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Improving the Efficiency of Protein-Based Baits by Adding Ammonia for Controlling Fruit Flies (Diptera: Tephritidae)

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ABSTRACT

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) and the peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) are among the most economically damaging pests of citrus orchards in Egypt. Proprietary aqueous protein baits that incorporate ammonia derivatives have been developed for pestiferous fruit flies. An approach combining lures (protein baits and olfactory ammonia attractants) sprayed with insecticides has been used to combine detection and monitoring in the management programs of fruit flies. Three ammonia derivatives [di-ammonium phosphate (DA), ammonium acetate (AA), and ammonium hydroxide (AH)] were used to improve the efficacy of protein hydrolysate Buminal, the most common protein-based bait used for monitoring tephritid flies in Egypt, in attracting *C. capitata* and *B. zonata* flies. Then, the spot-bait spray was applied either by incorporating the 1% DA with one of the three insecticides (lambda-cyhalothrin, abamectin, and malathion) or without. The results of this study are essential for developing an effective pest management program for controlling *C. capitata* and *B. zonata*. The addition of DA, but not AA or AH, to the commercially available Buminal bait magnified its attractiveness for both tephritid species. The pyrethroid, lambda-cyhalothrin, had high potency especially when applied together with the Buminal bait and the olfactory attractant, DA, which renders them potential agents for controlling *C. capitata* and *B. zonata*. These findings can potentially increase the effectiveness of protein baits in spot-spray applications for fruit fly monitoring and suppression.

INTRODUCTION

Proprietary aqueous protein baits developed for pestiferous fruit flies typically incorporate ammonia derivatives (Epsky *et al.*, 2014, Hemeida *et al.*, 2017). Such baits are commercially available in various countries under the brand names Buminal, Nulure, Solbait, Corn Steep Water, Pinnacle, or Hymlure. Buminal is a bait spray based on protein hydrolysate that is commonly used against the olive fruit fly, *Bactroceraoleae* (Gmelin) (Prota 1983) and various *Ceratitis* spp. (Quilici 1993). Over the years, various studies have explored additives to improve the attractiveness of these baits (Piñero *et al.*, 2022). For example, the addition of ammonium acetate, but not ammonium carbonate, to GF120®, Nu-Lure®, Buminal, and Bugs for Bugs® improved their attractiveness to *Bactrocera cucurbitae* (Coquillet) and *Bactroceradorsalis* (Hendel) (Piñero *et al.*, 2022), and *C.*

capitata (Piñero *et al.*, 2015). Similarly, the addition of ammonia compounds to GF120® resulted in significantly higher catches of *C. capitata* (El-Metwally 2018) and *B. zonata* (Hemeida *et al.*, 2017, Ghanim 2018). The addition of ammonium acetate to protein hydrolysate increased captures of *C. capitata* when compared to protein hydrolysate alone (Piñero *et al.,* 2015). In contrast, the addition of 1% ammonium acetate to a mixture solution of hydrolyzed protein and borax significantly reduced captures of *Anastrepha obliqua* Macquart and *Anastrepha serpentina* (Wiedemann), (Diptera: Tephritidae) (Lasa and Williams 2021). Thus, ammonia derivatives have the potential to improve the attractiveness of Buminal-based baits for *C. capitata* and/or *B. zonata* species, as reported for borax-based baits used against other tephritid species (Heath *et al.,* 1994).

Nitrogen-rich food sources have strong impacts on the physiology and behavior of tephritid flies (Hemeida *et al.*, 2017, El-Metwally (2018) and various nitrogenous compounds serve as effective attractants (e.g., Lasa and Williams 2021, Kouloussis et al. (2022). Immature female flies require a protein meal for sexual maturation and ovarian development and retain a high protein demand during egg production (Perez-Staples *et al.*, 2007). Mixing a toxicant with a protein bait can be an effective 'attract-and-kill' tactic for fruit fly management (Róssler 1989, Mangan 2014, Epsky *et al.*, 2014), in part because protein stimulates flies to ingest a lethal dose of the toxicant (Mangan 2014). Protein baits combined with insecticides were first used for the control of fruit flies in Hawaii (Steiner 1952) and have been used successfully in many management and eradication programs since (e.g., Vargas *et al.,* 2010). Attraction occurs because female fruit flies use ammonia and ammonia derivatives as olfactory cues to locate protein-rich food sources (Piñero *et al.*, 2015, Bayoumy *et al.*, 2021). Thus, protein-based baits combined with insecticides have enabled region-wide control programs to be effective against tephritids (e.g., Piñero *et al.*, 2015), whereas previously they were used primly for detection and monitoring (Hendrichs *et al.,* 1995, Stonehouse et al. 2002) and have become indispensable for suppression or eradication of fruit flies worldwide, while also reducing the amount of insecticide required in such programs (Barzman *et al.*, 2015).

The present work aims first to improve the potential attractiveness of protein Buminal bait, a commercially and locally available bait, for improved monitoring and detection of *C. capitata* and *B. zonata* through mixing with some ammonia additives, and secondly to evaluate the efficacy of the mixture consisting of the enhanced protein lure (i.e Buminal + ammonium) and certain insecticides in controlling *C. capitata* and *B. zonata* through spot- spray application.

MATERIALS AND METHODS

Baits:

Buminal (hydrolyzed protein 39.78%, NABA GmbH Company, Germany), the most commonly used bait for monitoring tephritid flies in Egypt, was obtained from the Plant Protection Research Institute, Egyptian Ministry of Agriculture, and was used in all trials at a 5% (vol/vol) concentration, as per recommendations. Three ammonium compounds were tested as amendments (at 1, 2, and 3% concentrations) to improve the attractiveness of Buminal to *C .capitata* and *B. zonata* flies; di-ammonium phosphate [$(NH_4)_2$ HPO_4], ammonium acetate [CH_3COONH_4], and ammonium hydroxide [NH_4OH] (El-Naser Drugs and Chemicals Company, Egypt). Concentrations of each were calculated as wt/vol, except for ammonium hydroxide, which was vol/vol. Ten different bait formulations were then prepared: 5% Buminal (Bu), 5% Buminal + 1, 2, or 3% di-ammonium phosphate (Bu+DA1, 2, 3); 5% Buminal + 1, 2, or 3% ammonium acetate (Bu+AA1, 2, 3), and 5% Buminal + 1, 2, or 3% ammonium hydroxide (Bu+AH1, 2, 3).

Bait Evaluation In Traps:

A field trial was conducted in a 2.43 ha mandarin orange orchard at the experimental farm of the Faculty of Agriculture, Mansoura University, Egypt using modified Nadel traps (Hanafy et al. 2001), each loaded with 200 ml of bait formulation. Traps (n = 8 traps per formulation) were hung within tree canopies on the south shady side at a height of 2 meters, 100 m apart, with baits of each type randomly assigned among locations. Trap catches were collected five times (once every three days for 15 days), with no replacement of baits during the trial, and the numbers of *C. capitata* and *B. zonata* flies caught in each trap were recorded and expressed as 'no. flies/trap/day' (FTD).

Spray Trial With Spot Application Of The Enhanced Buminal Bait:

Based on the results of the trap evaluation trial, an amendment of 1% di-ammonium phosphate was most effective in enhancing the attractiveness of Buminal for both *C. capitata* and *B. zonata*. Therefore, a spot-application bait trial was conducted in the mandarin orchard from 1 December to 31 December 2019, to assay the efficacy of the enhanced bait when combined with one of three insecticides that differed in mode of action; the synthetic pyrethroid lambda-cyhalothrin (Catron® 5% emulsifiable concentrate = Lc), the synthetic organophosphate malathion (Malatox® 57% emulsifiable concentrate = Ma), and the actinomycete fermentation product abamectin (Nasractine® 1.08% emulsifiable concentrate = Ab). The experiment was conducted in a '3 x 3' randomized complete block design, with each insecticide tested in Buminal baits once with, and once without the 1% di-ammonium phosphate amendment, with corresponding control blocks that did not receive any insecticide treatment. Thus, nine blocks were evaluated (Lc + Bu+ 1% DA, Lc + Bu, control; Ma + Bu+ 1% DA, Ma + Bu, control; Ab + Bu + 1% DA, Bu+ 1% DA, Ma + Bu, control). For each treatment, eight traps were used.

The orchard was divided into nine blocks, each containing 36 trees, with a buffer of four rows of trees between treatments. One week prior to the first bait application, eight Nadel traps loaded with 1% di-ammonium phosphate amended Buminal lures were arranged randomly across equally-spaced locations in each block, and hung within tree canopies on the shady side at a height of 2 meters. Bait sprays were applied three times, at 10-day intervals (1st, 10th, and 20th day of December). Traps in control trees were placed on 23rd of November and pre-treatment trap catches were collected one week later on 30th of November, and again 3 and 10 days after each bait application. Numbers of captured *C. capitata* and *B. zonata* flies were counted and the catch was expressed as 'no. flies/trap/day' (FTD). The percentage reduction in fly numbers as a result of each bait application was calculated according to the Henderson and Tilton (1955) formula as follows:

$$Corrected\ \% = \left(1 - \frac{No.\ in\ C\ before\ treatment}{No.\ in\ C\ after\ treatment} \times \frac{No.\ in\ T\ after\ treatment}{No.\ in\ T\ before\ treatment}\right) \times 100$$

Where C = control blocks and T = treatment blocks.

Statistical Analysis:

Trap catches of both fruit fly species were analyzed by a General Linear Model using three ammonia compounds, four concentrations for each ammonia compound, and five inspection times, as independent variables. Because there is an a priori expectation of increasing trap catches with increasing concentration of ammonia compounds - we expect a change in one direction only when concentration increases compared to the basic bait, the K-independent non-parametric test, Jonckheere-Terpstra, for ordered alternatives, which does not require the data conform to any distribution was applied. Means were separated using Dunn's test (α <0.05). Given significant departures from normality (Shapiro–Wilks test), trap catch data were analyzed using non-parametric techniques (Kruskal-Wallis one-way ANOVA on Ranks and Mann-Whitney *U* tests). Means were separated using Dunn's test (α <0.05). All tests were accomplished by Systat Sigma Plot 14.5 (Systat Software, San Jose, CA; Systat Software 2020).

RESULTS

A general linear model revealed that there were significant effects of ammonia compounds and their concentrations, and time of trap inspection as well as all of the available interactions between two or three of the independent variables on *C. capitata* and *B. zonata* captures (Table 1).

Table 1: A general linear model summary of the main and interactive fixed effects, of three ammonia compounds (AA: ammonium acetate; AH: ammonium hydroxide; DA: di-ammonium phosphate), four Buminal- ammonia mixture concentrations (0, 1, 2, and 3%), and five times (3, 6, 9, 12, and 15 days) of trap captures, on numbers of *C. capitata* and *B. zonata* flies captured in traps.

Source of variation	**df**	***Ceratitis capitata***		***Bactrocera zonata***	
		F	***P***	***F***	***P***
Ammonia compounds	2	97.67	<0.001	108.74	<0.001
Compound concentration	3	50.59	<0.001	63.17	<0.001
Inspection time	4	215.01	<0.001	15.49	<0.001
Compounds * Concentration	6	51.24	<0.001	138.22	<0.001
Compounds * Time	8	33.89	<0.001	16.78	<0.001
Concentration * Time	12	19.66	<0.001	17.11	<0.001
Compounds * Concentration * Time	24	12.76	<0.001	8.09	<0.001

df of error = 420 for all variations

Without taking into account the time of trap catches a Jonckheere-Terpstra test revealed that there was a significant increase in the trend of trap catches of *C. capitata* and *B. zonata* flies with increasing concentrations of ammonium acetate and di-ammonium phosphate, but not with increasing ammonium hydroxide concentration (Table 2).

Table 2: Results of K-independent non-parametric test, Jonckheere - Terpstra test for order alternatives between different mixtures of Buminal-ammonia concentrations (1, 2, and 3%) including control treatment (Buminal + 0 % ammonia) and trap catches of *C. capitata* and *B. zonata*.

Species	**Olfactory stimuli**	**Jonckheere - Terpstra Test**			
		χ^2	***Df***	***Std T-J***	***P***
C. capitata	Ammonium acetate	31.20	3	5.45	< 0.001
	Ammonium hydroxide	12.00	3	1.88	0.06
	Diammonium phosphate	22.40	3	2.10	0.05
B. zonata	Ammonium acetate	58.18	3	8.66	< 0.001
	Ammonium hydroxide	8.00	3	0.073	0.05
	Diammonium phosphate	41.21	3	2.09	< 0.001

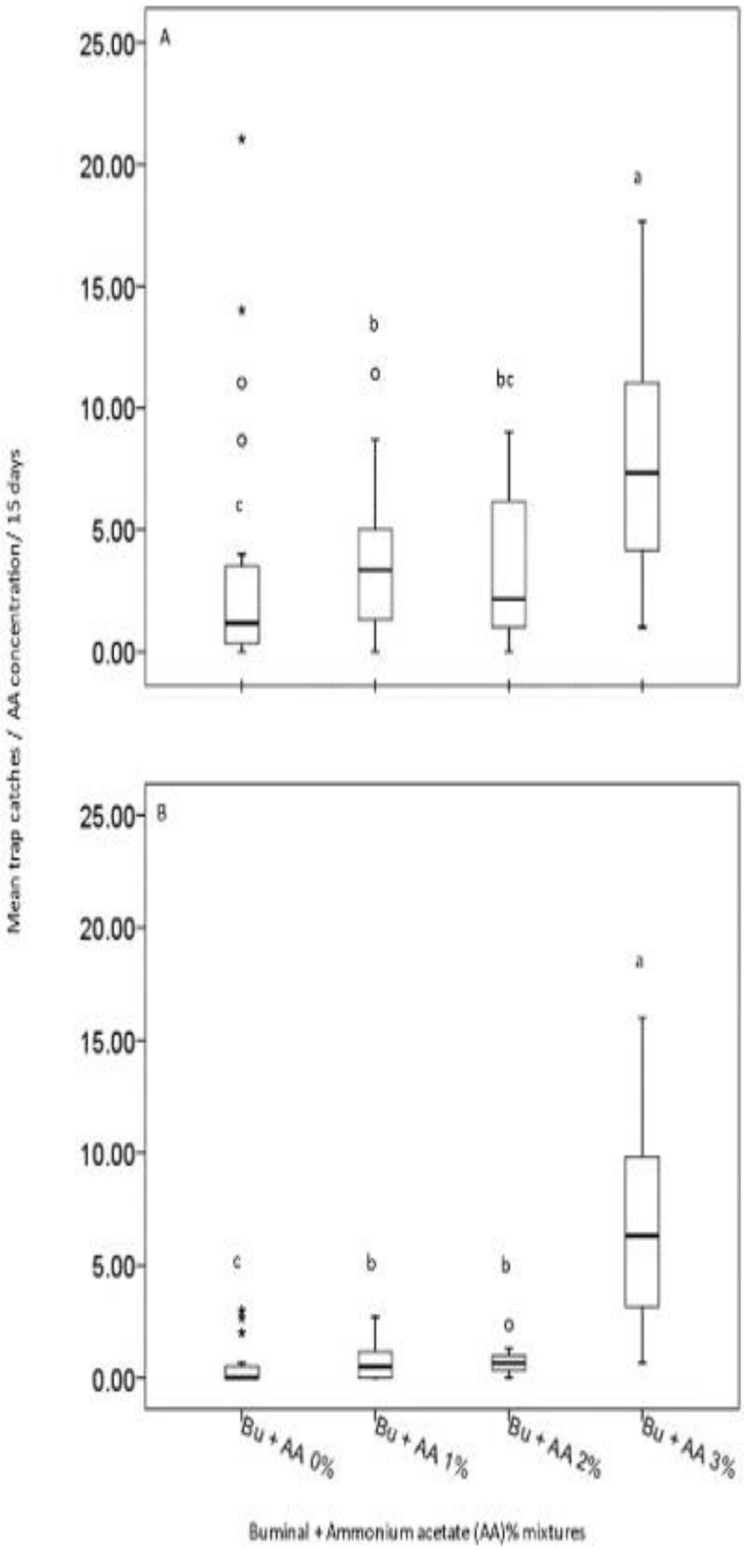

Fig. 1. Box-whisker plots of median trap catches of *C. capitata* (A) and *B. zonata* (B) at different lure mixtures Buminal – ammonia acetate concentrations. Thin black lines in the box = medians. The lower and upper limits of the box represent the 25th and 75th percentiles, lower and upper whiskers denote the 10th and 90th percentiles while the lower and upper dots stand for the 5th and 95th percentiles. Stars above and below the lower and upper boundaries are outliers. Box plots bearing the same letters are not significantly different (Pairwise comparisons, Mann-Whitney, $\alpha = 0.05$).

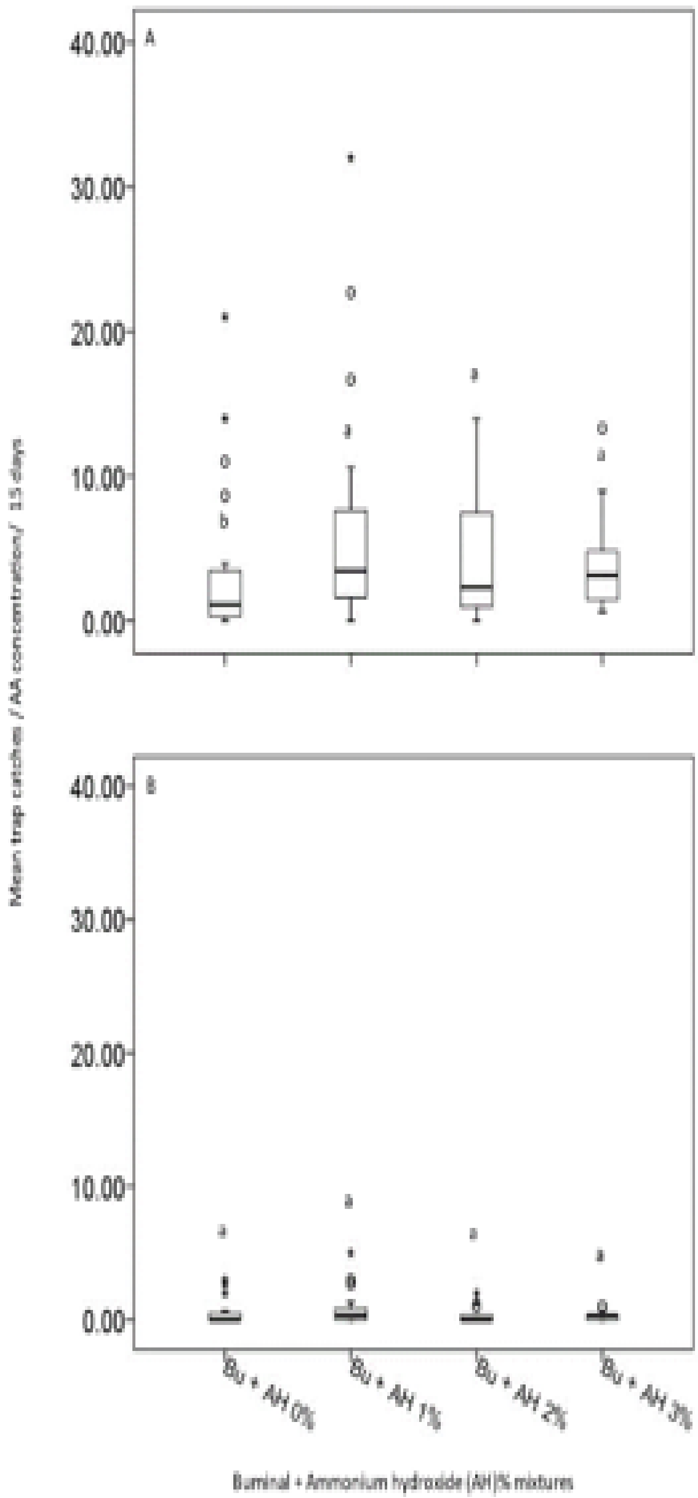

Fig. 2. Box-whisker plots of median trap catches of *C. capitata* (A) and *B. zonata* (B) at different lure mixtures of Buminal – ammonia hydroxide concentrations. Thin black lines in the box = medians. The lower and upper limits of the box represent the 25th and 75th percentiles, lower and upper whiskers denote the 10th and 90th percentiles while the lower and upper dots stand for the 5th and 95th percentiles. Stars above and below the lower and upper boundaries are outliers. Box plots bearing the same letters are not significantly different (Pairwise comparisons, Mann-Whitney, $\alpha = 0.05$).

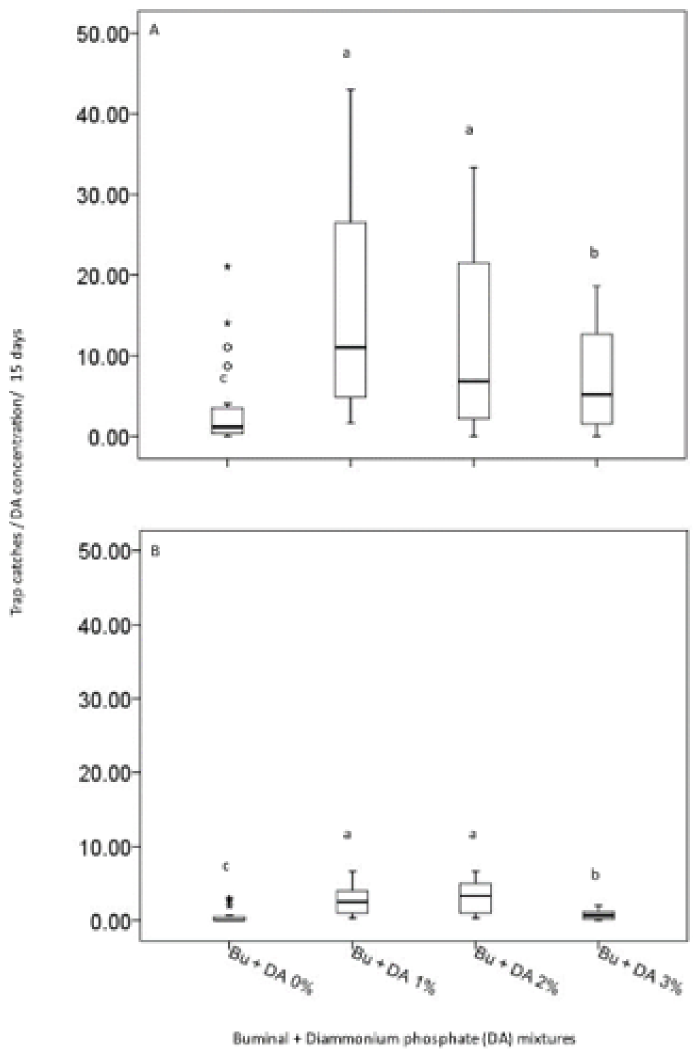

Fig. 3. Box-whisker plots of median trap catches of *C. capitata* (A) and *B. zonata* (B) at different lure mixtures Buminal – diammonium phosphate concentrations.
Thin black lines in the box = medians. The lower and upper limits of the box represent the 25th and 75th percentiles, lower and upper whiskers denote the 10th and 90th percentiles while the lower and upper dots stand for the 5th and 95th percentiles. Stars above and below the lower and upper boundaries are outliers. Box plots bearing the same letters are not significantly different (Pairwise comparisons, Mann-Whitney, $\alpha = 0.05$).

The highest medians of trap catches of both fruit fly species by buminal bait were at the highest concentration of ammonium acetate (3%) and at 1 and 2% di-ammonium phosphate (Figs. 1 and 2), whereas there were no significant differences between ammonium hydroxide concentrations in trap catches of both fly species (Fig. 3) (Tables 3 and 4).

Kruskal-Wallis one-way ANOVA on ranks revealed that there were significant differences among all treatments of Buminal and ammonia compounds including those that had Buminal only (control) within each time of trap check in the medians of trap catches of *C. capitata* with the highest medians of trap catches were at a mixture of 1% di-ammonium phosphate-Buminal for most of times (Table 3). The same findings were obtained for *B. zonata* with the highest trap catches by adding 1 or 2% di-ammonium phosphate to the Buminal attractant without significant differences between both concentrations **(**Table 4**).**

Table 3: Daily medians (25th - 75th quartiles) of *Ceratitis capitata* flies captured in traps baited with mixtures of Buminal and ammonia compounds (AA: ammonium acetate; AH: ammonium hydroxide; DA: di-ammonium phosphate) that prepared in three concentrations. Only Buminal is used in the control traps. Traps were checked in three-day intervals for 15 days in the field with no renewal of the solutions in the traps during the period of trials.

Treatment	No. *Ceratitis capitata* flies / trap/day				
	3 days	6 days	9 days	12 days	15 days
Bu (Check)	0.67 c (0.49-0.84)	12.52 ab (9.26-1.27)	2.33 ab (1.17-3.75)	0.83 c (0.33-1.83)	0.17 b (0.00-1.08)
Bu + AA1%	4.20 c (3.35-6.87)	4.35 b (3.42-9.80)	4.69 ab (1.68-6.44)	1.84 ab (0.83-3.62)	0.83 b (0.08-1.34)
Bu + AA2%	7.68 bc (5.51-8.68)	6.17 ab (3.50-6.83)	1.50 b (1.08-2.92)	1.17 c (0.49-3.08)	1.00 ab (0.25-1.25)
Bu + AA3%	9.02 b (6.35-11.04)	14.52 ab (8.99-17.0)	8.35 a (4.84-13.61)	4.50 bc (3.25-6.75)	3.34 a (1.34-4.83)
Bu + AH1%	2.17 c (1.33-3.50)	19.70 a (12.15-29.68)	4.00 ab (3.08-6.92)	4.17 bc (2.42-6.92)	1.16 ab (0.17-1.66)
Bu + AH2%	0.83 c (0.33-1.84)	9.85 ab (8.41-13.26)	2.33 ab (1.09-3.08)	6.34 a (2.75-8.94)	1.00 ab (0.17-1.83)
Bu + AH3%	2.67 c (1.67-3.50)	7.67 ab (4.83-12.25)	4.17 ab (2.84-6.50)	2.33 b (0.84-4.33)	1.33 ab (0.75-1.66)
Bu + DA1%	31.50 a (25.0-38.0)	26.50 a (17.92-29.09)	5.00 ab (4.17-10.84)	7.17 a (4.00-14.08)	4.50 a (2.09-5.92)
Bu + DA2%	25.84 a (21.50-31.34)	21.50 a (12.75-23.26)	3.99 ab (2.08-7.42)	3.83 bc (1.83-9.09)	1.34 ab (0.17-3.25)
Bu + DA3%	14.67 ab (13.67-17.17)	7.83 ab (4.99-9.42)	8.84 a (2.75-13.17)	2.67 b (1.58-3.75)	0.50 b (0.00-1.25)
H	73.25	55.25	36.89	40.94	34.92
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

DF = 9 for all. Medians bearing the different letters within a column are significantly different (Kruskal Wallis one-way ANOVA on Ranks). Medians separated using Dunn's test (α = 0.05).

Table 4: Daily medians (25th - 75th quartiles) of *B. zonata* flies captured in traps baited with mixtures of Buminal and ammonia compounds (AA: ammonium acetate; AH: ammonium hydroxide; DA: di-ammonium phosphate) that prepared in three concentrations. Only Buminal is used in the control traps. Traps were checked in three-day intervals for 15 days in the field with no renewal of the solutions in the traps during the period of trials.

Treatment	No. *B. zonata* flies / trap/day				
	3 days	6 days	9 days	12 days	15 days
Bu (Check)	3.00 ab (3.00-3.00)	2.33 ab (0.49-2.91)	0.17 b (0.00-0.58)	0.00 c (0.00-0.00)	0.00 c (0.00-0.00)
Bu + AA1%	0.17 b (0.00-0.58)	0.49 b (0.33-1.16)	0.66 ab (0.08-0.99)	0.00 c (0.00-0.49)	0.00 c (0.00-0.49)
Bu + AA2%	2.35 ab (1.49-2.70)	0.66 b (0.08-0.99)	0.66 ab (0.41-1.16)	0.99 ab (0.74-1.99)	0.17 b (0.00-0.83)
Bu + AA3%	0.49 b (0.08-0.66)	5.83 a (4.39-8.28)	5.33 a (2.78-10.18)	9.67 a (5.83-15.00)	9.82 a (8.86-11.80)
Bu + AH1%	0.17 b (0.00-0.33)	2.84 ab (1.66-4.50)	0.17 b (0.00-0.83)	0.17 b (0.00-0.33)	0.50 b (0.08-0.67)
Bu + AH2%	0.00 b (0.00-0.24)	1.17 ab (0.25-1.83)	0.00 b (0.00-0.25)	0.17 b (0.00-1.08)	0.17 b (0.00-0.33)
Bu + AH3%	0.00 b (0.00-0.25)	0.33 b (0.08-0.83)	0.33 b (0.33-0.83)	0.33 b (0.08-0.59)	0.00 c (0.00-0.00)
Bu + DA1%	3.50 ab (1.67-5.34)	3.67 a (2.09-6.00)	3.34 a (1.42-5.50)	0.67 ab (0.33-2.25)	1.17 b (0.75-2.08)
Bu + DA2%	5.50 a (3.50-5.34)	5.00 a (3.75-5.75)	2.50 ab (1.08-4.17)	2.00 ab (0.42-4.58)	0.67 b (0.33-1.75)
Bu + DA3%	0.17 b (0.00-0.59)	1.34 ab (0.75-1.67)	0.83 ab (0.08-1.82)	0.67 ab (0.08-1.75)	0.67 b (0.08-1.00)
H	64.67	59.29	54.82	51.91	56.52
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

DF = 9 for all. Medians bearing the different letters within a column are significantly different (Kruskal Wallis one-way ANOVA on Ranks). Medians separated using Dunn's test (α = 0.05).

The field spot-spray applications of adding 1% di-ammonium phosphate (DA) to baits, composited of Buminal (Bu) and each of lambda-cyhalothrin (Lc), abamectin (Ab) or malathion (Ma) achieved different reductions in *C. capitata* populations, in both times of trap inspection during the three successive sprays. In the first spot-spray, one-way ANOVA on Ranks revealed that there were significant differences in fly population reductions among the different treatments after three (H = 15.74; P = 0.008) and ten (H = 13.09; P = 0.02) days from the treatment with the highest reduction achieved by a mixture of Lc + Bu + DA during the first and second inspection times (medians: 100.0 and 85.72%, respectively). In the second spot-spray, there were significant differences among the different treatments in population reduction after three days from application (H = 18.41; P = 0.002), but not after ten days (H = 5.46; P = 0.36), with the highest reduction achieved by a mixture of Lc + Bu + DA during the first inspection time (medians: 100.0 %) and with a mixture of Ab + Bu + DA during the second inspection time (medians: 81.25%). In the third spot-spray, there were no significant differences neither after three days (H = 9.78; P = 0.08) nor after ten days (H = 4.86; P = 0.43), however the highest reduction attained by a mixture of Lc + Bu + DA during the first and second inspection times (medians: 100.0 and 95.0%, respectively).

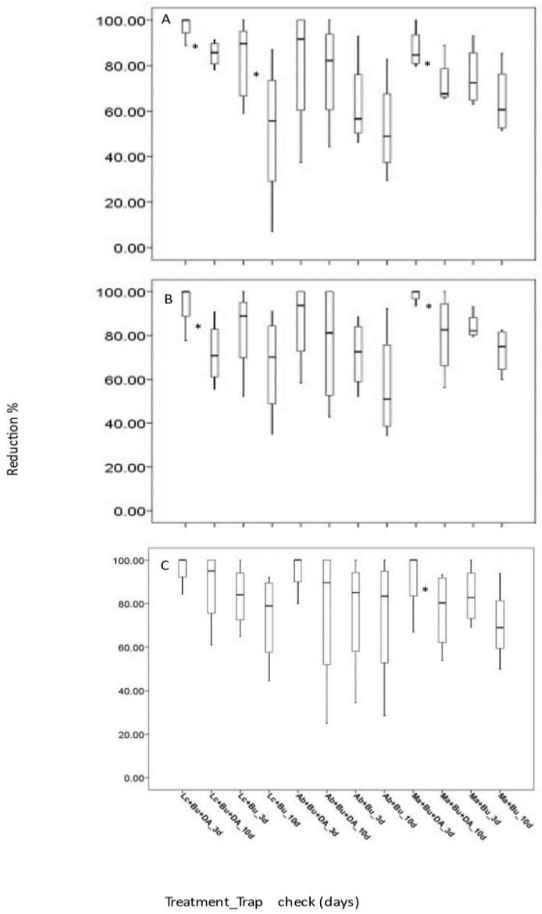

Fig. 4. Effect of adding 1% di-ammonium phosphate (DA) to baits composited of Buminal and each of lambda-cyhalothrin (Lc), abamectin (Ab), or malathion (Ma), that used in spot-spray of *C. capitata*. The reduction % was estimated based on Henderson-Tilton's formula. Three successive partial-spray applications (A, B, and C), in ten-day intervals, were applied based on recommendations of the Egyptian Ministry of Agriculture. Traps were examined after 3 and 10 days from each spray event in both treated and control treatments (x-axis). Thin black lines in the box = medians. The lower and upper limits of the box represent the 25th and 75th percentiles, lower and upper whiskers denote the 10th and 90th percentiles while the lower and upper dots stand for the 5th and 95th percentiles. One-way ANOVA on Ranks. Pairwise bars with a star in the middle indicate a significant difference (Dunn's test, α = 0.05).

As shown in Figure (4), pairwise comparisons between both inspection times (i.e. three and ten days) within each spray time in population reductions of *C. capitate* revealed that there were significant differences between both inspection times for the enhanced composition "Buminal bait + lambda cyhalothrin" by adding 1% di-ammonium phosphate in the first (U = 4.00, P = 0.002) and second (U = 4.00, P = 0.002) spot-sprays, but were not in the third spray application (U = 24.0, P = 0.44). With respect to the non-enhanced lure of Buminal + lambda-cyhalothrin, there was a significant difference between both inspection times during the first spray application only (U = 8.0, P = 0.01), but not in the second (U = 20.0, P = 0.23) and third (U = 24.0, P = 0.44) sprays. With respect to both the enhanced composition of Buminal + abamectin by adding 1% di-ammonium phosphate and the non-enhanced lure of Buminal + abamectin, there were no significant differences in *C. capitata* population reductions between both times of trap check in the three successive spray times (U = 28.0, P = 0.72; U = 26.0, P = 0.27; and U = 20.0, P = 0.23, respectively). As well, there was no significant difference between both inspection times in fly population reductions by the non-enhanced lure of Buminal + lambda-cyhalothrin during the three successive spray applications (U = 16.0, P = 0.11; U = 20.0, P = 0.21; and U = 28.0, P = 0.72, respectively). The population reduction in *C. capitate* by the spot-spray of the enhanced composition of "Buminal bait + malathion" by adding 1% di-ammonium phosphate differed significantly between the two inspection times of the first (U = 12.0, P = 0.04), second (U = 10.0, P = 0.02), and third(U = 12.0, P = 0.04) spray applications, whereas the population reductions by the non-enhanced Buminal bait + malathion did not significantly differ between both inspection times of the three spray applications(First: U = 20.0, P = 0.23; Second: U = 12.0, P = 0.05; and third: U = 14.0, P = 0.07).

The field spot-spray applications of adding 1% di-ammonium phosphate (DA) to baits, composited of Buminal (Bu) and each of lambda-cyhalothrin (Lc), abamectin (Ab) or malathion (Ma) achieved different reductions in *B. zonata* populations, in both times of trap inspection during the three successive sprays. In the first spot-spray, one-way ANOVA on Ranks revealed that there were significant differences in fly population reductions among the different treatments after three (H = 27.14; P< 0.001) and ten (H = 16.03; P = 0.007) days from the application with the highest reduction achieved by a mixture of Lc + Bu + DA during the first and second inspection times (medians: 100 and 92.22 %, respectively). In the second spot-spray, there were significant differences among the different treatments in population reduction after three (H = 21.34; P<0.001) and ten (H = 13.42; P = 0.02) days from application, with the highest reduction achieved by a mixture of Lc + Bu + DA during the first and second inspection times (medians: 100 and 94.17 %, respectively). In the third spot spray, there were significant differences after three (H = 24.81; P< 0.001) and ten (H = 26.31; P< 0.001) days from treatment, with the highest reduction attained by a mixture of Lc + Bu + DA during the first and second inspection times (medians: 100.0 and 100.0 %, respectively).

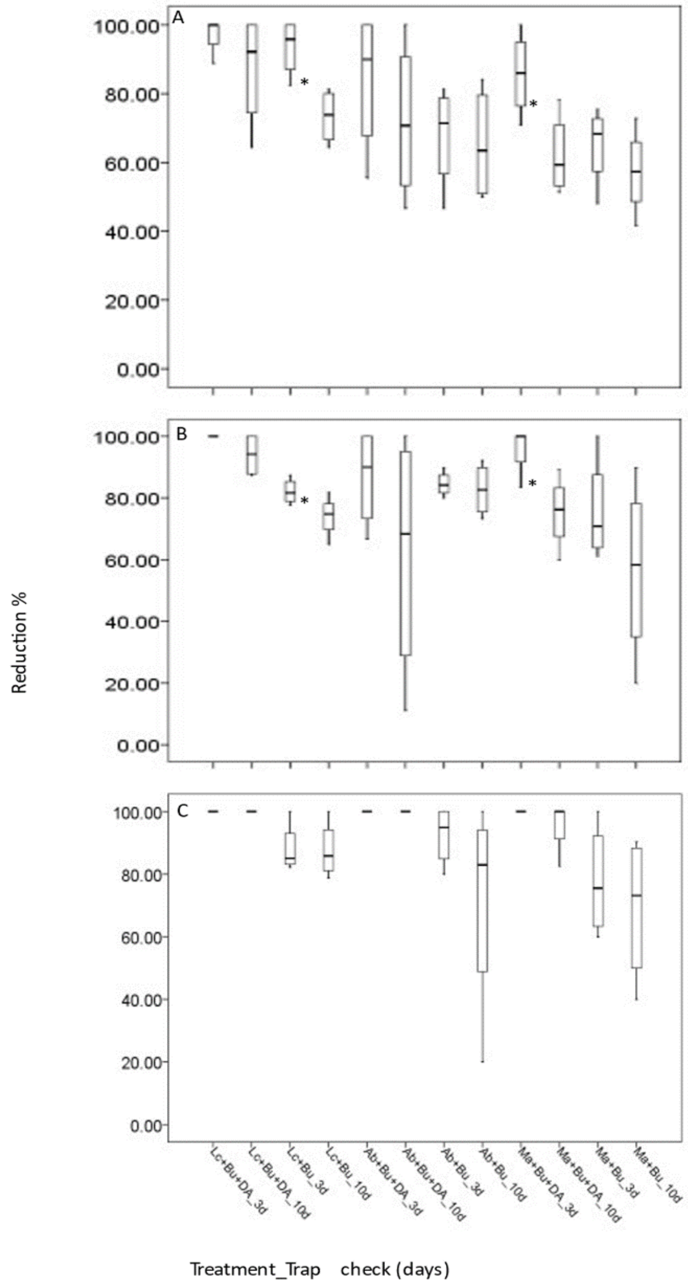

Fig.5. Effect of adding 1% di-ammonium phosphate to baits composited of Buminal and each of lambda-cyhalothrin (Lc), abamectin (Ab), or malathion (Ma), that used in spot-spray of *B. zonata*. The reduction % was estimated based on Henderson-Tilton's formula. Three successive partial-spray applications (A, B, and C), in ten-day intervals, were applied based on recommendations of the Egyptian Ministry of Agriculture. Traps were examined after 3 and 10 days from each spray event in both treated and control treatments (x-axis). Thin black lines in the box = medians. The lower and upper limits of the box represent the 25^{th} and 75^{th} percentiles, lower and upper whiskers denote the 10^{th} and 90^{th} percentiles while the lower and upper dots stand for the 5^{th} and 95^{th} percentiles. One-way ANOVA on Ranks. Pairwise bars with a star in the middle indicate a significant difference (Dunn's test, $\alpha = 0.05$).

The difference in population reductions of *B. zonata* between both inspection times within each spray application time revealed that there were no significant differences between both inspection times for the enhanced "Buminal bait + lambda-cyhalothrin" by adding 1% di-ammonium phosphate in the first (*U* = 20.0, *P* = 0.23), second (*U* = 16.0, *P* = 0.11), and third spray (*U* = 32.0, *P* = 0.1) applications. With respect to the non-enhanced lure of Buminal + lambda-cyhalothrin, there was a significant difference between both inspection times during the first (*U* = 0.0, *P*< 0.001) and second (*U* = 8.0, *P* = 0.01) spray applications, but not in the third spray application (*U* = 30.0, *P* = 0.88). With respect to both the enhanced composition of Buminal + abamectin by adding 1% di-ammonium phosphate and the non-enhanced lure of Buminal + abamectin, there were no significant differences in *C. capitata* population reductions between both times of trap check in the three successive spray times (*U* = 24.0, *P* = 0.44; *U* = 20.0, *P* = 0.23; and *U* = 23.0, *P* = 0.10, respectively). As well, there was no significant difference between both inspection times in fly population reductions by the non-enhanced lure of Buminal + lambda-cyhalothrin during the three successive spray applications (*U* = 32.0, *P* = 0.10; *U* = 28.0, *P* = 0.72; and *U* = 16.0, *P* = 0.11, respectively). The population reduction in *C. capitata* by the spot-spray of the enhanced "Buminal bait + malathion" by adding 1% di-ammonium phosphate differed significantly between the two inspection times of the first (*U* = 4.0, *P* = 0.003) and second (*U* = 4.0, *P* = 0.002), spray applications, but not in the third spray application (*U* = 24.0, *P* = 0.44). Whereas the population reductions by the non-enhanced Buminal bait + malathion did not significantly differ between both inspection times of the three spray applications (First: *U* = 20.0, *P* = 0.21; Second: *U* = 18.0, *P* = 0.16; and third: *U* = 26.0, *P* = 0.57) (Fig. 5).

DISCUSSION

The release of semiochemicals from foods plays a major role in the location and utilization of nutrition resources by tephritid flies. Ammonia is considered to be a key component of semiochemical profiles for these flies (Bateman and Morton 1981, Epsky *et al.*, 2014). However, the interactions of ammonia with other bait constituents are largely unknown (Lasa and Williams 2021), even in the case of the Buminal protein-ammonia mixtures that are commonly used to trap these fruit flies. According to Leblanc *et al.* (2010), a variety of baits take advantage of the key role that ammonia plays in fruit fly attraction by including ammonia in their formulations. For example, adding ammonium acetate to GF120®, Buminal and other protein baits significantly attracted *B. cucurbitae*, *B. dorsalis* (Hendel), and *C. capitata* flies (Piñero et al. 2011, Piñero *et al.*, 2015, El-Metwally 2018, Bayoumy *et al.*, 2021, Piñero *et al.*, 2022) and *B. zonata* (Hemeida *et al.*, 2017, Ghanim (2018). These findings support the present study, in which the addition of ammonia compounds (i.e. di-ammonium phosphate, ammonium acetate and ammonium hydroxide) to the commercial product of Buminal (the protein-based bait) led to an increase in attracting *C. capitata* and *B. zonata* flies. However, di-ammonium phosphate was the most effective additive for enhancing Buminal as an attractant for *C. capitata* and *B. zonata* adults. Hemeida et al. (2017), El-Metwally (2018), Ghanim (2018), and Ragab and Youssef (2021)found that di-ammonium phosphate was more efficient than ammonium acetate component in increasing the effectiveness of the protein-based baits, Agrinal, Amadene and GF-120, in attraction of *B. zonata* and *C. capitata*flies.On the other hand, ammonium hydroxide was the weakest compound for enhancing the attractiveness of Buminal for *B. zonata* or *C. capitate* flies. Buminal mixture that contained di-ammonium phosphate was superior to Buminal- ammonium acetate mixture and Buminal alone in attracting *B. zonata*, whereas ammonium hydroxide was a less effective compound than ammonium acetate in attracting both *C. capitata* and *B. zonata* adults (Abd El-Kareim et al. 2008, Moustafa and Ghanim 2008, Hemeida *et al*. 2017). Application of 1 and 2% (wt: vol) di-ammonium phosphate to protein baits enhanced their effectiveness in attracting both fruit flies under the conditions of this study. This amount corresponds to the amount of ammonium acetate used in GF-120 (Moreno and Mangan 2002, Piñero *et al.*, 2011), a protein bait that is very effective against fruit flies in Hawaii and other regions of the world (Mangan 2014).

Similar to many studies, increasing ammonium acetate concentrations in the Buminal bait solution, that was applied in this study, led to an increase in bait attractiveness to *C. capitata* adults in the earlier catches, whereas this was evident in later catches of *B. zonata*, where the application of 1% (wt: vol) ammonium acetate attracted higher significant numbers of *B. zonata* at the end of the experiment than of 2 and 3% concentrations. In a reverse direction, increasing the concentration of di-ammonium phosphate resulted in increased bait attraction to *B. zonata* flies in the earlier catches, but not *C. capitata*. This variation between both fruit flies in the time of their response to Buminal-ammonium mixtures may suggest that the protein-based odors eliminate or overwhelm fly attraction to ammonia and the olfactory sense organs on the fly antenna of both fly species may differ in their response to such odors. A previous study by Kendra *et al.* (2005) recorded a positive relationship between the antennal response (measured by electroantennography) of male and female *A. suspensa* and the amount of ammonia released up to a certain dose. Although attraction appears to vary across species, these findings reinforce previous observations that have indicated that the amount of ammonia released by food-based substances is less influential on the attraction of tephritid flies when other protein-derived odors are also present (Lasa and Williams 2021). The current results are also consistent with those of Moustafa and Ghanim (2008), El-Metwally (2018), Ghanim (2018), and Ragab and Youssef (2021); they found that increasing concentration of ammonium hydroxide in protein baits decreased their attractiveness to *C. capitata*, *B. zonata* and *C. incompleta* flies. In completely opposite studies to ours, Abd El-Kareim et al. (2008) and Moustafa and Ghanim (2008) found a negative relationship between concentrations of ammonium acetate and di-ammonium phosphate and bait attractiveness for *B. zonata* flies, while an opposite relationship is determined for ammonium hydroxide.

Almost, the efficiency of Buminal either alone or mixed with di-ammonium phosphate, ammonium acetate and ammonium hydroxide. Declined after six days of hanging the traps. These findings are close to those obtained by Abd El-Kareim *et al.* (2008), and Moustafa and Ghanim (2008).

Many fruit fly pests can successfully be controlled by AChE inhibitors, such as organophosphates, dimethoate, malathion, and carbamates. For example, malathion, administrated together with the attractant Naziman, is frequently used in bait sprays for the control of *C. capitata* (Róssler 1989) and *Bactrocera tryoni* (Froggatt) and *Rhagoletis* spp. (Bellas 1996). The present study discovered that lambda-cyhalothrin was a more efficient insecticide than abamectin and malathion in spot-spray applications against *C. capitata* and *B. zonata*. This toxic effect of lambda-cyhalothrin is maximized, by using Buminal baits that are enhanced by adding 1% di-ammonium phosphate, against *C. capitata* and *B. zonata*. Further, the enhanced Buminal bait almost increased the effect of spot-sprays by both abamectin and malathion against both fruit fly species. The bait application technique (BAT) consists of protein hydrolysate-insecticide bait sprays that have been used for many years against fruit flies (e.g., Róssler1989, Ruiz *et al*. 2008, Gazit and Akiva 2017). Pyrethroids have killing ability, rapid and massive knockdown effect, and prevention of oviposition for controlling *Dacus ciliatus* Loew compared with organophosphates (Maklakov *et al*. 2001). The pyrethroid, lambda-cyhalothrin showed a novel disabling effect on surviving *C. capitata* in Spain (Couso-Ferrer *et al.*, 2011) and against *B. zonata* in Egypt (El-Gendy *et al.*, 2021). While malathion was the lowest effective insecticide against *B. zonata* and *Bactrocera oleae* (Gmelin) in Tunisia (Braham et al. 2007), against *B. zonata* in Egypt (El-Aw *et al.*, 2008), and against *C. capitata*, *B. zonata* and *Bactrocera dorsalis* (Hendel) in China (Wang *et al.*, 2013). Couso-Ferrer *et al.*, (2011)

The results of this study are essential for developing an effective pest management program for controlling *C. capitata* and *B. zonata*. The addition of DA, but not AA or AH, to the commercially available Buminal bait improved bait attractiveness for both tephritid species. Nonetheless, the three olfactory attractive additives of food lures which are ammonium acetate (AA), ammonium hydroxide (AH), and di-ammonium phosphate (DA) were proved to magnify the effect of Buminal bait in trapping both fruit fly insects in citrus orchards. These findings can potentially increase the effectiveness of protein baits for fruit fly monitoring and suppression. The high potency of the pyrethroid, lambda-cyhalothrin, especially when applied together with the protein-based bait, Buminal, mixed with the olfactory attractant, DA, renders them potential agents for controlling *C. capitata* and *B. zonata*.

Declarations:

Ethical Approval: Not applicable

Competing interests: The authors declare that they have no duality of interest associated with this manuscript.

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