to obtain a direct correlation between capture of the fruit fly female, *C. capitata* and ammonia concentration.

Also, direct relationships were reported between ammonia release rate and fruit flies (*C. capitata*; Heath *et al.*, 1994 and *Anastrepha suspensa* (Loew); Epsky *et al.*, 1993) [5].

Attractants of fruit flies are used for two objectives;(1) to detect and monitor populations of fruit flies(2) control of fruit flies (Abd El-Kariem *et al* 2008, Ghanim *et al* 2004 Ghanim, 2018 and El- Metwally, 2018) [10, 24]. There for, the present study aimed to blared the solutions of ammonium acetate and di- ammonium phosphate to obtain new treatments which may be more effective in attracting *C. capitata* under field conditions.

Material and Methods

Tested Compounds

Ammonium acetate (Aa) and di-ammonium phosphate (Da) were obtained from El-Naser for Drugs and Chemicals Company were evaluated as olfactory attractants for Mediterranean fruit fly, *C. capitata* under field conditions at Mansoura district. Each ammonium compound were investigated by using three concentrations (1, 2 and 3%), and interchangeably mixing the concentrations of ammonium acetate and di- ammonium phosphate.

Bioassay Experiments

To evaluate the efficacy of ammonium compounds as olfactory attractants for *C. capitata* adults, an experiment was carried out in mandarin orchards of the experimental at Mansoura district, during the period from the 9thof September till 25th of September 2020.

The modified Nadel traps (described by Hanafy *et al.*, 2001) [12] were used in this experiment; by putting 300 ml of each treatment in the trap. Each treatment was replicated four times. All prepared traps were distributed in a completely randomized design. The traps were hanged at about 1.5 meters in a shady site of the trees. To avoid interference among traps loaded with different treatments the distance between every two successive hanged traps was not less than 20 meters.

The traps were inspected every 4 days (as intervals) along a period of 16 days with no renewal of solutions. Captured females and males of *C. capitata* were counted and recorded as FTD (number of flies per trap per day).

Data Analysis

Statistical analysis was done as one way ANOVA and means comparison was conducted by using L.S.D. test at the probability of 5% (CoStat, 1990) [3] in addition to the regression analysis was applied for treatments.

Results

Data in table (1) showed that Medetranian fruit fly, *C. capitata* adults exhibited different preferability to the tested ammonium compounds. Data illustrated in Table (1) showed that, the highest attractive treatment to *c. capitata* after 4 days was that of ammonium acetate 3% mixed with di- ammonium phosphate 2% (FTD= 1.31). After 8 days, ammonium acetate 2% mixed with di- ammonium phosphate 2% was the most effective treatment in attracting *C. capitata*. The mixture of ammonium acetate and di- ammonium phosphate as 3% of each exhibited the highest attraction to this pest. While, ammonium acetate 2% mixed with di- ammonium phosphate 1% was the highest attractant to C. capitata after 16 days.

On the other hand all of the tested treatments attracted females of C. capitata obviously more than males (Table, 1).

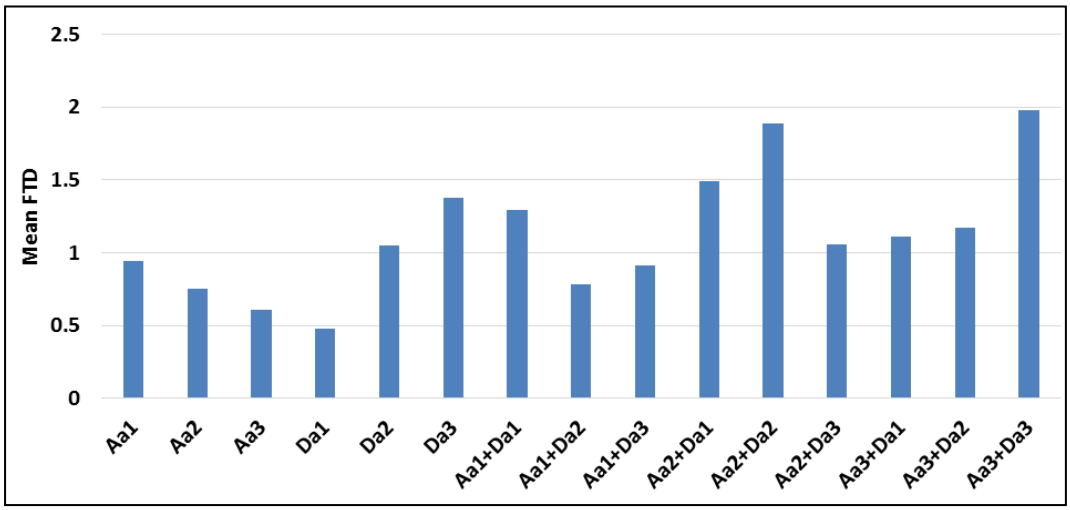
Table 1: Mean attracted *C. capitata* adults (females (F) and males (M)) to different preparations of ammonium compounds all over the 16 days under field conditions.

Time	FTD after 4 days			FTD after 8 days			FTD after 12 Days			FTD after 16Days		
	F	M	F+M	F	M	F+M	F	M	F+M	F	M	F+M
Aa1	0.38	0	0.38	0.44	0	0.44	1.63	0.25	1.88	0.94	0.125	0.81
Aa2	0.19	0	0.19	0.63	0	0.63	0.5	0	0.5	1.44	0.25	0.81
Aa3	0.19	0	0.19	0.38	0	0.38	0.56	0	0.56	1.125	0.19	1.31
Da1	0.63	0.06	0.69	0.75	0	0.75	0.38	0	0.38	0.44	0.06	0.5
Da2	0.88	0	0.88	1	0	1	0.88	0.125	0.94	1.13	0.25	1.38
Da3	1.19	0	1.19	1.19	0	1.19	1.5	0.125	1.63	1.25	0.25	1.5
Aa1+Da1	0	0	0	0.94	0.125	1.06	2.81	0.125	2.94	1.06	0.125	1.19
Aa1+Da2	0.56	0	0.56	0.56	0	0.56	0.94	0.44	1.38	0.63	0	0.63
Aa1+Da3	0.44	0	0.44	0.81	0	0.81	0.88	0	0.88	1.19	0.31	1.5
Aa2+Da1	0.19	0	0.19	1.63	0	1.63	0.81	0.06	0.88	2.81	0.44	3.25
Aa2+Da2	0.5	0.125	0.63	1.88	0	1.88	1.88	0	1.88	2.81	1.5	3.19
Aa2+Da3	0.56	0	0.56	1.19	0.38	1.56	0.81	0	0.81	1.44	0.125	1.31
Aa3+Da1	0.94	0.19	1.125	0.69	0	0.69	1.125	0.06	1.19	1.125	0.31	1.44
Aa3+Da2	1.31	0	1.31	1.44	0	1.44	1.06	0.06	1.125	0.75	0.06	0.81
Aa3+Da3	1.25	0	1.25	1.63	0	1.63	2.88	0	2.88	1.94	0.25	2.19
LSD	0.359	0.112	0.338	0.446	0.075	0.440	0.485	0.181	0.502	0.095	0.251	0.464

Not : 1,2,3 in all treatments mean concentrations of 1%, 2%, 3% respectively.

With respect to ammonium acetate alone, concentration of 1% was the most attractive to *C. capitata* (mean FTD was 0.94). while the concentration of 3% was the highest of di-ammonium phosphate (mean FDT was 1.38).

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 *C. capitata* (mean FTD was 1.98) followed by the mixture of 2% of each ammonium compound (mean FTD was 1.89) (Fig., 1).



Not: 1, 2, 3 in all treatments mean concentrations of 1%, 2%, 3% respectively.

Fig 1: Mean numbers of attracted *C. capitata* (as females + males) to different concentrations of ammonium acetate (Aa) and di- ammonium phosphate (Da) allover 16 days under field conditions.

To evaluate the potency of the tested compounds (as lures for *C. capitata* adults) against concentrations of ammonium compounds, regression analysis has been done between the FTD and concentrations (Table, 2).

As shown as in Table (2), the concentrations of ammonium acetate showed adverse effect on the attractancy of *C. capitata* when it was alone or mixed with di- ammonium phosphate 1%. However each increase of ammonium acetate concentration by 1%; the attracted *C. capitata* (as FTD of females+ males) decreased by 0.96 and 0.56 flies all over 16 days when ammonium acetate was alone or mixed with di-ammonium phosphate 1% respectively. In contrast, the concentration of ammonium acetate showed positive effect on the attractancy of *C. capitata* when it mixed with di-ammonium phosphate 2 or 3%. However, each increase of ammonium acetate concentration by 1%; the attracted *C. capitata* (as FTD of females + males) increased by 1.15 and 3.17 flies all over the tested period when ammonium acetate mixed with di- ammonium phosphate 2 and 3% respectively (Table, 2).

Table 2: The effect of ammonium acetate (Aa) concentrations alon or mixed with di- ammonium phosphate (Da) on attraction of *C. capitata* under field conditions.

Duration	Sex	Aa alone		Aa + Da1%		Aa + Da2%		Aa + Da3%	
		b	R^2	b	R^2	B	R^2	b	R^2
After 4 days	♀	-0.01	0.75	0.05	0.892	0.04	0.688	0.04	0.862
	♂	0	0	0.25	0.75	0.11	0	0	0
	♀+♂	0.01	0.75	0.06	0.871	0.04	0.811	0.04	0.410
After 8 days	♀	-0.002	0.056	-0.01	0.066	0.03	0.429	0.03	0.998
	♂	0	0	-0.13	0.75	0	0	0.25	0
	♀+♂	-0.002	0.057	-0.01	0.157	0.03	0.428	0.03	0.807
After 12 days	♀	-0.03	0.705	-0.05	0.614	0.003	0.015	0.05	0.727
	♂	-0.11	0.75	-0.03	0.75	-0.16	0.628	0	0
	♀+♂	-0.03	0.713	-0.04	0.619	-0.01	0.107	0.05	0.727
After 16 days	♀	0.004	0.136	0.001	0.001	0.003	0.002	0.02	0.75
	♂	0.01	0.25	0.03	0.355	0.01	0.024	-0.01	0.107
	♀+♂	0.01	0.158	0.01	0.012	0.004	0.004	0.02	0.559
General mean	♀	-0.89	0.995	-0.75	0.353	1.49	0.182	3.49	0.824
	♂	-1.81	0.964	1.81	0.963	-3.01	0.604	-1.21	0.158
	♀+♂	-0.96	0.994	-0.56	0.252	1.15	0.936	3.17	0.884

Not: 1, 2, 3 in all treatments mean concentrations of 1%, 2%, 3% respectively.

As shown in Table (3), the concentration of di- ammonium phosphate showed positive effect on the attractancy of *C. capitata* when it was alone or mixed with ammonium acetate 3%. However each increased of di- ammonium phosphate concentration by 1%; the attracted *C. capitata* (as FTD of females + males) increased by 2.35 and 2.58 flies all over the 16 days when di- ammonium phosphate was alone pr mixed with ammonium acetate 3%, respectively.

In contrast, the concentration of di- ammonium phosphate showed adverse effect on the attractancy of *C. capitata* when it mixed with ammonium acetate 1 and 2%. However, each increase of di- ammonium phosphate concentration by 1%; the attracted *C. capitata* (as FTD of females + males) decreased by 1.15 and 1.24 flies all over the tested period when di- ammonium phosphate mixed with ammonium acetate 1 and 2%, respectively (Table, 3).

Table 3: The effect of di- ammonium phosphate (Da) concentrations alone or mixed with ammonium acetate (Aa) on attraction of *C. capitata* under field conditions.

Duration	Sex	Da alone		Da + Aa1%		Da + Aa2%		Da + Aa3%	
		B	R^2	b	R^2	b	R^2	b	R^2
After 4 days	♀	0.03	0.995	0.02	0.548	0.02	0.871	0.02	0.605
	♂	-0.08	0.75	0.0	0	0.11	0	-0.25	0.75
	♀+♂	0.03	0.980	0.02	0.547	0.01	0.085	0.01	0.426
After 8 days	♀	0.01	0.133	-0.004	0.105	-0.01	0.395	0.03	0.892
	♂	0.0	0	0.13	0.75	0.38	0.75	0.0	0
	♀+♂	0.01	0.993	-0.01	0.25	-0.002	0.035	0.03	0.892
After 12 days	♀	0.03	0.995	-0.05	0.774	0.06	0	0.05	0.722
	♂	0.05	1	-0.05	0.076	-0.03	0.75	-0.03	0.75
	♀+♂	0.03	0.996	-0.05	0.919	-0.001	0.002	0.04	0.722
After 16 days	♀	0.02	0.862	0.003	0.044	-0.04	0.75	0.02	0.45
	♂	0.03	0.75	0.03	0.355	-0.05	0.892	-0.01	0.057
	♀+♂	0.02	0.841	0.01	0.124	0.04	0.774	0.02	0.296
General mean	♀	2.34	0.992	-1.19	0.470	-1.35	0.260	3.04	0.879
	♂	2.41	0.923	7.63	0	9.64	0	-3.01	0.480
	♀+♂	2.35	0.989	-1.15	0.523	-1.24	0.259	2.58	0.879

Not: 1, 2, 3 in all treatments mean concentrations of 1%, 2%, 3% respectively.

Mathematically, the elapsed time showed its highest effect on the mixture of ammonium acetate 3% + di- ammonium phosphate 2% (R^2= 0.975) followed by di- ammonium phosphate1% alone (R^2= 0.867), ammonium acetate 3% alone (R^2 = 0.793) and di- ammonium phosphate 3% alone (R^2= 0.728). In contrast, the elapsed time exhibited its lowest effect on the mixture of ammonium acetate 2% + di- ammonium phosphate 3% and ammonium acetate 3% + di-ammonium phosphate 3% where, R^2- values were 0.123 and 0.131, respectively (Table, 4).

Table 4: Effect of passed time on the attractancy of ammonium acetate, di- ammonium phosphate and the mixtures between them towards *C capitata* under field conditions

Treatment	♀		♂		♀+♂	
	B	R^2	b	R^2	b	R^2
Aa1%	0.001	0.157	0.008	0.180	0.002	0.149
Aa2%	0.003	0.622	0.006	0.6	0.003	0.614
Aa3%	0.002	0.821	0.004	0.6	0.002	0.793
Da1%	-0.004	0.878	0.01	0.497	-0.004	0.867
Da2%	-0.001	0.051	0.007	0.897	-0.002	0.597
Da3%	-0.004	0.782	0.008	0.67	0.004	0.728
Aa1% + Da1%	0.006	0.265	0.00	0.000	0.01	0.244
Aa2% + Da1%	0.007	0.478	0.01	0.852	0.01	0.504
Aa3% + Da1%	-0.003	0.397	0.03	0.421	0.003	0.358
Aa1% + Da2%	-0.001	0.538	0.01	0.066	-0.001	0.163
Aa2% + Da2%	0.01	0.666	-0.02	0.276	0.004	0.624
Aa3% + Da2%	0.01	0.967	0.002	0.342	-0.01	0.975
Aa1% + Da3%	0.00	0.409	0.01	0.6	0.001	0.464
Aa2% + Da3%	-0.001	0.123	-0.02	0.048	0.003	0.123
Aa3% + Da3%	-0.001	0.113	0.01	0.6	0.002	0.131

Not: 1, 2, 3 in all treatments mean concentrations of 1%, 2%, 3% respectively.

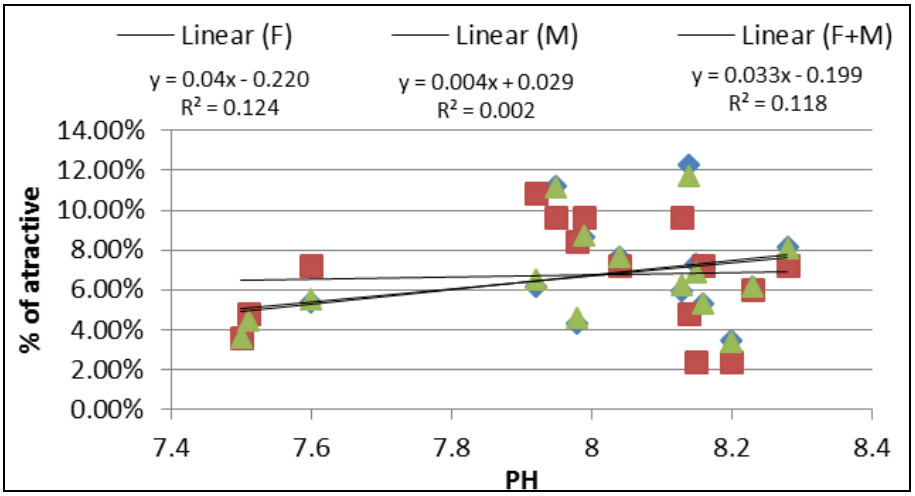


Fig 2: Effect of pH-level of the tested compounds on the attracted *C. capitata* (females, males and total adults) under field conditions.

Data represented in Fig(2) showed that pH- level showed positive effects on female and male of *C. capitata* captured adults. However, each increase of pH- level by one degree, the relative attractance of females, males and total of them (as FTD) increased by 0.04, 0.004s and 0.03 flies, respectively.

Discussion

Data indicated that medetranian fruit fly, *C. capitata* adults exhibited different prefer ability to the tested ammonium compounds. Concentration1% of ammonium acetate was recorded the heist attraction females males of *C. capitata* when ammonium acetate used alone, while Concentration 3% of Dai ammonium phosphate was recorded the heist attraction females and males of *C. capitata* when di- ammonium phosphate was used alone. These findings agree with Jones (1987) [17], Abd El-Kareim *et al*, (2008) [1] and Moustafa & Ghanim (2008) [23] and Ghanim *et al*. (2014) [9], they 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 as stated by Saafan (2001) [27].

Data indicated that mixture of ammonium acetate 1% and Dai ammonium phosphate 1%was recorded the highest attraction females and males of *C. capitata* after 12 days, in respect to mixture of ammonium acetate 2% and Dai ammonium phosphate 1% was recorded the highest FTD after 16 days, while the highest attractancy of mixture of ammonium acetate 3% and Dai ammonium phosphate 3% was recorded after12 days. Alsof, Mostafa and Ghanim (2008) [23], Abd El-Kareim *et al*. (2008) [1] and Ghanim *et al*. (2014) [9] recorded that the highest attraction of ammonium acetate was recorded after 12-15 days; while, the highest attraction of di- ammonium phosphate was recorded afterv 3 – 6 days of hanging traps. The differences between the present study and others may be attributed to the variation of fruit fly species, weather factors and/ or host plants.

The present results showed that the attractancy of ammonium acetate showed negative response of its concentration, in contrast, the attractance of di- ammonium phosphate showed positive response to increase of its concentration. The findings are in agreement with the studies of Abd El-Kareim *et al*. (2008) [1], Ghanim *et al*. (2014) [9] and Ghanim and El-Metwally (2019) [11]. Adverse results were obtained by Mostafa and Ghanim (2008) [23]. Addining di- ammonium phosphate to ammonium acetate and vice versa changed these findings. This may be attributed to interaction between the mixed compunds.

Data showed that ammonium acetate and di- ammonium phosphate significantly attracted more females than males of *C. capitata* agree with Ghanim *et al*., 2014 [9], they found that ammonium compounds significantly attracted more females of *Carpomya incomplete*(Beeker) than males.

According to Hanafy *et al*. (2001) [12], Saafan (2005) [28]; Abd El-Kareim *et al*. (2008) [1] and Moustafa and Ghanim (2008) [23], females of *B. zonata* and *C. capitata* were more attracted to ammonium compounds than males. In addition, Delrio and Orto (1989) stated that ammonium acetate attracted a high proportion of females. Landolt and Davis-Hernandez (1993) [19] hypothesized that antennal response to ammonia would be higher for females than for males because of the greater need for protein by females for egg development. Probable, the increased need for protein 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 (Arn *et al*., 1975 and Mayer *et al*., 1987) [2, 22].

Data recorded that FTD increased in ammonium acetate 1, 2 and 3% by the passed time. When it recorded gradually decreased by the time based in di- ammonium phosphate 1 and 2% and increased in concentration 3%. Similar results were recorded on *Bacrtocera. zonata* (Abd El-Kareim *et al*., 2008) [1] and *C. capitata* (Moustafa & Ghanim, 2008) [23]. Ghanim *et al*., (2014) [9] they recorded that the highest efficiency of tri-ammonium phosphate was recorded after 8 days; while, the highest efficiency of ammonium carbonate was recorded after 16 days. With respect to ammonium acetate, ammonium chloride and ammonium thiocyanate, the highest efficiency was recorded earlier (after 4 days).

Data represented showed that the highest number of attracted females and males of *C. capitata* when pH-levels at 8.14 These results came in the same line with those of Ghanim and El- Metwally (2019) [11]; they recorded that the highest attraction of C. capitata were occurred between 6.32 and 8.29 of pH- level.

El-Metwally (2018) [4] and Ghanim (2018) [10]; mentioned that adding ammonium acetate, ammonium carbonate or di-ammonium phosphate to the insecticidal protein-based bait, GF-120 increased its pH-level; and attractiveness to *C. capitata* and *B. zonata*.

It could be suggested that the present results may be useful in applying integrated pest management control programs by using mixtures of ammonium acetate and di- ammonium phosphate at concentrations of 3 or 2% of each ammonium compounds because of its good attractancy for *C. capitata* adults and its potency along elapsed time.

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