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EVALUATION OF SOME AMMONIUM COMPOUNDS AS OLFACTORY STIMULANTS FOR ZIZYPHUS FRUIT FLY *Carpomya incompleta* (DIPTERA:TEPHRITIDAE) IN CHRIST'S THORN ORCHARDS AT QASSIM, SAUDI ARABIA

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ABSTRACT

The present investigation aims to evaluate the efficacy of six ammonium compounds (tri-ammonium phosphate, ammonium carbonate, ammonium acetate, ammonium chloride, ammonium thiocyanate and ammonium dihydrogen phosphate) as lures for the adults of zizyphus fruit fly, *Carpomya incompleta* (Beeker) in Christ's thorn orchards under field conditions of Qassim area, Saudi Arabia. Each ammonium compound was tested by using five concentration (1, 2, 3, 4 and 5%). Trap tests indicated that *C. incompleta* adults showed different degrees of preference for the different tested ammonium compounds. However, tri-ammonium phosphate attracted the highest numbers of *C. incompleta*. While, all concentrations of ammonium dihydrogen phosphate did not attract any females or males of *C. incompleta*. Tri-ammonium phosphate and ammonium chloride exhibited their highest attractant at 5% concentration; however, mean CTDs were 6.30 and 0.47 adults. While, ammonium carbonate, ammonium acetate and ammonium thiocyanate exerted their highest efficiency at 1% concentration (CTDs were 3.63, 0.79 and 0.80 adults, respectively). On another hand, tri-ammonium phosphate, ammonium carbonate and ammonium acetate attracted females more than males. While, the rest of tested compounds' concentrations attracted both sexes with no significant differences between them.

Keywords: Attractants, fruit flies, *Carpomya incompleta*, *Ziziphus spina-christi*, Qassim

INTRODUCTION

Christ's thorn trees, *Zizyphus spina-christi* (L.) (Family: Rhamnaceae) is a wild tree commonly available in Saudi Arabia. It has very nutritious fruits that are usually eaten fresh and its flowers are important source for honey bee. For a long time, in folklore medicine, *Z. spina-christi* has been used for the treatment of several human diseases (Kirtikar and Basu, 1984 and Han and Park, 1986). Tephritid fruit flies are a group of the most destructive pests since they directly infest the fruits causing great damages (White and Elson-Harris, 1994). Zizyphus fruit fly, *Carpomya incompleta* (Beeker) is one of tephritid fruit flies which attacks *Z. spina-christi* (Carroll *et al.*, 2004).

Effective insect detection systems are essential for survey of fruit flies and preventing the establishment of exotic pests (Lance and Gates, 1994). The efficiency of many attractants (olfactory, food and sex attractant compounds) was previously evaluated against fruit flies by several authors

(Gopaul and Price, 1999; Hanafy *et al.*, 2001; Saafan, 2005; Abd El-Kareim *et al.*, 2008 and Moustafa and Ghanim, 2008). Olfactory stimulants released from the fermentation of protein including ammonia (Bateman and Morton, 1981 and Mazor *et al.*, 1987), putrescine and methyl-substituted ammonia (Robacker and Warfield, 1993 and Heath *et al.*, 1995) are the primary compounds responsible for attracting fruit flies. According to Keiser *et al.* (1976), Bateman and Morton (1981) and Mazor *et al.* (1987), 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 (Moore, 1969), ammonium carbonate (Liquido *et al.*, 1993), ammonium bicarbonate (Robacker and Warfield, 1993), and ammonium hydroxide (Stills, 1964 and Boucher *et al.*, 2001).

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, *Ceratitis capitata* (Wiedeman) 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).

There are two objectives of using the attractants of fruit flies, the first is detecting and monitoring fruit flies populations, while the second objective is for fruit flies control (Abd El-Kareim *et al.*, 2008). There are shortage in researches related to attractants of zizyphus fruit fly, *C. incompleta* under Saudi Arabia conditions. Therefore, the present investigation aims to evaluate the efficacy of some ammonium compounds as lures for detecting and monitoring *C. incompleta* populations in Christ's thorn orchards in Qassim district, Saudi Arabia.

MATERIAL AND METHODS

Tested Compounds

Six ammonium compounds (tri-ammonium phosphate, ammonium carbonate, ammonium acetate, ammonium chloride, ammonium thiocyanate and ammonium dihydrogen phosphate) were evaluated as olfactory attractants for zizyphus fruit fly, *C. incompleta* under field conditions at Qassim area, Saudi Arabia. Each ammonium compound was investigated by using five concentrations (*i.e.* 1, 2, 3, 4 and 5%).

Bioassay Experiments

To evaluate the efficacy of ammonium compounds as olfactory attractants for *C. incompleta* adults, an experiment was carried out in Christ's thorn orchards (*Z. spina-christi*) of the experimental farm of Faculty of Agriculture and Veterinary Medicine, Qassim University at Qassim district, Saudi Arabia during the period from the 7thof March till 4th of April 2013.

The modified Nadel traps (described by Hanafy *et al.*, 2001) were used in this experiment; by putting 600 ml of each concentration of every ammonium compound in the trap. Each treatment was replicated five times. All prepared traps were distributed in a completely randomized design. The traps were hanged at about 1.5 meters on the trees. To avoid interference among traps loaded with different ammonium compounds and/ or concentrations, 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 28 days. Captured females and males of *B. zonata* were counted and recorded and CTD values (captured flies /trap/day) were calculated.

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) in addition to the regression analysis was applied for treatments.

RESULTS

Zizyphus fruit fly, *C. incompleta* adults exhibited different preferability to the tested ammonium compounds. All tested concentrations of ammonium dihydrogen phosphate did not attract any females or males of *C. incompleta*, all over the tested period. Tri-ammonium phosphate attracted the highest numbers of *C. incompleta* in comparison with the other tested ammonium compounds. Tri-ammonium phosphate at 5% concentration significantly attracted the highest numbers of *C. incompleta* adults all over the tested period. Attractency of *C. incompleta* adults to tested ammonium compounds could be arranged in descending order as follows: Tri-ammonium phosphate at 5% concentration> tri-ammonium phosphate at 4% concentration> ammonium carbonate at 1% concentration> tri-ammonium phosphate at 3% concentration> tri-ammonium phosphate at 2% concentration> tri-ammonium phosphate at 1% concentration>, ammonium thiocyanate (Table, 1).

With respect to the tested concentrations of each ammonium compound, data illustrated in Table (1) indicated that tri-ammonium phosphate and ammonium chloride exhibited their highest attraction at 5% concentration; where general mean CTD values were 6.3 and 0.5 adults. While, ammonium carbonate, ammonium acetate and ammonium thiocyanate exerted their highest efficiency at 1% concentration (CTDs were 3.6, 0.8 and 0.8 adults, respectively).

Despite efficiency of tri-ammonium phosphate and ammonium carbonate decreased by lapse of time, attractency of adult *C. incompleta* extended to 28 days, while ammonium chloride and ammonium thiocyanate lost their efficiency after 2 weeks from start of experiment. Attractency of adult flies in case of ammonium acetate almost terminated after 24 days from start of experiment.

Table (1). Mean numbers of captured *C. incompleta* adults/trap/day(CTD) in traps loaded with different concentrations of the tested ammonium compounds in Christ's thorn orchards.

Compound	Concentration	CTD after (days)							Mean
		4	8	12	16	20	24	28	
Tri-ammonium phosphate	1	3.8±1.4	3.2±0.9	1.6±0.6	5.0±2.1	3.2±1.2	1.1±0.42	1.2±0.5	2.7±1.0
	2	3.8±0.8	3.1±0.3	3.3±0.4	3.0±0.5	2.6±0.9	2.0±0.76	1.6±0.6	2.8±0.4
	3	3.9±0.9	3.4±1.1	3.0±0.7	3.6±1.2	4.0±1.3	2.8±0.99	1.5±0.6	3.2±0.9
	4	6.0±1.5	6.5±1.7	3.4±1.5	4.4±1.1	4.8±1.1	2.8±1.17	0.8±0.6	4.1±1.2
	5	8.5±1.5	9.0±1.6	5.0±1.5	7.5±1.5	8.0±1.6	4.5±1.38	1.6±0.6	6.3±1.4
Ammonium carbonate	1	3.8±1.2	5.2±1.0	1.5±0.8	5.8±1.5	6.5±1.5	2.5±1.20	0.4±0.3	3.6±1.0
	2	1.1±0.7	1.3±0.8	1.5±0.9	3.0±1.1	2.5±1.3	1.3±0.83	0.6±0.3	1.6±0.9
	3	2.5±1.0	3.0±1.2	1.8±0.9	1.4±0.6	1.3±0.5	0.6±0.22	0.5±0.3	1.6±0.6
	4	1.3±0.8	1.8±0.7	1.0±0.6	1.0±0.6	0.7±0.4	0.4±0.29	0.3±0.3	0.9±0.5
	5	2.6±1.2	3.1±1.1	1.6±0.6	3.8±1.1	3.0±1.4	0.4±0.42	0.0±0.0	2.0±0.8
Ammonium acetate	1	1.3±0.6	1.0±0.4	1.3±0.8	0.6±0.3	0.6±0.3	0.3±0.18	0.1±0.1	0.8±0.4
	2	0.9±0.5	1.0±0.6	1.1±0.7	0.7±0.5	0.4±0.3	0.2±0.27	0.0±0.0	0.6±0.4
	3	0.8±0.4	0.5±0.3	0.4±0.2	0.5±0.3	0.2±0.2	0.1±0.11	0.0±0.0	0.3±0.2
	4	2.1±0.8	1.0±0.5	0.7±0.4	0.5±0.4	0.3±0.3	0.1±0.14	0.0±0.0	0.7±0.3
	5	1.4±1.0	0.9±0.6	0.7±0.6	0.5±0.5	0.3±0.4	0.2±0.21	0.1±0.1	0.6±0.5
Ammonium chloride	1	1.8±0.8	1.0±0.7	0.5±0.3	0.0±0.0	0.0±0.0	0.0±0.00	0.0±0.0	0.5±0.3
	2	1.3±0.5	0.5±0.4	0.1±0.1	0.0±0.0	0.0±0.0	0.0±0.00	0.0±0.0	0.3±0.1
	3	2.0±0.9	1.0±0.6	0.3±0.3	0.1±0.1	0.0±0.0	0.0±0.00	0.0±0.0	0.5±0.2
	4	1.8±0.8	0.5±0.3	0.3±0.2	0.4±0.4	0.0±0.0	0.0±0.00	0.0±0.0	0.4±0.2
	5	3.0±0.9	1.3±0.7	0.3±0.4	0.1±0.1	0.0±0.0	0.0±0.00	0.0±0.0	0.5±0.3
Ammonium thiocyanate	1	3.0±0.6	1.9±0.6	0.4±0.3	0.3±0.2	0.1±0.1	0.0±0.00	0.0±0.0	0.8±0.1
	2	0.7±0.3	0.3±0.3	0.1±0.1	0.0±0.0	0.0±0.0	0.0±0.00	0.0±0.0	0.2±0.1
	3	0.8±0.3	0.3±0.2	0.1±0.1	0.1±0.1	0.0±0.0	0.0±0.00	0.0±0.0	0.2±0.1
	4	0.8±0.7	0.6±0.5	0.2±0.2	0.1±0.2	0.1±0.2	0.0±0.00	0.0±0.0	0.3±0.2
	5	0.8±0.7	0.5±0.4	0.2±0.2	0.1±0.1	0.0±0.0	0.0±0.00	0.0±0.0	0.2±0.2
$LSD_{(P=5\%)}$		**1.1**	**1.0**	**0.8**	**1.0**	**1.0**	**0.7**	**0.4**	**0.8**

To evaluate the potency of the tested compounds (as lures for *C. incompleta* adults) against time, regression analysis has been done between the CTDs and time (days).

Tri-ammonium phosphate 5% decreased sharply (b = -0.24) followed by tri-ammonium phosphate 4% (b = -0.19). Ammonium carbonate2%, ammonium thiocyanate 2, 3, 4 & 5% and ammonium acetate 3% decreased slowly by the time passed (b ranged between 0.01 and 0.03). While, the other tested treatments decreased with moderate levels (Table, 2).

Regression analysis had been done between CTDs and concentrations of each ammonium compound to evaluate the effect of concentration on the efficiency of the tested compounds (as lures for *C. incompleta*).

Table (2). Regression analysis between the captured adults (CTD) of *C. incompleta* (y) by different concentrations (Con.) of the tested compounds and the lapsed time (in days) after hanging traps (x).

Con. (%)	Tri-ammonium phosphate	Ammonium carbonate	Ammonium acetate	Ammonium chloride	Ammonium thiocyanate
1	y = -0.09x + 4.19 (R^2 = 0.30)	y = -0.09x + 5.15 (R^2 = 0.13)	y = -0.05x + 1.52 (R^2 = 0.85)	y = -0.07x + 1.55 (R^2 = 0.76)	y = -0.12x + 2.68 (R^2 = 0.74)
2	y = -0.09x + 4.31 (R^2 = 0.97)	y = -0.01x + 1.66 (R^2 = 0.01)	y = -0.04x + 1.30 (R^2 = 0.84)	y = -0.04x + 0.94 (R^2 = 0.61)	y = -0.02x + 0.53 (R^2 = 0.69)
3	y = -0.06x + 4.20 (R^2 = 0.43)	y = -0.10x + 3.19 (R^2 = 0.89)	y = -0.03x + 0.80 (R^2 = 0.92)	y = -0.07x + 1.62 (R^2 = 0.70)	y = -0.03x + 0.57 (R^2 = 0.65)
4	y = -0.19x + 7.19 (R^2 = 0.72)	y = -0.05x + 1.75 (R^2 = 0.81)	y = -0.07x + 1.86 (R^2 = 0.82)	y = -0.06x + 1.34 (R^2 = 0.65)	y = -0.03x + 0.75 (R^2 = 0.79)
5	y = -0.24x + 10.11 (R^2 = 0.59)	y = -0.10x + 3.69 (R^2 = 0.39)	y = -0.05x + 1.37 (R^2 = 0.94)	y = -0.11x + 2.36 (R^2 = 0.66)	y = -0.03x + 0.70 (R^2 = 0.75)

Data illustrated in Figure (1) shows that tri-ammonium phosphate and ammonium chloride exhibited a positive relationship between trap catches and concentrations. However, increasing the concentration of tri-ammonium phosphate and ammonium chloride by 1% increased the CTDs by 0.85 and 0.02 adults, respectively. While, the captured flies by ammonium carbonate, ammonium acetate and ammonium thiocyanate had an adverse relationship between trap catches and concentrations. However, the CTDs decreased by 0.39, 0.04 and 0.11 adults as the concentration of ammonium carbonate, ammonium acetate and ammonium thiocyanate, respectively increased by 1%.

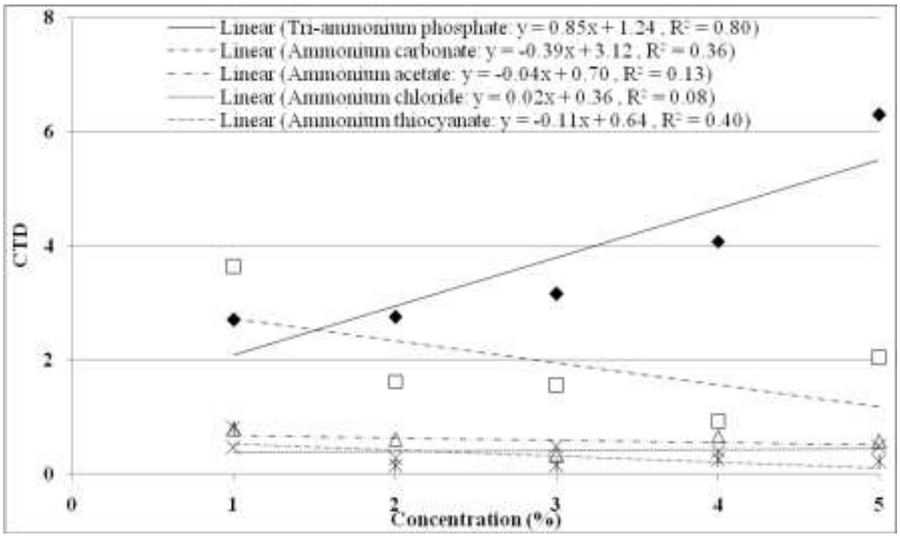

Figure (1). Relationships between the captured adults (CTD) of *C. incompleta* and the concentrations of the tested compounds.

Figure (2) showed that tri-ammonium phosphate, ammonium carbonate and ammonium acetate significantly attracted more females than males of *C. incompleta*. While, no significant differences in number of attracted males and females were observed among the rest of tested compounds.

DISCUSSION

The present study indicated that *C. incompleta* adults (male and female) exhibited significantly high positive response to tri-ammonium phosphate, ammonium carbonate, ammonium acetate, ammonium chloride and ammonium thiocyanate. On the contrary, *C. incompleta* showed no response to ammonium dihydrogen phosphate. These findings agree with Jones (1987), Abd El-Kareim *et al.* (2008) and Moustafa & Ghanim (2008) who 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).

Our data revealed that tri-ammonium phosphate had the highest attraction to *C. incompleta* at Qassim area, Saudi Arabia. While, di-ammonium phosphate was the best compound in attracting *Bactrocera zonata* (Saunders) in Egypt (Hanafy *et al.*, 2001). Also, ammonium acetate and ammonium carbonate exhibited a moderate efficiency in attracting *C. incompleta*. This data matched with results obtained by Abd El-Kareim *et al.* (2008) and Moustafa & Ghanim (2008) who mentioned that ammonium acetate and ammonium carbonate were the best compounds in attracting *B. zonata* and *C. capitata*. These differences may be attributed to the variation between climatic factors and/or fruit fly species.

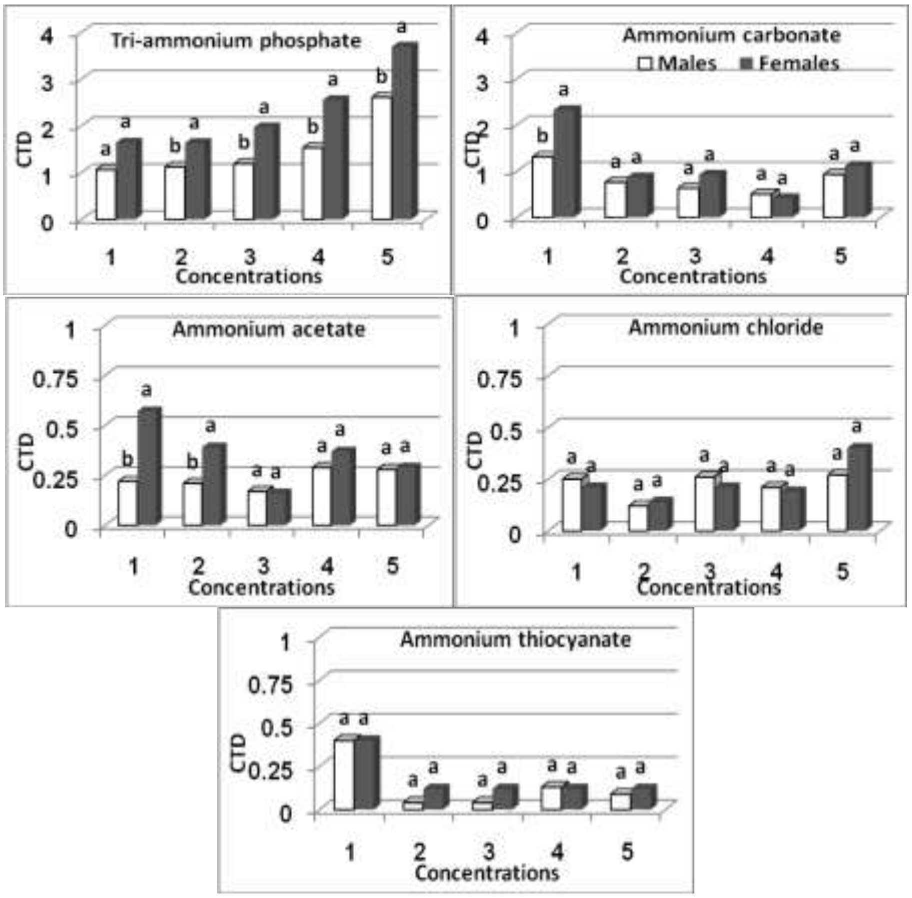

Figure (2). Mean CTD values of *C. incompleta* females and/or males captured by different concentrations of the tested ammonium compounds over 28 days (in each concentration of each compound, columns had the same litters did not differ significantly at P = 5%).

The tested ammonium compounds in the present study showed differences in their attractiveness toward zizyphus fruit fly (*C. incompleta*) with elapsed time. However, 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). Similar results were recorded on *B. zonata* (Abd El-Kareim *et al.*, 2008) and *C. capitata* (Moustafa & Ghanim, 2008).

Decrement in adult flies attractency with higher concentrations of some ammonium compounds (ammonium carbonate and ammonium thiocyanate) may be attributed to the release rate of ammonia (which could act as repellent for adults of fruit flies) in preparations. This data is in agreement with results obtained by Thomas *et al.* (2008) who found that the lower dosages of ammonium acetate and ammonium bicarbonate had significantly greater captures of fruit flies (*Anastrepha* spp.), demonstrating that release rate of ammonia from the formulations is critical. Very high dosage of ammonia may actually be repellent as has been shown in flight tunnel bioassays (Kendra *et al.*, 2005).

In our findings, tri-ammonium phosphate, ammonium carbonate and ammonium acetate significantly attracted more females than males. While, the rest of tested compounds' concentrations attracted females and males with no significant differences between them. According to Hanafy *et al.* (2001), Saafan (2005); Abd El-Kareim *et al.* (2008) and Moustafa and Ghanim (2008), 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) 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. Presumably, 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). Electroantennographic (EAG) studies with the Caribbean fruit fly, *A. suspensa*, indicated that antennal response to ammonia varies with dose (Kendra *et al.*, 2005).

It could be suggested that the present results may be useful in applying integrated pest management control programs by using tri-ammonium phosphate (at concentrations 4 or 5%) because of its good attractency for *C. incompleta* adult flies and its potency along elapsed time (three weeks).

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تقييم بعض مركبات الآمونيوم كمنبهات غذائية لذبابة ثمار النبق في مزارع النبق بمنطقة القصيم بالمملكة العربية السعودية

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تهدف هذه الدراسة إلى تقييم كفاءة ستة مركبات آمونيوم (تراي آمونيوم فوسفات، آمونيوم كربونات، آمونيوم اسيتات ، آمونيوم كلوريد، آمونيوم ثيوسيانات وآمونيوم دايهيدروجين فوسفات) كجاذبات للحشرات الكاملة من ذبابة ثمار النبق تحت الظروف الحقلية لمزارع النبق بمنطقة القصيم بالمملكة العربية السعودية. وقد تم تقييم خمسة تركيزات من كل مركب من هذه المركبات (1، 2، 3، 4 و5%). وقد أظهرت ذبابة النبق اختلافات واضحة في استجابتها لمركبات الآمونيوم المدروسة؛ حيث وجد أن مركب تراي آمونيوم فوسفات كان أكثر المركبات جذباً للذبابة. بينما لم تنجذب أي أفراد من الذبابة للمركب آمونيوم دايهيدروجين فوسفات في تركيزاته المختلفة. ظهرت أعلى كفاءة لمركبي تراي آمونيوم فوسفات وآمونيوم كلوريد في حالة التركيز العالي (5%) حيث بلغ متوسط الجذب اليومي للمصيدة (CTD) 6.30 و 0.47 ذبابة، أما أعلى كفاءة لمركبات آمونيوم كربونات، آمونيوم اسيتات وآمونيوم ثيوسيانات فقد تم تسجيلها في التركيز المنخفض (1%) حيث بلغ متوسط الجذب اليومي للمصيدة 3.63، 0.79 و0.80 ذبابة، على التوالي. ومن ناحية أخرى لوحظ أن مركبات تراي آمونيوم فوسفات، آمونيوم كربونات وآمونيوم اسيتات تجذب الإناث أكثر من الذكور، بينما لم يكن هناك اختلاف بين اعداد الإناث والذكور في حالة المركبات الأخرى.

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