https://dx.doi.org/10.4314/ejppri.v8i3.7

Egypt. J. Plant Prot. Res. Inst. (2025), 8 (3): 276-286

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Field evaluation of certain ammonium compounds as attractants and bait enhancers for *Bactrocera zonata* (Diptera: Tephritidae) adults

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ARTICLE INFO

Article History

Received:23/7 /2025

Accepted:24/9 /2025

Keywords

Bactrocera zonata, adults, field evaluation, ammonium compounds and bait enhancers.

Abstract

Bactrocera zonata (Saunders) (Diptera: Tephritidae), the peach fruit fly (PFF), is a polyphagous insect pest that poses a significant threat to a variety of fruits in Egypt. The present study aimed to evaluate certain ammonium compounds as attractants for PFF adults, in addition to the enhancement of the recommended protein-based bait (Buminal) for attracting this pest under field conditions of a mandarin orchard. The obtained results showed that ammonium hydroxide (at the concentration of 3%) was superior in attracting PFF, followed by ammonium chloride (at 5%), ammonium acetate (at 3%), and ammonium sulfate (at concentrations between 4 and 5%). Adding ammonium hydroxide, ammonium chloride, or ammonium acetate (with 1 or 2% concentrations) to the protein-based bait (Buminal 5%) significantly increased its efficiency as an attractant to PFF adults, while adding ammonium sulfate to Buminal 5% did not differ significantly from the control treatment (Buminal 5% without additives).

Introduction

Native to tropical Asia, the peach fruit fly (PFF), *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), is a polyphagous insect pest that had spread to many regions of the world (Agarwal *et al*., 1999 and El-Minshawy *et al*., 1999). Due to this insect's infestation, commercial fruits are subject to protection and quarantine treatments before export, and quarantine limitations are implemented on the affected areas (Vargas *et al*., 2008). According to Hashem *et al*. (2001) and Ghanim (2009), PFF established itself as a significant pest in Egypt, affecting a variety of fruits that ripen at different periods and endure virtually all year round.

Like other tephritid flies, PFF strongly responds to nitrogen-containing food sources (Kaspi *et al*., 2000; Yuval *et al*., 2007; Abd El-Kareim *et al*., 2008; Hemeida *et al*., 2017; Ghanim *et al*., 2021; Abd El-Salam and Youssef, 2023 and Hendawy *et al*., 2024). According to Epsky *et al*. (2014) and Pinero *et al*. (2015), the females' need protein to develop their eggs. In order to manage PFF, protein-based baits combined with insecticides have been employed (Ghanim, 2018; Hendawy *et al*., 2024 and El-Kelany, 2025). So, protein-based baits should have high levels of attraction and stimulate adults of PFF (Like other tephritids) to ingest the lethal doses of the used insecticides for good

suppression of the insect population (Mangan, 2009 and 2014). Epsky and Heath (1998) and Hull and Cribb (2001) reported that ammonia substances released from protein-based baits have a significant effect on their attraction. So, the development of effective ammonia-containing baits for PFF is aimed at by Hemeida *et al*. (2017), Ghanim (2018), and Hendawy *et al*. (2024). Additionally, Mazor *et al*. (2002) and Bateman and Morton (1981) noted that fruit flies' attraction to ammonia-based substances is referred to as dose-dependent, which revealed that the range of attractiveness is very narrow, while the range of repellence is much wider. So, adding ammonium compounds (At specific concentrations) to protein-based baits is important to enhance their effectiveness as attractants for monitoring and suppressing the populations of fruit flies (Pinero *et al*., 2015).

In order to improve the effectiveness of the suggested protein-based bait (Buminal) as an attractant for PFF adults in the field (Mandarin orchard), the current study first evaluated specific ammonium compounds as olfactory attractants for PFF adults (Using varying concentrations).

Materials and methods

1. The materials used:

Six ammonium compounds ammonium hydroxide (NH_4OH), ammonium chloride (NH_4Cl), ammonium acetate (CH_3COONH_4), ammonium sulfate $(NH_4)_2SO_4$, hydroxyl ammonium chloride ($HONH_2{\cdot}HCl$), and thiourea (H_2NCSNH_2) were obtained from Edwic Company, Egypt. In addition to the protein-based bait (Buminal) (39.78% Protein) and the modified Nadel traps (which were described by **Hanafy *et al*. (2001)** were obtained from the Plant Protection Research Institute, Agricultural Research Center, Egypt.

2. Area of study:

An area of 15 feddans (One feddan is equivalent to 4200 m^2) of mandarin (*Citrus reticulata* Blanco) orchard in Mansoura district, Dakahlia governorate, Egypt, was used for the current studies.

3. Field evaluation of the efficiency of ammonium compounds as attractants for *Bactrocera zonata*:

During the present study, the five concentrations of 0.5, 1, 2, 3, and 5% of each ammonium compound were prepared by diluting with water. A modified Nadel trap was filled with 250 ml of each treatment, which was then carried out four times (As duplicates). At a height of roughly two meters, traps were suspended from the trees in areas with shade. The traps were placed inside the orchard using a completely randomized design, with a 20-meter gap between each subsequent trap to prevent trap interference.

Traps were filtered every three days to remove fruit fly adults from the solution during the 15 days from October 7 to October 22, 2024. Fruit flies were then taken to the lab for examination after the solutions were put back in the traps. For every trap, the number of PFF adults was counted and recorded as captured flies/trap/day (FTD values).

4. Enhancement of ammonium compounds' protein-based bait (Buminal) for attraction in *Bactrocera zonata* adults:

Buminal was diluted with water to produce a 5% concentration. For this experiment, ammonium compounds were produced in concentrations of 1 and 2%. Each concentration of ammonium compound was mixed with Buminal 5% and then 250 ml of the mixture of one treatment was put inside the modified Nadel trap. As a control, Buminal 5% without mixing any of the

ammonium compounds was used for comparison and put inside each of the modified traps as the other treatments. Every treatment was duplicated four times, along with the control. In the chosen mandarin orchard, the prepared traps were dispersed using a completely randomized design, with a 20-meter gap between each subsequent trap to prevent trap interference.

As previously noted, traps were inspected every three days over the 15-day period from October 27 to November 11, 2024. As previously stated, the number of PFF adults that were captured was counted.

5. Statistical analysis:

After analyzing the data collected using the CoHort Software (2004) statistical analysis program, the least significant difference (LSD) was calculated at a 5% probability level. They performed a one-way ANOVA. Additionally, the same application was used to perform regression analysis.

Results and discussion

1. Attractancy of certain ammonium compounds to *Bactrocera zonata* adults:

1.1. Ammonium hydroxide:

According to the data in Table (1), the attractant of the tested ammonium hydroxide concentrations after 3, 6, 9, and 12 days of hanging traps, as well as their means during the tested time, did not differ significantly. On the other hand, the significant differences between concentrations were recorded after 15 days only, when the attractants of 0.5, 2, and 3% decreased significantly in comparison with the concentrations of 1 (The first rank) and 5% (The second rank) of ammonium hydroxide.

Table (1): The effectiveness of ammonium hydroxide concentrations (Con.) as attractants for *Bactrocera zonata* adults in a mandarin orchard over a period of 15 days.

Con.	Flies/trap/day (FTD) after (In days)					Mean
	3	6	9	12	15	
0.5	0.66±0.30	0.91±0.36	0.82±0.28	0.91±0.21	0.08±0.04	0.67±0.11
1	0.83±0.42	1.16±0.21	0.83±0.28	0.50±0.28	1.91±0.28	1.04±0.10
2	0.58±0.15	1.49±0.09	1.57±0.37	1.32±0.23	0.33±0.13	1.06±0.10
3	0.99±0.23	1.33±0.16	1.33±0.23	1.33±0.23	0.50±0.21	1.09±0.13
5	0.83±0.21	0.92±0.13	1.00±0.23	0.91±0.28	1.16±0.16	0.96±0.16
LSD	0.84^{ns}	0.65^{ns}	0.86^{ns}	0.75^{ns}	0.57**	0.37^{ns}

1.2. Ammonium chloride:

The tested concentrations of ammonium chloride (As attractants for PFF adults) varied significantly during the period of the 15-day test, except after 3 days of hanging traps (Table 2). The concentration of 1% was superior, followed by the concentration of 5% (With no significant differences between their means over the entire testing period). The concentrations of 0.5, 2, and 3% of ammonium chloride ranked third as attractants for PFF adults in the mandarin orchard.

Table (2): Efficiency of ammonium chloride concentrations (Con.) as attractants for *Bactrocera zonata* adults over 15 days in a mandarin orchard.

Con.	Flies/trap/day (FTD) after (In days)					Mean
	3	6	9	12	15	
0.5	0.83±0.16	0.41±0.08	0.33±0.13	0.25±0.08	0.17±0.09	0.39±0.07
1	1.00±0.13	1.41±0.31	1.00±0.30	0.91±0.34	1.25±0.21	1.11±0.11
2	1.08±0.25	1.50±0.16	0.08±0.04	0.08±0.04	0.08±0.04	0.56±0.14
3	1.00±0.30	1.08±0.16	0.49±0.16	0.49±0.16	0.41±0.15	0.69±0.10
5	1.41±0.31	1.91±0.20	0.42±0.09	0.08±0.04	0.25±0.04	0.81±0.18
LSD	0.73^{ns}	0.60**	0.52*	0.54*	0.51**	0.34**

1.3. Ammonium acetate:

As shown in Table (3), there were significant differences between the ammonium chloride concentrations (As attractants for PFF adults) all over the tested period (15 days), except after 3 and 6 days of hanging traps. The concentration of 0.5% was superior, followed by the concentration of 2% (With no significant differences between their means overall for the tested period). The concentrations of 1, 3, and 5% of ammonium acetate ranked third as attractants for PFF adults in the mandarin orchard, with no significant differences between them.

Table (3): Efficiency of ammonium acetate concentrations (Con.) as attractants for *Bactrocera zonata* adults over 15 days in a mandarin orchard.

Con.	Flies/trap/day (FTD) after (In days)					Mean
	3	6	9	12	15	
0.5	0.25±0.15	0.41±0.08	1.08±0.25	1.00±0.13	0.91±0.08	0.73±0.09
1	0.17±0.09	0.25±0.08	0.08±0.04	0.17±0.09	0.33±0.13	0.20±0.04
2	0.41±0.08	0.66±0.13	0.25±0.15	0.17±0.04	1.08±0.25	0.51±0.09
3	0.17±0.09	0.25±0.08	0.25±0.15	0.17±0.06	0.41±0.08	0.32±0.04
5	0.08±0.04	0.25±0.04	0.58±0.25	0.49±0.09	0.08±0.04	0.29±0.06
LSD	0.32^{ns}	0.34^{ns}	0.57*	0.36***	0.46**	0.26**

1.4. Ammonium sulfate:

Statistically, there were no significant differences between the attractant of the tested ammonium sulfate concentrations after 3, 6, 12, and 15 days of hanging traps, as well as their means over the tested period (Table 4). On the other hand, the significant differences between concentrations were recorded after 9 days only, when the attractions of 0.5, 2, 3, and 5% decreased significantly (With no significant differences between them) in comparison with the concentration of 1 (Which recorded the first rank).

Table (4): Efficiency of ammonium sulfate concentrations (Con.) as attractants for *Bactrocera zonata* adults over 15 days in a mandarin orchard.

Con.	Flies/trap/day (FTD) after (In days)					Mean
	3	6	9	12	15	
0.5	0.25±±0.31	0.41±0.31	0.00±0.00	0.00±0.00	0.00±0.00	0.13±0.07
1	0.58±0.31	0.75±0.49	0.41±0.16	0.25±0.15	0.33±0.26	0.46±0.09
2	0.17±0.09	0.25±0.15	0.17±0.09	0.17±0.09	0.17±0.09	0.19±0.01
3	0.49±0.19	0.66±0.27	0.00±0.00	0.08±0.04	0.17±0.09	0.28±0.12
5	0.74±0.33	1.07±0.62	0.17±0.09	0.17±0.09	0.08±0.04	0.45±0.19
LSD	0.56^{ns}	0.64^{ns}	0.21**	0.35^{ns}	0.27^{ns}	0.28^{ns}

1.5. Thiourea and hydroxyl ammonium chloride:

Data represented in Table (5) showed that the efficiencies of thiourea and hydroxyl ammonium chloride (At the tested concentrations) were low throughout the tested period (15 days). Where the mean FTDs of PFF adults attracted to thiourea and hydroxyl ammonium chloride at all the tested concentrations over the tested period (15 days) were 0.02 and 0.01 flies, respectively.

Table (5): Mean Flies/trap/day (FTDs) of thiourea and hydroxyl ammonium chloride (HAC) concentrations as attractants for *Bactrocera zonata* adults over 15 days in a mandarin orchard.

Compound	Concentration					Mean
	0.5	1	2	3	5	
Thiourea	0.02±0.01	0.03±0.01	0.05±0.03	0.00±0.00	0.00±0.00	0.02±0.01
HAC	0.00±0.00	0.03±0.01	0.00±0.00	0.00±0.00	0.03±0.001	0.01±0.007

2. The relationship between ammonium compound concentrations and their attraction to *Bactrocera zonata* adults:

The correlation between the tested ammonium compounds (Ammonium hydroxide, ammonium chloride, ammonium acetate, and ammonium sulfate) concentrations and their efficiency as attractants to PFF was explained in Figure (1). It can be observed that as ammonium hydroxide concentration increased to 3.0%, its efficiency increased as well after that, the attractant to PFF decreased with the increase of its concentration. When the concentration of ammonium chloride increased to 5.0%, it also improved its efficiency. Furthermore, ammonium sulfate's efficiency increased in parallel with its concentration, reaching a range of 4.0 to 5.0%. The efficacy of ammonium acetate as an attractant to PFF in adults increased with its concentration until it reached 2.0%, at which point the attractant to PFF reduced as the concentration increased.

The statistical correlation between the ammonium compound concentration during the test and their efficiencies as attractants to PFF was represented as follows:

A. Hydroxide: $FTD = 0.61 + 0.34C - 0.05C^2$ ($R^2 = 0.72$)
A. Chloride: $FTD = 0.64 + 0.05C - 0.01C^2$ ($R^2 = 0.02$)
A. Acetate: $FTD = 0.32 + 0.17C - 0.04C^2$ ($R^2 = 0.41$)
A. Sulfate: $FTD = 0.08 + 0.14C - 0.14C^2$ ($R^2 = 0.68$)

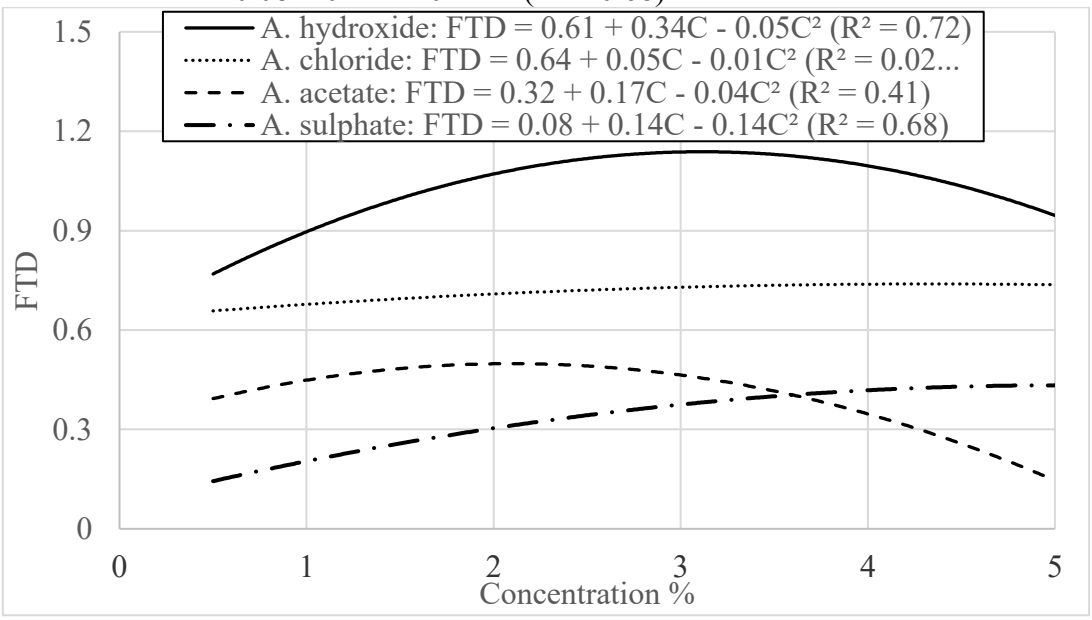

Figure (1): The correlation between the tested ammonium compounds' concentrations and how well they attract *Bactrocera zonata* adults in a mandarin orchard.

3. Effect of elapsed time on the attractancy of the tested ammonium compounds to *Bactrocera zonata* adults:

The efficiency of elapsed time on the attractancy of ammonium compounds (at different concentrations) to PFF adults is illustrated in Table (6). As shown in this table, all concentrations of ammonium acetate and 0.5% of ammonium hydroxide increased with the increase of elapsed time (As b-regression values were positive). For the rest of the treatments (Whether compounds or concentrations), their efficiencies as attractants to PFF males decreased with the increase of the elapsed time (As b-regression values were negative).

Table (6): Effect of elapsed time on the attractancy of the tested ammonium compounds towards *Bactrocera zonata* adults in a mandarin orchard.

Ammonium compound	Concentration of ammonium compounds									
	0.5%		1%		2%		3%		5%	
	b	R^2	B	R^2	B	R^2	b	R^2	b	R^2
Ammonium hydroxide	0.02	0.67	-0.03	0.18	-0.02	0.04	-0.04	0.28	0.05	0.19
Ammonium chloride	-0.05	0.83	0.00	0.00	-0.11	0.63	-0.06	0.77	-0.14	0.67
Ammonium acetate	0.06	0.65	0.01	0.16	0.03	0.13	0.002	0.003	0.01	0.03
Ammonium sulfate	-0.03	0.58	-0.03	0.62	-0.002	0.13	-0.041	0.47	-0.07	0.65

4. Enhancement of protein-based bait (Buminal) by ammonium compounds for attraction *Bactrocera zonata* adults:

As shown in Table (7), adding ammonium hydroxide, ammonium chloride, or ammonium acetate (With 1 or 2% concentrations) significantly increased Buminal 5%'s effectiveness as a PFF adult attractant. Adding 1% ammonium hydroxide was superior in enhancing the attractant of Buminal 5%, while adding 2% ammonium hydroxide and 1% ammonium chloride significantly ranked second, followed by 2% ammonium chloride and ammonium acetate 1% and 2, respectively. Concerning adding ammonium sulfate to Buminal 5%, there were no significant differences between them and the control treatment (Buminal 5).

Table (7): Improvement of protein-based bait (Buminal 5%) by adding 1 and 2% concentrations (Con.) of the effective ammonium compounds to attract *Bactrocera zonata* adults in comparison with the non-enhanced one over 15 days in a mandarin orchard.

Compound	Con.	Flies/trap/day (FTD) after (In days)					Mean
		3	6	9	12	15	
Ammonium hydroxide	1%	0.19±0.08	1.20±0.22	1.20±0.08	1.44±0.19	1.20±0.16	1.05±0.21
	2%	0.00±0.00	0.80±0.00	0.00±0.00	0.79±0.28	1.90±0.23	0.69±0.34
Ammonium chloride	1%	0.14±0.06	0.90±0.17	0.90±0.06	1.10±0.15	0.90±0.12	0.79±0.16
	2%	0.00±0.00	0.59±0.24	0.00±0.00	0.59±0.24	1.50±0.17	0.54±0.27
Ammonium acetate	1%	0.08±0.03	0.50±0.09	0.50±0.03	0.57±0.08	0.50±0.06	0.43±0.08
	2%	0.00±0.00	0.33±0.13	0.00±0.00	0.33±0.13	0.83±0.09	0.29±0.15
Ammonium sulfate	1%	0.00±0.00	0.33±0.13	0.08±0.04	0.08±0.04	0.42±0.15	0.18±0.08
	2%	0.00±0.00	0.50±0.06	0.00±0.00	0.17±0.09	0.30±0.08	0.19±0.09
Buminal 5%		0.00±0.00	0.08±0.04	0.08±0.08	0.17±0.09	0.00±0.00	0.07±0.03
LSD		0.14^{ns}	0.44***	0.21^{ns}	0.53***	0.52***	0.19***

5. Effect of time spent on the increased protein-based bait's ability to attract *Bactrocera zonata* adults (Buminal 5%):

Statistically, the efficiency of elapsed time on the attractancy of enhanced protein-based bait (Buminal 5) by adding ammonium hydroxide, ammonium chloride, and ammonium sulfate (At 1 and 2% concentrations) to PFF adults is illustrated in Table (8). As shown in this table, all enhanced treatments and control ones increased with the increase of elapsed time (As b-regression values were positive). The most affected treatment with the elapsed time was that enhanced with 2% ammonium sulfate (R^2 = 0.67), followed by the treatment enhanced with 2% ammonium hydroxide (R^2 = 0.59). The lowest affected treatments with the elapsed time were those of the treatment enhanced with 2% ammonium acetate and the control treatment (R^2-values were 0.04).

Table (8): Effect of elapsed time on the attractancy of the enhanced protein-based bait (Buminal 5%) towards *Bactrocera zonata* adults in comparison with a non-enhanced one over 15 days in a mandarin orchard.

Adding compound	Con.	b	R^2
Ammonium hydroxide	**1%**	0.07	0.53
	2%	0.12	0.59
Ammonium chloride	**1%**	0.06	0.53
	2%	0.09	0.52
Ammonium acetate	**1%**	0.02	0.26
	2%	0.01	0.04
Ammonium sulfate	**1%**	0.02	0.23
	2%	0.03	0.67
Control (Buminal 5%)		0.003	0.04

The data obtained revealed that ammonium hydroxide was the most effective in attracting PFF adults, followed by ammonium chloride, ammonium acetate, and ammonium sulfate, while thiourea and hydroxyl ammonium chloride showed no effects in attracting PFF adults. These findings agree with those of Abd El-Kareim *et al*. (2008), Hemeida *et al*. (2017), and Ghanim *et al.* (2021); they reported that ammonium acetate and ammonium hydroxide are the effective ammonium compounds in attracting PFF adults. Also, Ghanim *et al*. (2014) found that ammonium acetate and ammonium chloride showed obvious attraction to the zizyphus fruit fly, *C. incompleta*, in Christ's thorn orchards. Contrary to the present results, Abd El-Kareim *et al*. (2008) reported that ammonium acetate was more attractive to PFF adults than ammonium hydroxide. The variation between this study and the present one may be attributed to the variation of ecological factors, especially weather factors.

According to Bateman and Morton (1981) and Mazor (2009), increases in ammonia produced from protein-based baits (which included bacterial degradation) were correlated with increases in their efficiencies as attractants to adults of fruit flies. While Abd El-Kareim *et al*. (2008), Moustafa and Ghanim (2008), Ghanim *et al*. (2014), Hemeida *et al*. (2017), Ghanim *et al.* (2021), Ragab and Youssef (2021), Abd El-Salam and Youssef (2023), and Ghanim *et al*. (2023) reported that the highest attractive concentration of ammonium compounds varied according to the tested compound and fruit fly species. These findings supported the present study, where the high efficiency of ammonium hydroxide as an attractant to PFF adults was recorded at 3% concentration, while the highest attractant of ammonium acetate and ammonium chloride was recorded at 2 and 5%, respectively. The highest efficiency of ammonium sulfate as an attractant for PFF adults was recorded in the range between 4 and 5%. The lower attractiveness of the concentrations less than or more than the recorded highest attractive concentrations may be explained by Abd El-Salam and Youssef (2023) and Ghanim *et al*. (2023); they assumed that the lower efficiency of the concentrations less than the highest concentration in attracting PFF and the lower attractiveness of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), to their adults may be the cause of this; on the other hand, the fact that high concentrations are less effective than the highest concentration at attracting PFF and *C. capitata* adults

may be the result of their apparent relative repellency to their adults. Studies by Bateman and Morton (1981) and Mazor *et al*. (2002) may support these ideas; they noted that fruit flies' attraction level to ammonium compounds is dose-dependent, indicating that the range of repellence is much wider than the range of attractiveness.

The current study showed that, when applied alone, ammonium acetate (At doses of 1, 2, 3, and 5%) was the most effective therapy in terms of attracting PFF adults for 15 days in the field. Also, ammonium acetate (at the concentrations of 1 and 2%) used as an enhancer for the protein-based bait (Buminal) was the most stable treatment, while adding ammonium hydroxide (at the same concentrations) to Buminal tended to increase its attraction for PFF adults. These findings came in the same trends as Abd El-Kareim *et al*. (2008), Ghanim (2018), Ghanim *et al*. (2021), Abd El-Salam and Youssef (2023), Ghanim *et al*. (2023), and Hendawy *et al.* (2024). However, they stated that ammonium compounds' efficiencies (Which were used alone, mixed, or as enhancers for protein-based baits) in attracting adult PFF were either constant or tended to rise over time in the field.

Epsky *et al*. (2014) and Pinero *et al*. (2015) mentioned that females of fruit flies require sources of protein for consumption to mature their eggs, so this requirement may be the main cause for the strong female attraction to the protein-based baits. Fruit fly behavior and physiology were found to be significantly impacted by dietary sources high in nitrogen (Kaspi *et al*., 2000 and Yuval *et al*., 2007). According to Hemeida *et al.* (2017), Abd El-Salam and Youssef (2023), Ghanim *et al.* (2023), and Hendawy *et al.* (2024), protein-based baits that contain ammonia in their formulations have a comparatively high attractant for fruit fly adults due to the fact that ammonia attracts fruit flies. These findings supported the present study, which demonstrated that adding ammonium hydroxide, ammonium chloride, or ammonium acetate (With 1 or 2% concentrations) significantly increased the efficiency of Buminal 5% as an attractant to PFF adults, while adding ammonium sulfate to Buminal 5% did not differ significantly from the control treatment (Buminal 5 without additives). The findings of Hemeida *et al.* (2017), Ghanim (2018), Abd El-Salam and Youssef (2023), and Hendawy *et al.* (2024) are consistent with these findings; they discovered that adding ammonium compounds (Such as ammonium chloride and ammonium acetate) to different food attractants increases their attractiveness to PFF adults. Also, Pinero *et al*. (2011) mentioned that adding ammonium acetate to GF-120 (The insecticidal protein-based bait) increased its attractiveness to *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae).

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