Original Research Article

Toxicity of different dilutions of spinosad bait in adults of the fruit-flies *Anastrepha grandis* Macquart and *Ceratitis capitata* (Wied.)

ABSTRACT

Toxic bait is an alternative to manage fruit-flies (Tephritidae) in orchards and in wide-area programmes. The mortality caused by different dilutions of spinosad baits on adults of *Ceratitis capitata* (Wied.) and *Anastrepha grandis* (Macquart) (Tephritidae) were compared with a hydrolysed protein (BioAnastrepha) and water in laboratory. Females and males of *C. capitata* and *A. grandis* were exposed to Success® 0.02CB (GF-120 NF Naturalyte fruit-fly, 0.02% spinosad) diluted in distilled water (v/v) at 1:1.5; 1:4.5; 1:9.0 and 1:18.0. Spinosad dilutions at 1: 1.5 and 1: 4.5 showed similar effects and provided 100% adult mortality 24 hours after exposure to the baits. *Anastrepha grandis* was more susceptible to spinosad baits than *C. capitata.* Females of *C. capitata* were less susceptible to spinosad diluted at 1: 1.5, 1: 4.5 and 1: 90, than *C. capitata* males and both sexes of *A. grandis.* The values of lethal times (LT_{50}) varied for the different dilutions of spinosad for both fruit-fly species; however, with different patterns for each species. The LT_{50} values of the two highest concentrations (1: 1.5, 1: 4.5) of spinosad bait were similar for fruit-flies of both sexes of each species, but a significant difference was observed between species, with higher LT_{50} values for *C. capitata*. In general, the cumulative mortalities of spinosad baits increased at 240, 360,

480 minutes and 24 hours after exposure. BioAnastrepha was shown to be toxic to both species, especially for *A. grandis*, killing 82% of females and 72% of males at 24 hours after exposure. Spinosad bait may be used in different dilutions to manage *C. capitata* and *A. grandis,* with similar toxicity for the two highest spinosad concentrations, for both fruit-fly species

Keywords: Diptera, Tephritidae, toxic bait, mortality, adulticide.

1. INTRODUCTION

The Tephritidae fruit-fly *Ceratitis capitata* (Wied.) and *Anastrepha grandis* (Macquart) cause tremendous economic losses to horticultural crops and limit international trade because of quarantine restrictions. In Brazil, *C. capitata* (medfly) was found in 93 botanical hosts [1], while *A. grandis* has been registered in nine Cucurbit hosts [2].

In the last two decades, a high number of synthetic insecticides have been banned and this fact has affected the fruit-fly (Diptera: Tephritidae) management programmes in several countries. The reduced availability of authorized chemicals has changed the current scenario of fruit-fly control with the use of insecticides in cover spray or toxic baits [3, 4, 5] in Brazil.

The organic insecticide spinosad is often used in many countries to manage fruit-flies in organic and conventional crops [6]. Spynosyns initially cause muscle contractions and tremors by exciting neurons in the nervous system. After prolonged periods of hyperexcitation, insects show postural changes and became paralysed [7]. Spynosyn A, the main active ingredient of the insecticide spinosad, presents interaction

with the calcium channel and shows a novel mode of action [8]. Spinosad bait (GF-120) is a combination attractant, feeding stimulant, and spinosad insecticide [9] for controlling many fruit-fly species [3, 10, 11, 12, 13, 14, 15, 16, 17].

Although spinosad bait has been registered in Brazil for controlling fruit-flies since 2006, few fruit growers use the bait station technology, probably due to the financial cost of application of GF-120 at 1 litre of commercial product diluted with 1.6 litres of water per hectare. The objective of the present study was to evaluate the mortality of two fruit-flies species when treated with different dilutions of the commercial product of spinosad bait in the laboratory, aiming at reducing the cost of field applications.

2. MATERIALS AND METHODS

Adults of *C. capitata* and *A. grandis* were obtained from laboratory colonies that have been maintained at the Instituto Biológico, in Campinas, State of São Paulo (SP), Brazil, since 1993 and 2002, respectively. Both species were reared as described in earlier studies [18].

We tested Success® 0.02CB (GF-120 NF Naturalyte fruit-fly, 0.02% spinosad, Dow Agrosciences Industries Ltd.) diluted in distilled water (v/v) at 1:1.5, 1:4.5, 1:9.0 and 1:18.0 in comparison with a commercial hydrolysed protein at 5% (BioAnastrepha) and distilled water (untreated control). The pH, determined with a pH meter Alphalab (model PA 200, Piracicaba, SP, Brazil), showed values of 4.23; 4.16; 4.20; 4.27; 6.93 and 8.35, respectively.

Success® 0.02CB is registered for use in 10 fruit crops against *C. capitata, Anastrepha fraterculus* (Wied.), *A. obliqua* Macquart and *Bactrocera carambolae* Drew & Hancock in Brazil (MAPA, 2017).

Five females and five males of 4 - 5 day-old *C. capitata* and 20 - 23 day-old *A. grandis* were captured in glass tubes that were then closed with cotton wool. Prior to the exposure, the tubes were stored in refrigerator at approx. -15 ºC for 4 minutes, and the flies were transferred immediately to glass Petri dishes (150 mm diameter). The insecticide suspension (2 mL) was applied with volumetric pipettes into a plastic container filled with hydrophilic cotton (190 mg). Before the returned of fruit-fly activity, the cotton was transferred by tweezers to the middle of Petri dishes.

After the treatment, the flies were maintained at room temperature (25 ± 3 ºC) and ambient humidity (50 ± 10%). Evaluations of cumulative mortality were conducted at 15, 30, 45, 60, 90, 120, 150, 180, 240, 360, 480 minutes and 21 h after initial exposure. Irreversible knockdown followed by the death of the adults was the criterion to determine mortality [20].

Each Petri dish was considered one replication per treatment (in total 10 replicates). We performed ANOVA (Sisvar, version 5.6) [21]. Three-factor ANOVA was used to compare the mortality of fruit-flies. The LT_{50} (lethal time) values for each compound were estimated by Probit analysis [22] using the Polo Plus program [23].

3. RESULTS AND DISCUSSION

Anastrepha grandis was more susceptible to spinosad baits than *C. capitata* (F = 9.91; $P > 0.0018$). There was a significant difference between the control (water) and the remaining treatments (F = 488.2; $P < 0.0001$), irrespective to the fruit-fly species and sex. Spinosad dilutions at 1: 1.5 and 1: 4.5 showed a similar effect (Tukey's test) and provided 100% adult mortality 24 hours after exposure to the bait (Table 2).

The susceptibility of fruit-flies differed by sex ($F = 45.99$; $P < 0.001$). Females of *C. capitata* were less susceptible to spinosad diluted at 1: 1.5, 1: 4.5 and 1: 9.0, than *C. capitata* males and both sexes of *A. grandis* (Table 1). In the case of spinosad diluted at 1: 18.0, males of both fruit-fly species were more susceptible than the respective females. For *Bactrocera zonata* Saunders, the lethal concentration of spinosad for males was lower than for females [24]. The dilution of Success®0.02 CB at 1: 10 (v/v) was considered inadequate for *B. zonata* control in Israel [17]. In another study, more flies of *B. dorsalis* and *Bactrocera cucurbitae* (Coquillett) also responded to spinosad bait than to water, but the responses varied markedly between females of both species [25].

The medfly insects used in the present experiment were from a pesticide-susceptible population (S) (personal communication); thus, the formulated spinosad (Success®0.02 CB) at high insecticide concentrations may not have provided the appropriate feeding stimuli to induce the fruit-fly (*C. capitata* and *A. grandis*) insects to ingest enough toxic ingredient to cause mortality, considering the non-increasing mortality rates with the concentration increase in the toxic bait, mainly for the three lowest spinosad dilutions at 240 minutes after treatment (Table 2). This hypothesis should be elucidated in future studies because this effect on the medfly behaviour can favour the development of insecticide resistance, changes in biological parameters or induce population outbreaks [26, 27, 28], especially if bait stations are established in wide-area programmes.

For the control of *Anastrepha fraterculus* (Wied.), a concentration twice high as those provided by Success®0.02 CB was required [29]. Although no medfly resistance was detected to spinosad bait in the field, selected strains of *Bactrocera dorsalis* (Hendel) were obtained in the laboratory after a few generations when spinosad was provided by topical applications [30].

BioAnastrepha is a commercial hydrolysed protein for monitoring fruit-fly in Brazil. This product was shown to be toxic to both species, especially for *A. grandis*, killing 82% of females and 72% of males at 24 hours after initial exposure (Fig. 1). BioAnastrepha exhibited a difference with the control (water) at 24 hours, when the product was more toxic to *A. grandis* than to *C. capitata* (Table 2). No mortality of medfly was detected during the experiment, when the adults were exposed to distilled water.

We obtained interactions between species versus dilutions ($F = 3.92$; $P < 0.0016$), spinosad dilutions vs time of exposure ($F = 24.72$; $P < 0.0001$) and spinosad dilutions vs sex ($F = 4.24$; $P > 0.0008$).

Spinosad diluted at 1: 1.5, 1: 4.5 and 1: 9.0 caused similar mortalities to fruit-flies of both species at 240 min, 360 min and 24 hours after exposure. No statistical differences in mortalities for both species were obtained only 24 hours after exposure for spinosad diluted at 1: 18.0 (Table 2). In this evaluation, all spinosad dilutions exhibited a similar number of dead adults considering each isolated species or between species for each spinosad dilution.

The responses of both fruit-flies varied according to the time of exposure to baits ($F = 273.40$; $P < 0.0001$). The level of cumulative mortalities provided by spinosad dilutions increased during the different exposure periods (Figs. 1 and 2). In general, the cumulative mortalities increased at 240, 360, 480 minutes and 24 hours after exposure (Table 3).

The values of LT_{50} varied for the different dilutions of spinosad for both fruit-fly species; however, with different patterns for each species. In the case of *C. capitata* (females and males), the LT_{50} values of the two highest concentrations (1: 1.5 and 1: 4.5) were similar to each other, but differed from the remaining concentrations. The

highest contrast (up to 4.1 times) was observed between the dilutions of 1: 1.5 (186.8 – 272.6 min) and of 1: 18.0 (764.5 – 606.7 min) (Table 4).

In the case of *A. grandis* (females and males), the LT_{50} values of the three highest concentrations (1: 1.5, 1: 4.5 and 1: 9.0) were similar each other, but, differed from the dilution of 1: 18:0. The highest contrast (up to 3.1 times) was also observed between the dilutions of 1: 1.5 (192.2 - 210.8 min) and of 1: 18.0 (518.0 - 649.7 min) (Table 4).

Differences between fruit-fly species and sexes were detected for the lethal times of spinosad, with higher values of LT_{50} for *C. capitata* females than those for *A. grandis* females, for the two highest concentrations of the toxic bait. The *C. capitata* males were more susceptible than the medfly females, presenting shorter lethal times, for the two highest concentrations of spinosad; however, in the case of *A. grandis,* similar values of LT_{50} were observed for both sexes (of the same species), for all evaluated dilutions of the toxic bait (Table 4).

The LT_{50} values observed for BioAnastrepha were much higher (≥5.9 times) than those observed for spinosad bait at any concentration for both sexes of medfly. For females of *A. grandis*, the LT_{50} values of BioAnastrepha were also higher (≥ 3.0 times) than those verified for spinosad bait, except for the dilution of 1: 18.

A minimum of 98% of *A. grandis* died before 480 minutes after being exposed to spinosad diluted at 1: 1.5, 1: 4.5 and 1: 9.0, while for *C. capitata*, for the same dilutions and period of time, the mortalities reached 97%, 88% and 76%, respectively (Table 3). Clearly, the highest dilutions required more time to kill the insects (Figs. 1 and 2, Table 4), probably due to the ingestion of lower quantities of spinosad.

BioAnastrepha was less toxic to *C. capitata* than to *A. grandis*, but the lethal effect was clearly visible only at 24 hours after exposure (Table 3). This product is

derived from corn hydrolysed protein and also contains fructose, glucose and sucrose and stabilizers to increase the active lifespan in the field. The cause of the toxicity of BioAnastrepha to fruit-flies is still unknown, but this effect may increase the efficacy of toxic baits (formulated with BioAnastrepha) in the field.

Considering the insecticide concentration, an advantage of higher dilutions of spinosad (like 1: 4.5 and 1: 9.0) is related to the increasing number droplets per tree, providing more spots for fruit-flies for feeding on the toxic bait in the canopy. However, other insects of local fauna, like other Diptera (predators and parasites), parasitoids (Hymenoptera), and ants, may feed on spinosad bait [31] and higher insecticide dilutions may cause a lower impact on non-target organisms in the field. Probably, spinosad treatments do not irritate or repel flies [32] of different species and may cause toxic effect on them, depending on the concentration.

Spinosad bait (89.0 ppm AI) killed females of *Rhagoletis indifferens* Curran (Diptera: Tephritidae) before they oviposit in cherry fruits [11]. However, this strategy may be affected when natural food sources or fruit exudate is available [32]. Thus, the possibility of using different dilutions of Success 0.02CB for killing fruit-flies, and consequently, to protect the fruits from oviposition in the field, deserves further investigation.

4. CONCLUSION

Spinosad bait may be used in different dilutions to manage *C. capitata* and *A. grandis* populations, with similar toxicity for the two highest spinosad concentrations (1: 1.5 and 1: 4.5), for both fruit-fly species. Higher dilutions of spinosad bait may provide similar efficacy against to fruit-flies (Tephritidae), and also may reduce the risk of repellency and application costs.

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Table 1 – Number of dead adults (mean ± SEM) of fruit flies exposed to different dilutions of spinosad bait (Success 0.02 CB) in laboratory (n=5)

Treatment		*A. grandis*		*C. capitata*	
		Female	Male	Female	Male
Success	1 : 1.5	4.23 ± 0.92aA	4.45 ± 1.04aA	3.53 ± 1.96aB	4.38 ± 1.00aA
	1 : 4.5	4.18 ± 1.11aA	4.23 ± 1.21aA	3.50 ± 1.57aB	4.25 ± 1.50aA
	1 : 9.0	3.60 ± 1.48bB	4.23 ± 1.00aA	2.80 ± 1.90bC	3.90 ± 1.28aA
	1 : 18.0	1.65 ± 2.05cB	2.08 ± 1.87bA	1.65 ± 2.02cB	2.25 ± 1.75bA

BioAnastrepha	1.35 ± 1.73cA	1.38 ± 1.50cA	1.40 ± 1.68cA	1.75 ± 1.72bA
Water	0.20 ± 0.56dA	0.15 ± 0.36dA	0.00 ± 0.00dA	0.00 ± 0.00cA

Mean numbers (±SE) in the same column followed by the same lower case are not significantly different (one-way ANOVA; $P > 0.05$). Means within rows followed by the same upper case are not significantly diffferent (one-way ANOVA; $P > 0.05$)

Table 2 – Cumulative mortality of *Anastrepha grandis* (*Ag*) and *Ceratitis capitata* (*Cc*) during four times of exposure to different dilutions of spinosad bait in laboratory (n=10)

Treatment		240 min		360 min		480 min		24 h	
		Ag	***Cc***	***Ag***	***Cc***	***Ag***	***Cc***	***Ag***	***Cc***
Success	1 : 1.5	7.10 ± 1.85aA	3.70 ± 1.25abB	7.80 ± 1.48aA	8.20 ± 1.99aA	9.90 ± 0.42aA	9.70 ± 0.67aA	10.00 ± 0.00aA	10.00 ± 0.00aA
	1 : 4.5	6.3 0 ± 2.41aA	4.40 ± 0.97aB	7.50 ± 1.96abA	7.80 ± 1.62aA	9.80 ± 0.63aA	8.80 ± 0.79abA	10.00 ± 0.00aA	10.00 ± 0.00aA
	1 : 9.0	5.60 ± 1.34aA	3.20 ± 1.13abB	5.80 ± 1.32bA	6.00 ± 2.17bA	9.80 ± 0.31aA	7.60 ± 1.58bA	10.00 ± 0.00aA	10.00 ± 0.00aA
	1 : 18.0	0.70 ± 0.82bB	2.80 ± 2.02abA	1.10 ± 0.74cB	2.80 ± 0.82dA	3.20 ± 1.14bA	2.80 ± 2.20cA	9.90 ± 0.32aA	9.30 ± 0.82aA
BioAnastrepha 5%		0.60 ± 0.70bB	2.4 ± 2.17bA	0.80 ± 0.88cB	2.90 ± 2.84cA	1.80 ± 1.23bcB	3.10 ± 2.81cA	7.70 ± 1.25bA	4.20 ± 2.6 bB
Water		0.00 ± 0.00bA	0.00 ± 0.00cA	0.00 ± 0.32cA	0.00 ± 0.00dA	0.10 ± 0.31cA	0.00 ± 0.00d	1.20 ± 1.23cA	0.00 ± 0.00cB

Mean numbers (±SE) in the same column followed by the same lower case are not significantly different (one-way ANOVA; $P > 0.05$). Mean numbers (±SE) within rows of respective transect followed by the same upper case are not significantly diffferent (one-way ANOVA; $P > 0.05$).

Table 3 - Cumulative mortality of *Anastrepha grandis* (*Ag*) and *Ceratitis capitata* (*Cc*) during four times of exposure to different dilutions of spinosad bait in laboratory (n=10)

Treatment		*Anastrepha grandis*			
		240min	360 min	480 min	24h
Success	1 : 1.5	7.10 ± 1.85 B	7.80 ± 1.48 B	9.80 ± 0.42 A	10.00 ± 0.00 A
	1 : 4.5	6.30 ± 2.41 B	7.50 ± 1.96 B	9.80 ± 0.63 A	10.00 ± 0.00 A
	1 : 9.0	5.60 ± 1.34 B	5.80 ± 1.32 B	9.90 ± 0.31 A	10.00 ± 0.00 A
	1 : 18.0	0.70 ± 0.82 C	1.10 ± 0.74 C	3.20 ± 1.14 B	9.90 ± 0.32 A
BioAnastrepha		0.60 ± 0.7 B	0.80 ± 0.8 B	1.80 ± 1.23 B	7.70 ± 1.25 A
Water		0.00 0.00 A	0.10±0.32 A	0.10 ± 0.31A	1.20 ± 1.23 A
		Ceratitis capitata			
Success	1 : 1.5	3.70 ± 1.25 C	8.20 ± 1.99 B	9.70 ± 0.67 AB	10.00 ± 0.00 A
	1 : 4.5	4.40 ± 0.97 C	7.80 ± 1.62 B	8.80 ± 0.79 AB	10.00 ± 0.00 A
	1 : 9.0	3.20 ± 1.13 D	6.00 ± 2.17 C	7.60 ± 1.58 B	10.00 ± 0.00 A
	1 : 18.0	2.80 ± 2.02 B	2.80 ± 0.82 B	2.80 ± 2.20 B	9.30 ± 0.82 A
BioAnastrepha		2.4 ± 2.17 C	2.90 ± 2.84 AB	3.10 ± 2.81 AB	4.20 ± 2.61 A
Water		0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A	0.00 ± 0.00 A

Mean numbers (±SE) within rows followed by the same upper case are not significantly diffferent (one-way ANOVA; $P > 0.05$).

Table 4. Comparison of lethal times (LT_{50}) obtained for both sex of *Ceratitis capitata* (*Cc*) and *Anastrepha grandis* (*Ag*) exposed to insecticides under cover spray in laboratory.

Treatment		Species	Sex	LT_{50} (min)	Slope ± SE	X^2	df
Success	1 : 1.5	*Cc*	Females	272.6 (253.8 – 294.3)	6.09 ± 0.55	2.54	4
		Ag	Females	210.8 (192.7 – 231.7)	3.90 ± 0.37	2.69	5
		Cc	Males	186.8 (168.9 – 204.9)	4.01 ± 0.45	2.95	4
		Ag	Males	192.2 (177.6 – 208.4)	4.69 ± 0.43	0.82	5
Success	1 : 4.5	*Cc*	Females	294.2 (268.9 – 324,6)	4.54 ± 0.50	2.11	4
		Ag	Females	214.0 (196.5 – 234.1)	4.20 ± 0.39	1.51	5
		Cc	Males	154.2 (118.6 – 181.9)	2.79 ± 0.46	1.40	4
		Ag	Males	193.4 (176.3 – 212.3)	3.80 ± 0.36	1.95	6
Success	1 : 9.0	*Cc*	Females	389.2 (352.1 – 439.2)	4.43 ± 0.49	1,57	4
		Ag	Females	282.3 (227.6 – 376.2)	4.74 ± 0.88	5.54	5
		Cc	Males	245.1 (221.0 – 271.1)	3.97 ± 0.48	1.01	4
		Ag	Males	207.4 (170.4 – 261.3)	3.56 ± 0.52	9.98	7
Success	1 : 18.0	*Cc*	Females	764.5 (674.2 – 884.9)	5.46 ± 0.60	3.04	2
		Ag	Females	649.7 (574.8 – 754.9)	4.80 ± 0.52	5.96	3
		Cc	Males	606.7 (531.8 – 712.1)	3.75 ± 0.36	4.23	5
		Ag	Males	518.0 (459.2 – 599.9)	3.99 ± 0.39	6.74	5
BioAnastrepha		*Cc*	Females	4526.7 (1719.4 – 34290.3)	0.63 ± 0.12	1.15	10
		Ag	Females	852.2 (726.7 – 1039.5)	3.36 ± 0.35	5.49	5
		Cc	Males	2770.3 (1059.3 – 25167.6)	0.50 ± 0.11	1.04	10
		Ag	Males	961.3 (768.0 – 1306.9)	2.25 ± 0.25	7.68	6

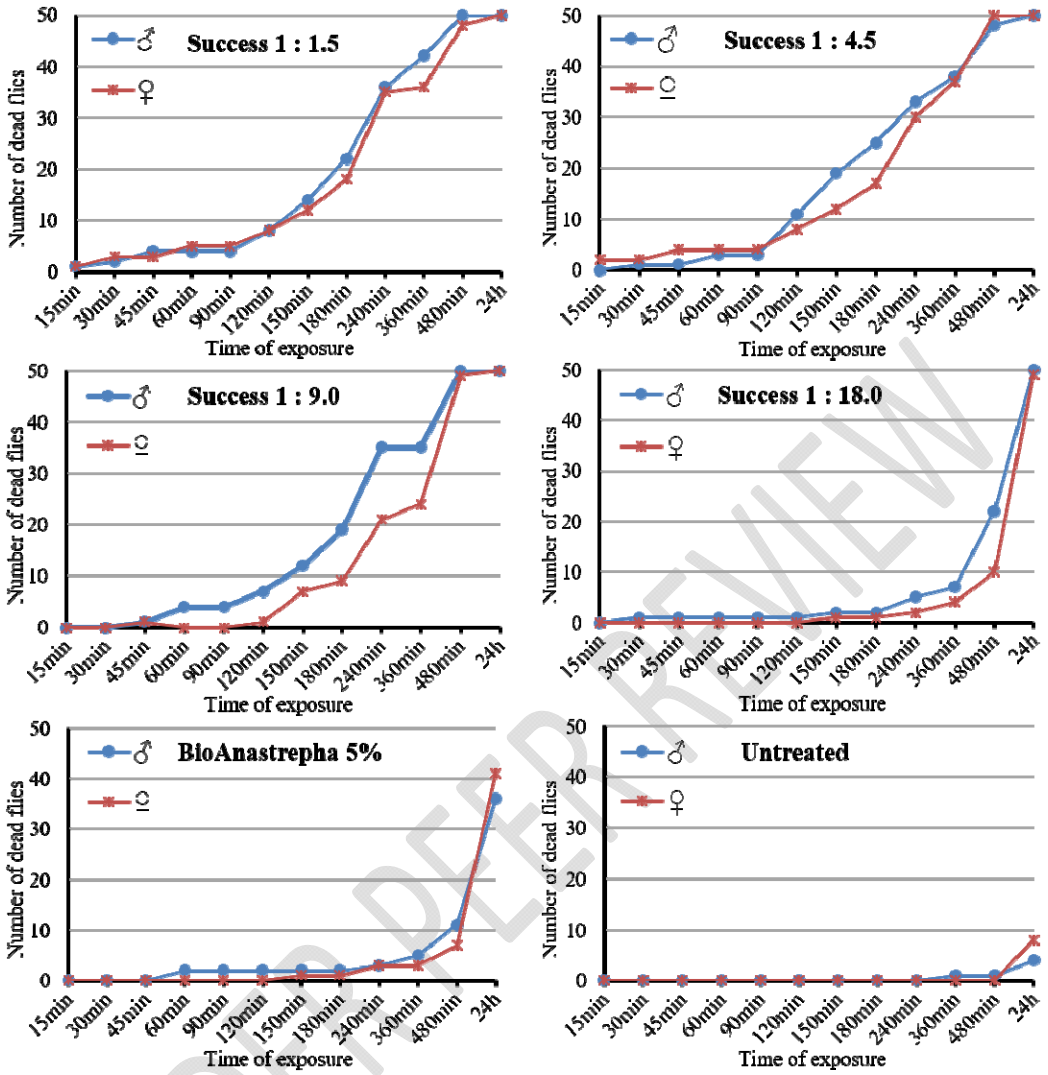

Fig. 1 – Cumulative mortality of *Anastrepha grandis* per treatment (n=50) up to 24 hours of exposure in laboratory.

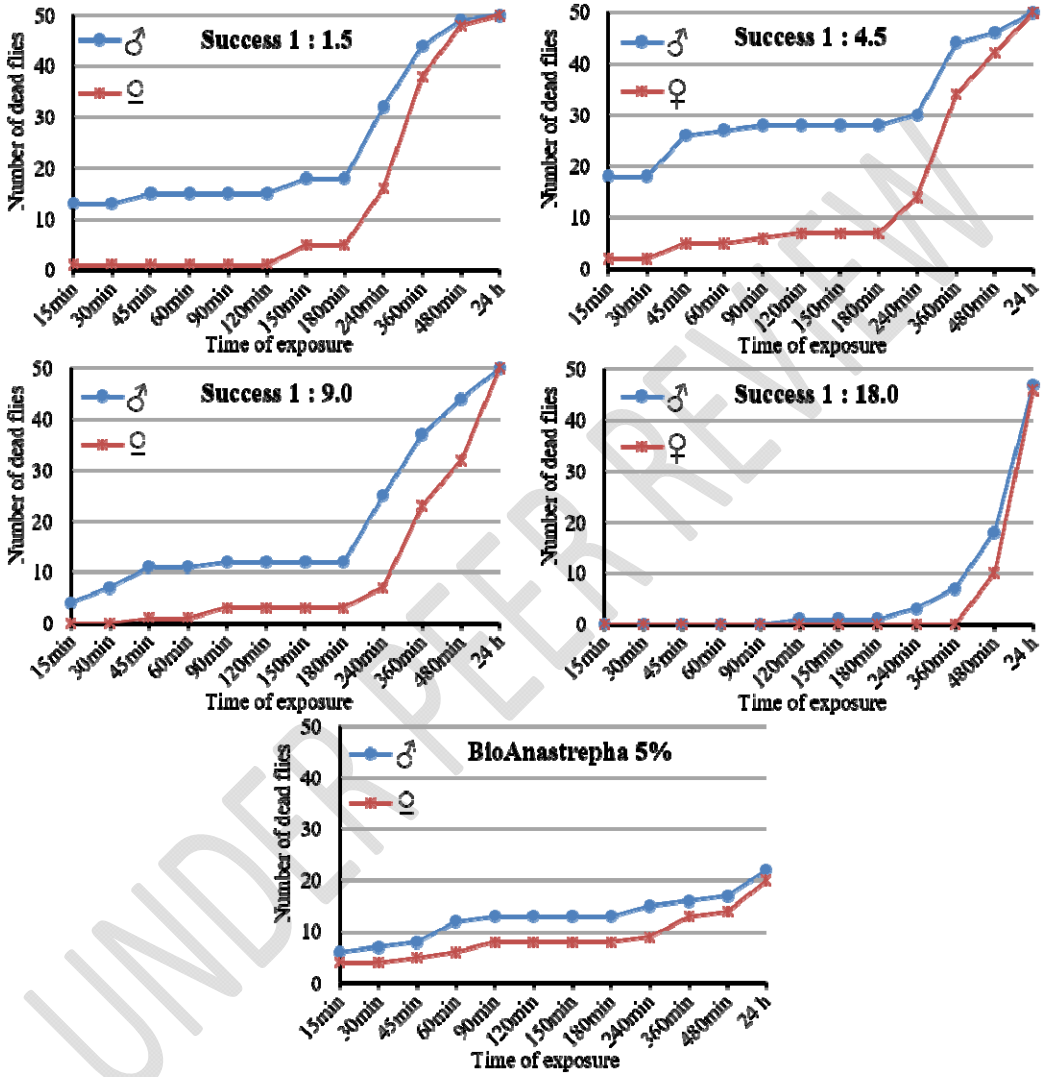

Fig. 2 - Cumulative mortality of *Ceratitis capitata* per treatment (n=50) up to 24 hours of exposure in laboratory.