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Improving the Efficiency of GF-120 Baits in Attracting *Bactrocera Zonata* by Adding Ammonium Compounds with Particular Emphasis on pH level

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Abstract

The peach fruit fly, *Bactrocera zonata* (Saunders) is a serious pest attacking a wide range of fruits. Field experiments were carried out, at Mansoura district, Dakahlia Governorate, Egypt to evaluate the efficiency of di- ammonium phosphate, ammonium carbonate and ammonium acetate in enhancing GF-120, as insecticidal bait, for *B. zonata* based on their pH level under high and low population levels of *B. zonata*. Results showed that di-ammonium phosphate enhanced the attractiveness of GF-120 the most, followed by ammonium carbonate and ammonium acetate. Without adding any of the ammonium compounds to the GF-120 bait, the bait attracted the fewest *B. zonata* flies regardless of population levels. As the concentrations of ammonium compounds increased, the pH-level increased as well in the prepared GF-120 solutions, resulting in increased numbers of *B. zonata* flies captured. In contrast to males, females of *B. zonata* were more responsive to increase concentrations of the three ammonium compounds tested. Accordingly, all treatments attracted females more than males. The sex ratio (as number of attracted females per one male) was generally higher under low than high fly population levels.

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Introduction

Phytophagous insects use a variety of sensory capabilities to orient and recognize the appropriate host plants [1]. Tephritid fruit flies (Diptera: Tephritidae) may use chemical stimuli in the form of nutrients to orient towards host plants [2]. Food sources that are rich in nitrogen have a strong influence on the physiology and behaviour of tephritid flies [3-4]. This behaviorally-based tactic targets female fruit flies primarily based on the female's need of protein for ovarian development and egg production [5-6].

Use of proteinaceous bait sprays (protein mixed with a toxicant) are one effective method for suppressing populations of many tephritid species [7,8,9]. Bait sprays mainly work on female fruit flies, which are strongly attracted to protein source from which ammonia emanate, causing flies to ingest a lethal dose of insecticide together with the protein [10-11]. Response of fruit flies to traps baited with synthetic lures versus those with liquid protein bait tends to be variable and host/population level may be the cause in such variation [5-12].

Baited insecticides are an attractive alternative to conventional pesticides that used in fruit fly control, in part because the environmental impact is reduced compared with broadcast foliar sprays. GF-120 [GF-120 NF Naturalyte® Fruit Fly Bait (Dow AgroSciences, Indianapolis, IN)] has been the most frequently tested bait against fruit flies [9,13,14]. It contains a much lower concentration of active ingredient than in broadcast spray formulations, such as in the unbaited SpinTor formulation of spinosad (0.2% compared with 22.8% in SpinTor) and it has a lower effect on non-target insects [7,13,15]. GF-120 contains 1% ammonium acetate (wt:vol) as an attractant [16]. Spinosad is an insecticide derived from fermentation products of the bacterium, *Saccharopolyspora spinosa* Mertz and Yao that has a high safety profile [17].

Bait sprays using GF-120 became the primary tool for covering a wide area and for suppressing the tephritid fruit fly populations [18,19,20]. Although protein baits do not attract flies from long distances, GF-120 apparently is attractive to some species and has been used to suppress larval infestations in many fruit orchards [21-22]. In contrast, some researchers reported that GF-120 is not highly attractive to some fruit flies (*i.e. Rhagoletis* flies) [14,18,23]. Accordingly, Pelz-Stelinski et al. [19] emphasized the need to improve the efficiency of GF-120 bait to be more attractive.

However, given the zero tolerance for fruit flies at fruit harvest, a higher level of control is required. Therefore, increasing attractiveness of GF-120 is required to increase its commercial use for attracting fruit flies. This increase would be expected to promote greater likelihood that flies ingest spinosad, thus improving the efficacy of this bait spray. On another hand, ammonia is associated with protein-rich foods and has long been known to attract fruit flies [24,25,26,27]. Yee and Landolt [28] found that increasing the concentration of ammonia in lures significantly increased their attraction to apple maggot fly, *Rhagoletis pomonella* (Walsh). Indeed, adding more ammonium acetate to GF-120 enhanced its attractiveness to some eastern fruit fly, *Rhagoletis cingulata* (Loew) [19]. On the other hand, pH-level of the baits plays a fundamental role in attracting fruit flies, since the effectiveness of bait is diminished as the pH-level decreased [29,30,31,32,33]. This information therefore could be used in chemical analysis for identification of new attractants from preferred bait formulations.

In Egypt, the peach fruit fly, *Bactrocera zonata* (Saunders), is a serious pest attacking a wide range of fruits that differ in their ripening time stage during the year. It is a polyphagus insect attacking more than 50 species of fruit and vegetable crops as well as wild host plants [34]. It causes 25-50% damage to fruits particularly in the summer season [35]. This fly could be effectively controlled using GF-120 enhanced with ammonium compounds, but the attractiveness of these baits needs to be determined.

The purposes of this study were to 1) enhance the ability of GF-120 to attract *B. zonata* by adding di- ammonium phosphate, ammonium carbonate and ammonium acetate, and to 2) study the relationship between pH-level and captures of *B. zonata* flies on traps.

Materials and Methods

Materials and Treatments

The GF-120 (Conserve 0.024% CB) was obtained from Dow AgroSciences, England, whereas di-ammonium phosphate ($(NH_4)_2\ HPO_4$), ammonium carbonate ($(NH_4)_2\ CO_3$) and ammonium acetate (CH_3COONH_4) were received from El-Naser for Drugs and Chemicals Company. Each of the three ammonium compounds was added to a 5% GF-120 solution (vol/vol) at 0.5, 1.0, 2.0 and 3.0% (wt/vol). All treatments (GF-120 + compound) were compared with a GF-120 bait only control.

Field trials

To evaluate the efficiency of di-ammonium phosphate, ammonium carbonate and ammonium acetate in enhancing the GF-120, as a bait for *B. zonata*, experiments were conducted in guava, *Psidium guajava* L. (as high *B. zonata* population level; whereas mean flies trapped per day values (FTDs) ranged between 1.75 and 10.33) and navel orange, *Citrus sinensis* L. (as low population level; whereas mean FTDs ranged between 0.44 and 2.40) orchards located in the experimental farm of Mansoura University, Dakahlia governorate, Egypt. The cultivated areas were about seven feddans for guava and eight feddans for navel orange (1 feddan = 4200 m^2). Experiments were carried out from 9-20 October 2017 in guava orchard and from 20-31 October 2017 in navel orange orchard.

Following the approach of Yee [36] to determine the effects of adding ammonium compounds to GF-120 on attraction of *R. pomonella* in Washington State using sticky yellow panel traps, this study used the modified Nadel traps [37] that baited with the GF-120 mixed with varying concentrations of ammonium compounds in capturing *B. zontata* flies. Each treatment consisted of 250 milliliters installed in a trap and replicated four times. Traps were distributed inside each orchard (guava or navel orange) in a completely randomized design. Traps were hanged at a height of 1.5-2.0 meters above the ground in the shade under trees. The distance between every two adjacent traps was about 20 meters to avoid the interaction between lures.

Traps were inspected every two days over 12 days after hanging for flies. Captured female and male flies were counted and recorded as FTDs. The captured flies were removed from traps with no renewal of the bait solution. To reduce trap position effects (*i.e.*, due to light, wind, heat, and other factors), traps were rotated at every inspection.

Estimating pH Levels

Fifty milliliters of each treatment were transferred to laboratory for estimating pH level. Samples were taken when preparing the treatments (as fresh bait) and at the end of experiment in guava and navel orange treatments. These samples were measured by Jenway 3510 pH meter.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by least significant difference (LSD) at probability level of 0.05. Regression analysis was also performed between concentration of ammonium compounds and pH in addition; regression analysis was performed between each of concentration and pH and FTDs. All analyses were performed using CoHort Software [38].

Results

By Adding Di-Ammonium Phosphate

Adding di-ammonium phosphate at the concentration of 2.0% to GF-120 resulted in the highest captures of *B. zonata* flies under high population (mean FTD was 10.33±0.59; $F = 77.78$ and $P < 0.01$). Under low population, adding this compound at the concentration of 1.0% resulted in the highest captures (FTD = 1.67±0.40; $F = 10.80$ and $P < 0.01$). In contrast, the GF-120 alone control caught the fewest *B. zonata* flies under high (FTD = 1.75±0.38) or low (0.44±0.14) populations. The attraction to traps baited with 1.0, 2.0 and 3.0% di-ammonium phosphate decreased two days after trap deployment (Table 1).

The highest attraction of *B. zonata* flies was recorded when pH level of the fresh bait was relatively high (more than 7.00). On the other hand, pH-levels were lower at the low than high population experiment (Table 1).

There was a positive relationship between concentration of di-ammonium phosphate and the resulted pH level in the prepared solution. By the end of experiment, each increase in the concentration of di-ammonium phosphate by one percent increased pH by 0.88, 0.71 and 0.86 degrees in fresh bait, under high population level and low population level, respectively (Figure 1).

The attracted *B. zonata* flies (females, males or total numbers) were more affected by pH levels in comparison with concentration of di-ammonium phosphate. The determination coefficient values (R^2) were generally higher in pH compared with those in concentrations of di-ammonium phosphate (Table 2).

On the other hand, *B. zonata* females were more responsive to the increase of pH level and concentration percentages of di-ammonium phosphate than males. Under high population level, each increase of pH level in fresh baits by one degree increased the attracted females and males (as FTDs) by 1.58 and 0.71. By the end of the experiment, each increase of pH level increased FTDs of females and males by 2.37 and 1.03. With respect to the concentration of di-ammonium phosphate, each increase by one percent increased the attracted females and males by 1.54 and 0.57 (Table 2).

Under low population level, each increase of pH level in fresh baits increased the attracted females and males by 0.16 and 0.05 FTDs; in the cases of pH at the end of experiment and concentration percentages, these relations were very weak (Table 2).

All of the tested treatments attracted more females than males of *B. zonata*. The highest number of females per one male was recorded when adding di-ammonium phosphate with the concentration of 3.0% under high population (2.62 females; *F = 7.63 and P <0.01*) and with the concentration of 1.0% under low population (3.74 females; *F = 23.52 and P <0.01*). In contrast, the lowest number of females per one male was recorded with the concentration of 0.5% under high (1.42 females) and low (1.37 females) populations. On the other hand, the number of attracted females per one male was generally high under low population in comparison with high population (Figure 2).

By Adding Ammonium Carbonate

Under high population level, adding ammonium carbonate with the concentration of 3.0% to GF-120 resulted in the highest number of *B. zonata* fly captured (mean FTD was 8.43±0.42; *F = 45.06 and P <0.01*); under low population, adding this compound at the concentration of 1.0% attracted the highest number of flies (FTD = 1.50±0.18; *F = 11.84 and P <0.01*). The GF-120 control attracted the fewest *B. zonata* flies under high and low population levels (Table 3).

The highest number of attracted *B. zonata* flies was recorded at 8.01 and 7.45 pH of the fresh bait. Also, the highest number of attracted flies was recorded at high pH levels at the end of experiment under high and low population levels; however, pH levels were 6.62 and 6.83 (Table 3).

Each increase in the concentration of ammonium carbonate by one percent increased pH by 0.94 degrees in the fresh solution, and increased it by 0.79 and 0.75 in the baits at the end of experiment under high and low population levels (Figure 3).

As in di-ammonium phosphate, the attracted *B. zonata* flies (females, males or total numbers) were more affected by pH level in comparison with concentration of ammonium carbonate; the determination coefficient values (R^2) were generally higher in pH-degrees compared with those in ammonium concentrations (Table 4).

Also, *B. zonata* females were more responsive to the increase of pH level and concentration percentage of ammonium carbonate in comparison with males (Table 4). The b-values in the mathematical relationship between pH levels and concentration percentages of ammonium carbonate and FTD were higher in the case of females in comparison with males in all cases.

The highest number of attracted females per one male was recorded with GF-120 alone under high population (2.13 females; *F = 1.19 and P = 0.357*) and when adding ammonium carbonate to the insecticidal bait with the concentration of 2.0% under low population (7.07 females; *F = 71.45 and P <0.01*). In contrast, the lowest number of attracted females per one male was recorded with the concentration of 1.0% under high population (1.49 females with no significant differences between concentrations) and with the concentration of 0.5% under low population (1.35 females). The number of attracted females per one male was generally higher under low population levels than under high population levels (Figure 4).

By Adding Ammonium Acetate

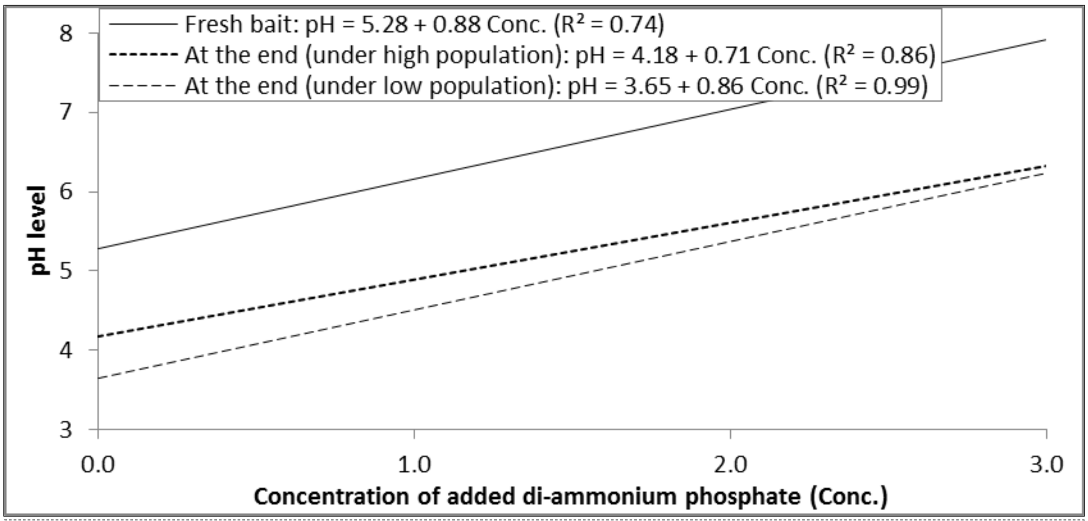

Figure 1. Relationship between concentrations of di-ammonium phosphate added to GF-120 and pH levels in fresh bait or at the end of experiment (under high and low levels of *B. zonata* population).

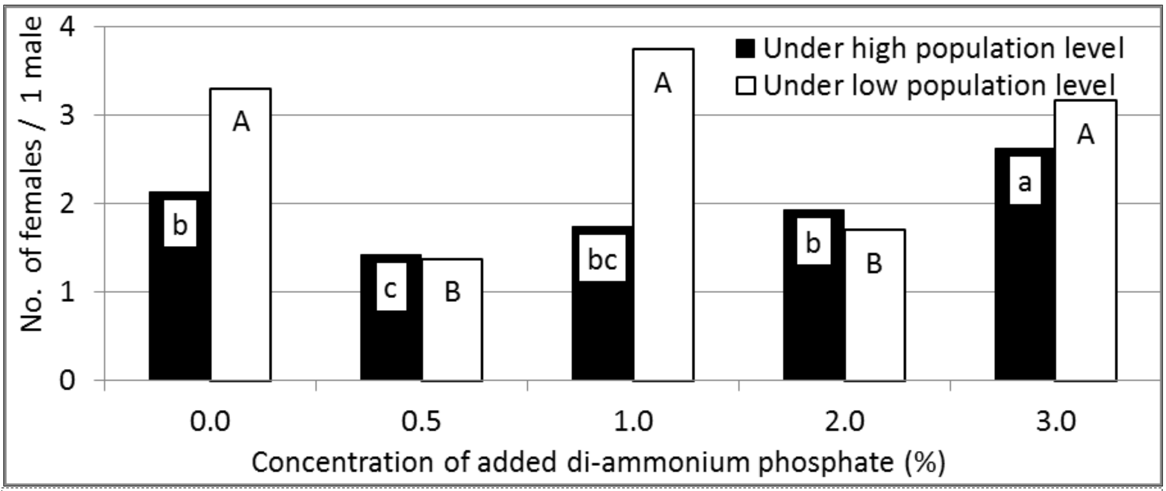

Figure 2. Sex ratio (as No. of females/ 1male) of attracted *B. zonata* flies to GF-120 enhanced by di-ammonium phosphate at different concentrations under two levels (high and low) of pest population (In each population level; means have the same letter did not differ significantly at the probability of 0.05).

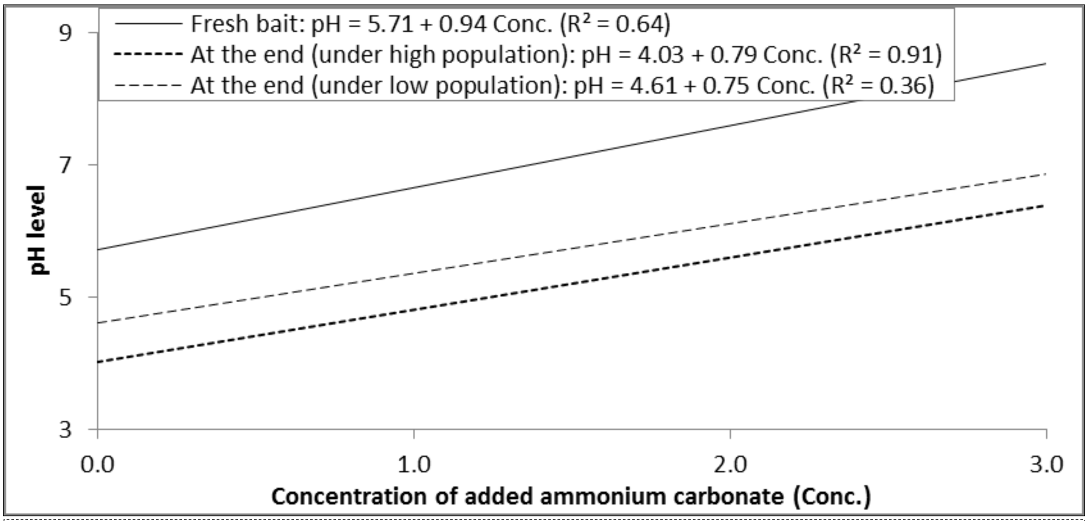

Figure 3. Relationship between concentrations of ammonium carbonate added to GF-120 and pH levels in fresh bait or at the end of experiment (under high and low levels of *B. zonata* population).

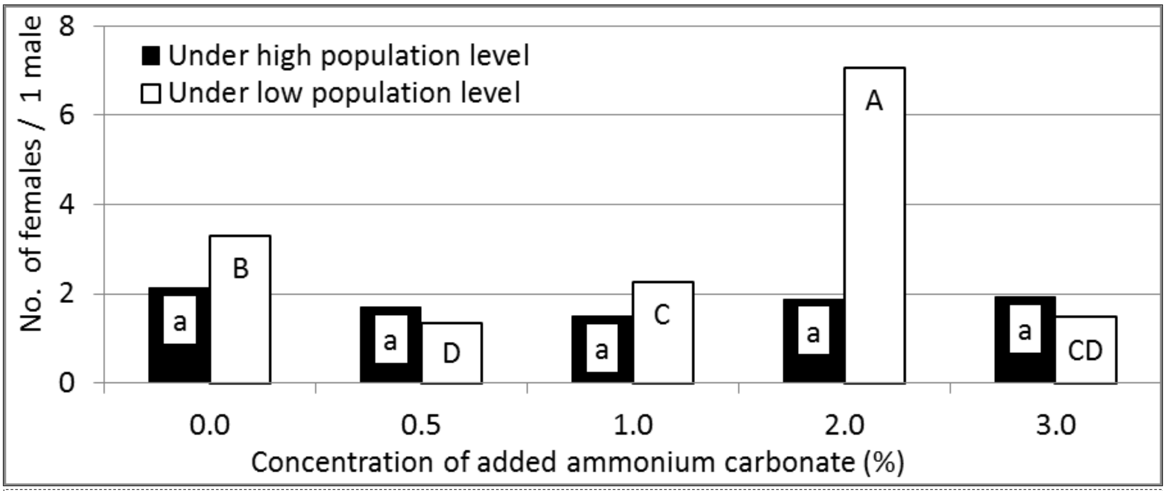

Figure 4. Sex ratio (as No. of females/ 1male) of attracted *B. zonata* flies to GF-120 enhanced by ammonium carbonate at different concentrations under two levels (high and low) of pest population (In each population level; means have the same letter did not differ significantly at the probability of 0.05).

Table 1. Mean number (±SD) of attracted *B. zonata* adults to GF-120 enhanced by di-ammonium phosphate at different concentrations under two levels (high and low) of pest population in relation to pH-level.

Conc. %	FTD after (in days)							pH	
	2	4	6	8	10	12	Mean	Fresh bait	At the end
Under high population level									
0.0	0.75±0.50c	0.88±0.48d	2.13±0.48c	2.25±0.29c	1.75±0.95b	2.75±0.29b	1.75±0.38c	4.51	3.92
0.5	1.63±0.48c	3.50±0.41c	3.75±0.65b	3.63±1.44b	2.75±1.55b	1.13±0.63c	2.73±0.79c	5.89	4.58
1.0	16.88±2.29b	9.25±1.19ab	4.25±1.19b	3.75±0.87b	1.50±1.08b	0.75±0.64c	6.06±0.66b	7.04	4.98
2.0	22.75±2.66a	9.75±1.32a	5.88±0.75a	7.38±0.25a	7.25±0.50a	9.00±1.22a	10.33±0.59a	7.18	6.12
3.0	23.25±3.28a	7.88±1.31b	2.38±0.63c	2.00±1.08c	1.63±0.75b	2.63±1.25b	6.63±1.20b	7.50	5.94
Under low population level									
0.0	0.13±0.25c	0.00±0.00d	1.63±0.48c	0.63±0.48a	0.00±0.00a	0.25±0.29b	0.44±0.14c	4.51	3.69
0.5	0.38±0.48c	0.38±0.25d	3.00±0.41b	0.25±0.50a	0.50±0.41a	0.88±0.48a	0.90±0.25b	5.89	4.02
1.0	1.75±0.71a	2.13±0.85a	4.38±0.63a	0.88±0.85a	0.63±0.48a	0.25±0.29b	1.67±0.40a	7.04	4.60
2.0	1.13±0.25ab	1.38±0.63bc	1.63±0.25c	0.75±0.64a	0.50±0.68a	0.00±0.00b	0.90±0.22b	7.18	5.22
3.0	0.75±0.50bc	1.13±0.25c	1.25±0.50c	0.63±0.48a	0.75±0.96a	0.25±0.29b	0.79±0.28bc	7.50	6.32

In each population level; means have the same letter in the same column did not differ significantly at the probability of 0.05.

Table 2. Relationships between attracted *B. zonata* flies (as FTD) and each of pH level (in fresh bait and at the end of experiment) and concentrations of di-ammonium phosphate added to GF-120 under two levels (high and low) of pest population.

Factor		Sex	At high population level		At low population level	
			Relationship	R^2	Relationship	R^2
pH level	Fresh bait	Females	FTD = -6.47 + 1.58 pH	0.70	FTD = -0.36 + 0.16 pH	0.27
		Males	FTD = -2.73 + 0.71 pH	0.60	FTD = -0.02 + 0.05 pH	0.22
		Total	FTD = -9.21 + 2.29 pH	0.68	FTD = -0.37 + 0.20 pH	0.31
	At the end of experiment	Females	FTD = -8.48 + 2.37 pH	0.89	FTD = 0.43 + 0.05 pH	0.02
		Males	FTD = -3.43 + 1.03 pH	0.72	FTD = 0.28 - 0.002 pH	0.0004
		Total	FTD = -11.92 + 3.41 pH	0.85	FTD = 0.73 + 0.04 pH	0.01
Concentration		Females	FTD = 1.65 + 1.54 pH	0.64	FTD = 0.62 + 0.03 pH	0.01
		Males	FTD = 1.11 + 0.57 pH	0.36	FTD = 0.27 + 0.003 pH	0.001
		Total	FTD = 2.76 + 2.11 Conc.	0.55	FTD = 0.90 + 0.03 Conc.	0.01

Table 3. Mean number (±SD) of attracted *B. zonata* adults to GF-120 enhanced by ammonium carbonate at different concentrations under two levels (high and low) of pest population in relation to pH level.

Conc. %	FTD after (in days)							pH	
	2	4	6	8	10	12	Mean	Fresh bait	At the end
Under high population level									
0.0	0.75±0.50c	0.88±0.48d	2.13±0.48d	2.25±0.29c	1.75±0.95b	2.75±0.29b	1.75±0.38c	4.51	3.92
0.5	23.50±2.65a	9.63±0.95a	4.00±0.71cd	2.13±1.55c	0.25±0.50c	0.38±0.25c	6.65±0.80ab	6.90	4.63
1.0	4.75±0.50b	5.38±1.31b	4.88±1.18bc	2.75±0.50c	1.38±0.85bc	0.75±0.29c	3.31±0.68bc	7.45	4.96
2.0	5.50±0.71b	5.38±1.31b	9.00±3.48a	6.25±1.76b	1.63±0.63b	1.38±0.48c	4.85±1.29b	7.80	5.13
3.0	5.38±1.11b	3.38±0.48c	6.88±0.75ab	8.95±0.71a	9.63±0.95a	16.38±1.75a	8.43±0.42a	8.01	6.62
Under low population level									
0.0	0.13±0.25ab	0.00±0.00c	1.63±0.48bc	0.63±0.48cd	0.00±0.00c	0.25±0.29a	0.44±0.14b	4.51	3.69
0.5	0.63±0.48a	0.75±0.29a	1.38±1.11c	1.25±0.65bc	0.25±0.29bc	0.13±0.25a	0.73±0.40b	6.90	4.48
1.0	0.50±0.58ab	0.38±0.25b	7.50±0.91a	0.13±0.25d	0.25±0.50bc	0.25±0.29a	1.50±0.18a	7.45	6.83
2.0	0.00±0.00b	0.00±0.00c	2.50±0.58b	1.63±0.25ab	3.00±0.71a	0.13±0.25a	1.21±0.26a	7.80	7.21
3.0	0.00±0.00b	0.00±0.00c	0.25±0.29d	2.13±0.95a	0.75±0.50b	0.00±0.00a	0.52±0.27b	8.01	5.72

Table 4. Relationships between attracted *B. zonata* flies (as FTD) and each of pH level (in fresh bait and at the end of experiment) and concentrations of ammonium carbonate added to GF-120 under two levels (high and low) of pest population.

Factor		Sex	At high population level		At low population level	
			Relationship	R^2	Relationship	R^2
pH level	Fresh bait	Females	FTD = -2.56 + 0.83 pH	0.47	FTD = -0.21 + 0.12 pH	0.20
		Males	FTD = -1.50 + 0.47 pH	0.53	FTD = -0.05 + 0.04 pH	0.18
		Total	FTD = -4.05 + 1.31 pH	0.49	FTD = -0.23 + 0.16 pH	0.25
	At the end of experiment	Females	FTD = -4.03 + 1.43 pH	0.67	FTD = -0.59 + 0.22 pH	0.73
		Males	FTD = -1.88 + 0.73 pH	0.61	FTD = 0.05 + 0.03 pH	0.13
		Total	FTD = -5.93 + 2.16 pH	0.66	FTD = -0.52 + 0.25 pH	0.67
Concentration		Females	FTD = 1.84 + 1.06 pH	0.54	FTD = 0.59 + 0.03 pH	0.01
		Males	FTD = 1.12 + 0.51 pH	0.46	FTD = 0.26 – 0.01 pH	0.006
		Total	FTD = 2.95 + 1.58 Conc.	0.52	FTD = 0.85 + 0.02 Conc.	0.003

Ammonium acetate at 0.5 and 3.0% in GF-120 were the most effective treatments for attracting *B. zonata* flies under high population level (mean FTDs were 4.33±0.44 and 4.23±0.87; *F = 26.17 and P <0.01*). Under low population, adding this compound at 3.0% attracted the highest number of flies (FTD = 2.40±0.24; *F = 72.95 and P <0.01*). In contrast, GF-120 alone and GF-120 with 1.0% ammonium acetate attracted the fewest *B. zonata* flies under high (1.75±0.38 and 1.65±0.31) or low (0.44±0.14 and 0.50±0.18) population levels (Table 5).

On the other hand, the highest numbers of attracted *B. zonata* flies were coincided with the high pH level of the fresh bait (pH = 6.02) and at the end of experiment under high and low population levels (pH = 5.23 and 5.16). Generally, pH levels decreased under low population more than that under high population (Table 5).

As with the other two ammonium compounds, there was a positive relationship between concentration of ammonium acetate and the resulted pH level in the prepared solution. Statistically, each increase in the concentration of ammonium acetate by one percent increased pH by 0.45, 0.37 and 0.44 degrees in fresh bait and at the end of experiments under high and low population levels, respectively (Figure 5).

B. zonata flies under high population level were generally more affected by pH level in comparison with concentration of ammonium acetate (Table 6); the determination coefficient values (R^2) were generally higher in pH levels compared with those in concentrations of di-ammonium phosphate. In contrast, the attracted *B. zonata* flies under low population level were generally more affected by concentration of ammonium acetate in comparison with pH levels.

On the other hand, *B. zonata* females were more responsive than males to the increase of pH levels and concentration percentages of ammonium acetate. This is explained by the b-values in the mathematical relationship between each of pH levels and concentration percentages of ammonium acetate and FTDs; which were higher in the case of females than males in all cases (Table 6).

The highest number of attracted females per one male was recorded when adding 1.0% ammonium acetate to GF-120 under high population (3.13 females; *F = 4.27 and P = 0.012*) and with GF-120 alone under low population (3.30 females; *F = 21.88 and P <0.01*). In contrast, the lowest number of attracted females per one male was recorded with the concentration of 2.0% under high population and when adding ammonium acetate with the concentration of 1.0% under low population; however, number of females was 1.69 and 0.61, respectively (Figure 6).

Discussion

It is likely that the ammonia emission rate is a significant determinant for the efficacy of candidate products, which is supported by the significantly higher catches of flies [39]. Results here suggest that a change to GF-120 compassion is warranted. According to Yee [36], addition of ammonium carbonate and ammonium acetate enhanced the ability of GF-120 to attract *R. pomonella* under field conditions. Similarly, in this study, the ability of GF-120 bait to attract *B. zonata* flies was enhanced by adding di-ammonium phosphate, ammonium carbonate or ammonium acetate to the bait. The addition of these ammonium compounds (especially with concentrations of 3 and 4%) to the bait significantly attracted more *B. zonata* flies than bait alone. Pelz et al. [18] and Pelz-Stelinski et al. [19] found that fruit flies spent more time around GF-120 bait with additional ammonium acetate than GF-120 without it. They suggest that higher levels of ammonium acetate in GF-120 can increase arrestment of foraging flies. In another study, adding ammonium acetate to protein baits potentially increased the bait's efficacy in attracting, monitoring and control of *Ceratitis capitata* (Wiedemann) [5]. The current results are further confirmed by El-Metwally [40] who reported that addition of di-ammonium phosphate, ammonium carbonate and ammonium acetate to GF-120 bait improved its ability to attract *C. capitata* flies.

Among the ammonium compounds tested, di-ammonium phosphate improved the ability of GF-120 to attractive *B. zonata* flies the most, followed by ammonium carbonate and ammonium acetate. The same results were obtained for *C. capitata* [40]. Furthermore, Hemeida et al. [41] reported that di-ammonium phosphate was more effective in enhancing the ability of protein-biased baits (Buminal,

Table 5. Mean number (±SD) of attracted *B. zonata* adults to GF-120 enhanced by ammonium acetate at different concentrations under two levels (high and low) of pest population in relation to pH-degree.

Conc. (%)	FTD after (in days)							pH	
	2	4	6	8	10	12	Mean	Fresh bait	At the end
Under high population level									
0.0	0.75±0.50b	0.88±0.48c	2.13±0.48c	2.25±0.29c	1.75±0.95bc	2.75±0.29c	1.75±0.38c	4.51	3.92
0.5	1.38±0.63b	8.13±2.93a	7.50±1.29a	5.38±1.31a	2.75±1.19b	0.88±0.48d	4.33±0.44a	5.18	4.47
1.0	0.88±0.25b	2.38±0.63bc	3.38±1.18bc	2.13±0.48c	0.75±0.29c	0.38±0.25d	1.65±0.31c	5.48	4.67
2.0	3.13±0.85a	3.00±0.58bc	3.50±0.41bc	3.50±1.08b	3.13±1.03b	4.25±0.50a	3.42±0.42b	5.77	4.76
3.0	4.13±1.31a	4.00±1.08b	3.75±1.04b	5.13±0.63a	4.88±1.25a	3.50±0.71b	4.23±0.87a	6.02	5.23
Under low population level									
0.0	0.13±0.25	0.00±0.00	1.63±0.48	0.63±0.48	0.00±0.00	0.25±0.29	0.44±0.14	4.51	3.69
0.5	0.75±0.29	0.50±0.41	1.25±0.50	1.00±0.41	0.38±0.25	0.25±0.29	0.69±0.08	5.18	4.33
1.0	0.00±0.00	0.00±0.00	2.13±0.48	0.50±0.41	0.13±0.25	0.25±0.29	0.50±0.18	5.48	4.53
2.0	0.25±0.29	0.25±0.29	4.38±0.75	0.38±0.48	0.25±0.29	0.75±0.29	1.04±0.25	5.77	4.82
3.0	0.13±0.25	0.00±0.00	11.00±1.08	1.75±0.29	0.63±0.63	0.88±0.48	2.40±0.24	6.02	5.16

Table 6. Relationships between attracted *B. zonata* flies (as FTD) and each of pH level (in fresh bait and at the end of experiment) and concentration of ammonium acetate added to GF-120 under two levels (high and low) of pest population.

Factor		Sex	At high population level		At low population level	
			Relationship	R^2	Relationship	R^2
pH level	Fresh bait	Females	FTD = -2.166 + 0.78 pH	0.28	FTD = -3.19 + 0.72 pH	0.44
		Males	FTD = -1.43 + 0.45 pH	0.30	FTD = -1.38 + 0.32 pH	0.87
		Total	FTD = -3.36 + 1.19 pH	0.29	FTD = -4.54 + 1.03 pH	0.55
	At the end of experiment	Females	FTD = -2.79 + 1.05 pH	0.34	FTD = -3.05 + 0.83 pH	0.52
		Males	FTD = -1.58 + 0.56 pH	0.31	FTD = -1.21 + 0.34 pH	0.90
		Total	FTD = -4.15 + 1.57 pH	0.33	FTD = -4.24 + 1.17 pH	0.64
Concentration		Females	FTD = 1.60 + 0.36 Conc.	0.25	FTD = 0.11 + 0.45 Conc.	0.71
		Males	FTD = 0.70 + 0.23 Conc.	0.34	FTD = 0.10 + 0.16 Conc.	0.98
		Total	FTD = 2.32 + 0.58 Conc.	0.28	FTD = 0.22 + 0.61 Conc.	0.82

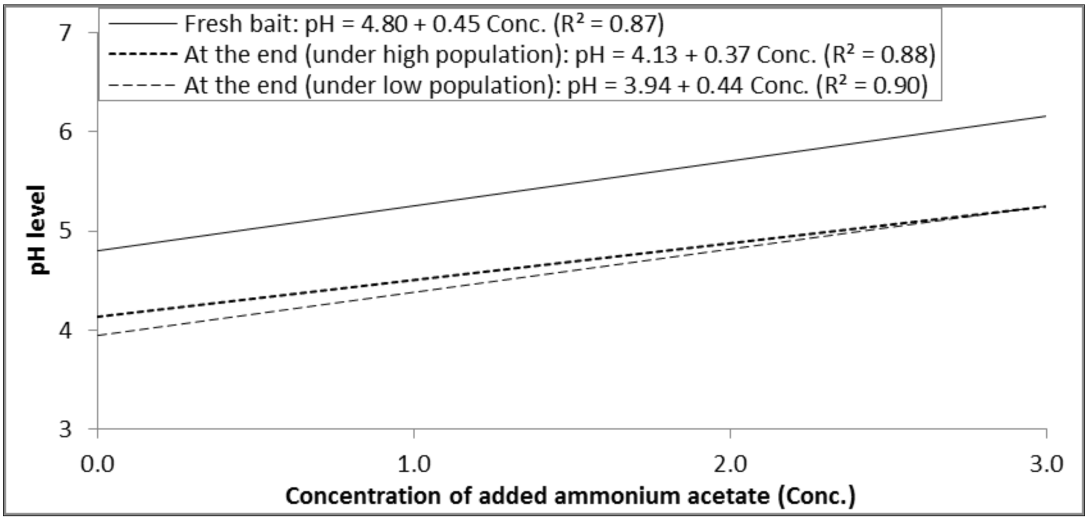

Figure 5. Relationship between concentrations of ammonium acetate added to GF-120 and pH levels in fresh bait or at the end of experiment (under high and low levels of *B. zonata* population).

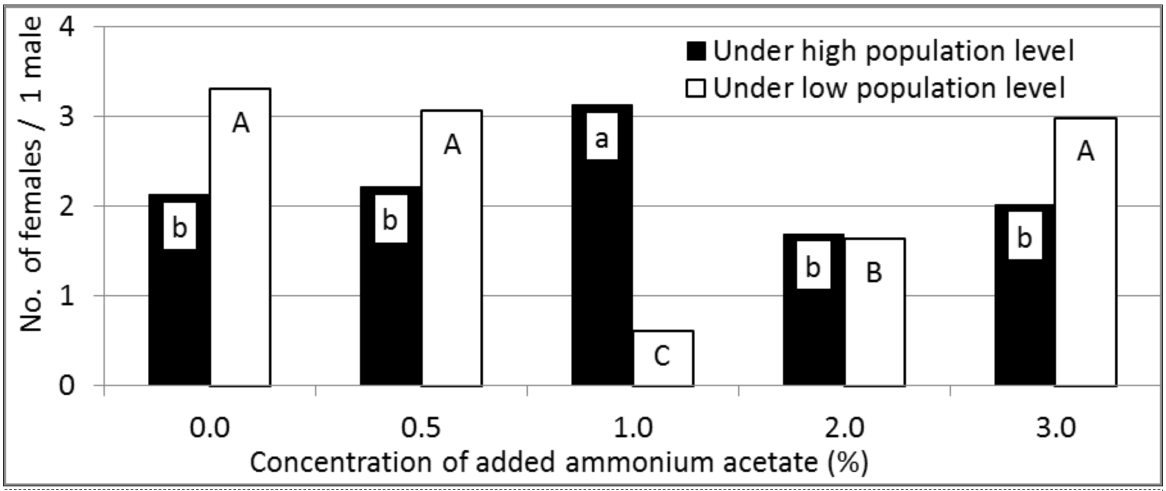

Figure 6. Sex ratio (as No. of females/ 1male) of attracted *B. zonata* flies to GF-120 enhanced by ammonium acetate at different concentrations under two levels (high and low) of pest population (In each population level; means have the same letter did not differ significantly at the probability of 0.05)

Agrinal and Amadine) to attract *B. zonata* flies than ammonium acetate. In contrast, attractiveness of *R. cingulata* flies to GF-120 bait increased as concentrations of ammonium acetate increased compared to di-ammonium phosphate [19]. But ammonium carbonate was more effective in improving the GF-120 bait to attract *R. pomonella* than ammonium acetate [36]. This variation may be specific to fruit fly species or compound concentrations. Yee and Landolt [28] found that captures of *R. pomonella* flies in traps increased as concentrations of ammonia in lures increased from 0 to 29.3%. Limited improvement in attractiveness to *R. pomonella* also was reported for ammonium bicarbonate added to the protein hydrolysate bait, NuLure [42].

Results showed that attractiveness of highly concentrated treatments (especially the highest concentrations of di-ammonium phosphate) decreased after two days of hanging traps. This was explained by Yee [36] who documented that the lack of differences between GF-120 alone and GF-120 enhanced by ammonium carbonate or ammonium acetate was likely due to ammonia release rates from enhanced drops decreased quickly after sprays, so after a few days or even less time the enhanced GF-120 was the same as GF-120 alone in attractiveness. Ammonium acetate dissipates rapidly because of its high volatility [9]. This volatilization is likely the cause of the loss of GF-120 attractiveness to the melon fly, *Bactrocera curcurbitae* Coquillett, observed within the first day after application. Further, the present results are consistent with the trends observed by Epsky et al. [43-44]. They mentioned that volatile attractant chemicals from Nulure/borax solution may be released at levels too high for effective fly capture when freshly prepared but are too low to have an effect on fly capture by 4-5 days after preparation. Vargas et al. [13] noted that aged baits (4 days-old) were as not attractive to *C. capitata* as were fresh baits.

The ability of GF-120 to attract *B. zonata* was found to be basically dependent on the ammonium compound, the concentrations, and pH with positive relationships. The degree of pH was positively affected by the concentration of added ammonium compound to GF-120. For example, each increase in the concentration of di-ammonium phosphate by one percent increased pH by 0.88, 0.71 and 0.86 degrees in fresh bait and at the end of treatment under high and low populations, respectively. These results are consistent with those of Bateman and Morton [45], Heath et al. [46], El-Gendy [32] and El-Metwally [40]; they mentioned that the attracted fruit flies to ammonium compounds, food baits and GF-120 (that enhanced by ammonium compounds) were found to be strongly dependent on concentration of ammonium compound and therefore on pH level. Further, there were positive relationships between pH level of some protein-based baits and the attracted flies of *B. zonata* and *C. capitata* [31]. El-Gendy [32] added that borax makes the solution of ammonium compounds more alkaline and therefore increases release of ammonia from the bait solution (which increased the attracted *B. zonata* flies). Also, addition of 1-10% borax to 10% Nulure solution (as food attractant) increased pH level, resulting in increased captures of *Anastrepha suspensa* (Loew) and *C. capitata* in traps under field conditions [29-43]. Mazor et al. [47] mentioned that the elevation of the pH of the liquid commercial baits, Buminal and Naziman, increased the efficacy of the latter for *C. capitata* but the increased stimulation could not be strictly correlated with the increased rate of ammonia release. The highest captures of *C. capitata* in McPhail traps occurred with Milhocina and borax adjusted to a pH of 8.5 [33]. In the present study, the highest attracted *B. zonata* flies were recorded when pH more than 7.00. On the other hand, *B. zonata* females were more responsive to the increase of pH and concentrations of ammonium compounds than males. Similar results were obtained by El-Metwally [40], who mentioned that females of *C. capitata* were more responded to the increase of pH in GF-120 preparations.

At the end of experiments, pH level decreased under high and low population levels; which resulted in decreased fly captures. These results are in agreement with El-Gendy [31], who mentioned that the properties of protein bait solutions in traps were changed along the elapsed time. Heath et al. [46] added that resultant pH affected by factors such as age of bait solution increased attraction of flies. According to the present study it may be affected by ecological factor such as air temperature degrees, relative humidity and/or population level of the attracted flies. Also, many authors showed through regression analysis that attraction of some ammonium compounds and food attractants decreased by time after treatment [24,25,26,48]. Epsky et al. [44] and El-Gendy [32] mentioned that there were no significant differences in pH levels of food baits and ammonium compounds over the time period of the test. Climatic and habitat differences may explain the inconsistency between the present results and others.

According to the relationships between concentrations of ammonium compounds and the resulted pH in the prepared solutions, there were positive relationships between them. The degrees of pH were more affected by the concentrations of ammonium compounds in fresh baits compared to those in baits at the end of experiment under field conditions. This may be attributed to the effects of ecological factors on the baits in the field. At the end of experiment under field conditions, pH varied from high to low pest population levels; whereas, the general pH-levels were higher in the high than low population experiment. This may be attributed to the high numbers of dead flies in traps in the high population experiment, which may have changed the chemical composition of the bait and therefore modified its pH level and therefore its attraction. This theory is agreement with that of Rousse et al. [30], who documented that the chemical composition of the bait may change because of dead flies, increasing its attraction to fruit flies, while living flies might emit attractive or repulsive volatiles.

The obtained results showed that GF-120 alone or enhanced by ammonium compounds attracted females more than males. These results are in agreement with those obtained by Yee [36] who reported that females of *R. pomonella* were more responsive to the GF-120 lures on traps than males. He added that the lures affected the sexes similarly in terms of relative responses. Also, many studies reported that ammonium compounds and protein-biased baits attracted more female than male flies [24,25,26,29,31,32,40,41,43,49]. Rousse et al. [30] showed that numerically more females than males of *B. cucurbitae* were caught in traps baited with food attractants especially when they were protein starved. Because fruit flies require a source of protein to complete egg maturation [50] this requirement is probably the main cause for the strong attraction of females towards decomposing proteinaceous substances [51].

The present results showed that the sex ratios of *B. zonata* (as number of attracted females per one male) obtained by GF-120 alone or enhanced by ammonium compounds were higher under low population level in comparison with high population level. This may be explained as follows: data in the high population level experiment were obtained in guava orchard at the end of the fruit ripening period (9-20 October 2017), where as in the low population level experiment they were obtained in navel orange orchard before the fruit ripening period (20-31 October 2017). Thus, many infested guava fruits were fallen under trees (as field observation) and most flies (females and males) were newly emerged; therefore both sexes of flies were attracted to food sources for nutrition. In navel orange orchard, females occurred as visitors searching for hosts to deposit their eggs. The present results are supported by El-Metwally [40], who mentioned that the sex ratios of attracted *C. capitata* to GF-120 enhanced with ammonium compounds tended to females in guava orchards more than those in navel orange orchards. Also, Pinero et al. [5] and Hemeida et al. [41] reported that adding di-ammonium phosphate, ammonium carbonate or ammonium acetate to a variety of protein baits and materials increased the numbers of attracted females of *C. capitata* and *B. zonata* in comparison with attracted males.

Conclusion

The ability of GF-120 to attract *B. zonata* flies can be enhanced by additing di-ammonium phosphate or ammonium carbonate at concentrations of 3 or 4%. In comparison with males, females of *B. zonata* were more responsive to the increase of pH. Attractiveness of GF-120 preparations is depending on pH; so, it can be used in chemical analysis for identification of new attractants from preferred bait formulations. Information regarding the effect of pH on the efficiency of different attractants in attracting fruit flies under various ecological situations should be studied.

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