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SUBLETHAL EFFECT OF TOXIC BAITS ON THE TEPHRITID FRUIT FLY PARASITOID *Fopius arisanus* (SONAN, 1932) (HYMENOPTERA: BRACONIDAE)

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ABSTRACT: Fruit flies of the Tephritidae family are among the most important invasive crop pests that have wide repercussions since their latent phase, with economic losses and rigorous quarantine limitations imposed by many countries to avoid their entry. The biological control of fruit flies using parasitoids of the order Hymenoptera has received great attention and is considered a promising control method in the integrated management of tephritid pests. Diets for feeding parasitoids are of great importance and can vary from a simple sugar solution to nutritionally complete diets, but the effect of the type of sugar may vary among parasitoids. This study aimed to evaluate the lethal and sublethal effects of different toxic bait formulations on *Fopius arisanus*, a fruit fly parasitoid. A bioassay was carried out in a completely randomized design, with four replicates using descendants of *F. arisanus* (F1) parasitoids according to the following treatments: (T1) 3% Biofruit® + malathion; (T2) 7% sugarcane molasses + malathion; (T3) 7% sugarcane molasses + Spinosad; (T4) 3% Biofruit® + Spinosad; (T5) 3% Biofruit®; (T6) 7% sugarcane molasses; (T7) commercial bait (Success® 0.02 CB); and (T8) honey (80%). The mortality rate did not reach more than 50%. In cages, the offer of treatments and the experimental conditions were the same as described in the bioassay on the effect of toxic baits on *F. arisanus*. In the F1 generation of the toxic bait bioassay for the control of fruit flies on *F. arisanus*, the parasitism percentage among treatments with offspring did not show any significant differences. Therefore, the sublethal effect of different pesticides on an organism depends on several factors, such as type of formulation, doses, ways in which the insect was exposed, and development stage.
KEYWORDS: Fruit growing, integrated pest management, biological control.

EFEITO SUBLETAL DE ISCAS TÓXICAS SOBRE O PARASITOIDE DA MOSCA DA FRUTA TEPHRITID Fopius arisanus (SONAN, 1932) (HYMENOPTERA: BRACONIDAE)

RESUMO: As moscas-das-frutas da família Tephritidae estão entre as pragas invasoras de culturas importantes, causam repercussões desde sua fase latente, causando danos econômicos diretos e necessitando de quarentena imposta por muitos países para neutralizar sua entrada. O controle biológico de moscas-das-frutas utilizando parasitoides da ordem Hymenoptera tem recebido maior atenção e é considerado um método de controle promissor no manejo integrado de pragas de tefritídeos. As dietas para alimentação de parasitoides são de grande importância e podem variar desde uma simples solução de açúcar até alimentos nutricionalmente completos, mas o efeito do tipo de açúcar pode variar entre os parasitoides. Este estudo teve como objetivo avaliar os efeitos letais e subletais de diferentes formulações de iscas tóxicas sobre o parasitoide *Fopius arisanus*. Foi realizado um bioensaio em delineamento inteiramente casualizado, com quatro repetições, utilizando os descendentes dos parasitoides *F. arisanus* (F1) dos tratamentos: (T1) Biofruit® 3% + malathion; (T2) melaço de cana 7% + malathion; (T3) melaço

de cana 7% + Spinosad; (T4) Biofruit® 3% + Espinosade; (T5) Biofruit® 3%; (T6) melaço de cana 7%; (T7) isca comercial (Success® 0,02 CB); e (T8) mel (80%), onde o percentual de mortalidade não ultrapassou 50%. Em gaiolas, a oferta de tratamentos e as condições experimentais utilizadas foram as mesmas descritas no bioensaio do efeito de iscas tóxicas sobre *F. arisanus*. Os resultados do efeito subletal na geração F1 do bioensaio demonstrou que a porcentagem de parasitismo dos tratamentos que tiveram descendentes não diferiu significativamente entre eles. Portanto, o efeito subletal de diferentes agrotóxicos em um organismo depende de vários fatores, como o tipo de formulação, as doses, a forma como o inseto foi exposto e o estágio de desenvolvimento.
PALAVRAS CHAVE: Fruticultura, manejo integrado de pragas, controle biológico.

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INTRODUCTION

Improved global trade and extended tourism and travel have led to more common incursions of quarantine plant pests. Increased trade on a global scale and the expansion of tourist travels have led to more common invasions of quarantine plant pests. Fruit flies of the Tephritidae family are among the most important invasive crop pests with wide repercussions, including direct economic losses and rigorous quarantine restrictions imposed by many countries to avoid their entry (Follett et al., 2021).

When tephritid fruit fly intrusions are identified, they typically trigger regulatory efforts to prevent the establishment or eradication of emerging populations. The discovery of an exotic fruit fly in a new region can disrupt both domestic and international trade in fresh commodities classified by quarantine National Plant Protection Organizations (NPPOs) as potential hosts of quarantine species (Paranhos et al., 2019; Szyniszewska et al., 2016)Ceratitis capitata (Wiedemann.

Among tephritids of economic importance in the Brazilian agricultural sector, the genus Anastrepha stands out, with 115 species, of which only six are particularly important. The species *Ceratitis capitata* (Wiedeman, 1824) (Diptera: Tephritidae) is also a significant pest, and its preferred hosts belong to Anacardiaceae, Myrtaceae, Rosaceae, Rutaceae, and Sapotaceae families (Reyes et al., 2012).

An option for the suppression of these species population is the use of toxic baits (Farah et al., 2020; Stoltzfus, 2008)originating from eight countries representing 51 % of US-resettled refugees for 2005-2011, living in 22 camps prior to potential US-resettlement. The corresponding camp-level nutritional survey data were evaluated. State Refugee Health Coordinators were surveyed on nutritional assessment, reporting and referrals for their US-refugee medical screenings. From 2004 to 2010, half of the camps (63 total surveys. This practice aims to reduce the infestation of adults who move into orchards from alternative hosts located close to crops (Biasazin et al., 2021)both native and invasive. Novel sustainable control methods need testing against the backdrop of smallholder-dominated farming of Africa. We evaluated the potential of male-specific attractants (parapheromones. Toxic baits are generally formulated by farmers by mixing hydrolyzed protein or sugarcane molasses with insecticides, usually organophosphates (Silva et al., 2013).

The biological control of fruit flies using parasitoids of the order Hymenoptera has received increasing attention and is considered a promising control method in the integrated management of tephritid pests. In Brazil, the most commonly collected tephritid parasitoids belong to the following families: Braconidae, Figitidae, Eulophidae, Pteromalidae, and Diapriidae, the majority belonging to the Braconidae family. In addition to these native parasitoids, in 2012, the *Fopius arisanus* parasitoid (Sonan, 1932) (Hymenoptera: Braconidae) was imported for the control of the egg and larval phases of *Bractocera carambolae* (Drew and Hancock, 1994) (Diptera: Tephritidae), a quarantine pest restricted to the states of Amapá and Roraima (Paranhos et al., 2019).

Diets for feeding parasitoids are of great importance and can vary from a simple sugar solution to nutritionally complete diets, but the effect of the type of sugar may vary among parasitoids. The use of selective products, by allowing the association with biological methods, allows the maintenance of beneficial organisms in agroecosystems, resulting in lower need for phytosanitary treatments in the field, which guarantees producers greater savings, better quality products, and less environmental impact.

However, little is known about the effects of toxic baits on biological control agents, including the egg-larvae *F. arisanus* parasitoid, and considering the potential use of this parasitoid, this study aimed to evaluate the lethal and sublethal effects of different toxic baits formulations over *F. arisanus*.

MATERIAL AND METHODS

Experiments were carried out at the Entomology Laboratory of Embrapa Clima Temperado, Pelotas -RS, and at the Integrated Pest Management Laboratory (LabMIP) of the Phytosanitary Department, at the 'Eliseu Maciel' Agronomy Faculty of the Federal University of Pelotas (UFPel).

Insects used in experiments were obtained from stocks kept in laboratory in acclimatized rooms at temperature of 25 ± 2°C, relative humidity of 70±10%, and photophase of 12 hours. *C. capitata* adults were housed in plastic cages (48 × 30 × 30 cm, length × width × height) with water and food (refined sugar, wheat germ, and brewer's yeast; 3: 1: 1) (Nunes et al., 2013) foram testadas as seguintes dietas: D1, original, com 10 g de ágar; D2, modificada, com 3,6 g de ágar; e, D3, modificada, com bagaço seco de cana-de-açúcar. Para os adultos, foram testadas quatro dietas: A, levedura de cerveja + mel (2:1. Eggs were collected, aerated, and seeded according to method proposed by Gonçalves et al. (2013). Approximately 9200 eggs per container (0.5 ml of solution) were seeded in a 300 ml artificial diet. The larval development diet and insect reproduction at the pupal stages followed the proposal of Salles (1992).

Data from Farah et al. (2020) were used to calculate the sublethal effect. Data from the effect of toxic baits and food-based attractants for *C. capitata* on *F. arisanus* were used to calculate the sublethal effect. The parasitism percentage after 24 hours using toxic baits was evaluated. Therefore, treatments consisted of:

T1: 3% Biofruit® + malathion
T2: 7% sugarcane molasses + malathion
T3: 7% sugarcane molasses + Spinosad
T4: 3% Biofruit® + Spinosad
T5: 3% Biofruit®
T6: 7% sugarcane molasses
T7: commercial bait (Success® 0.02 CB)
T8: honey (80%)

Treatments in the bioassay contained pesticides, as indicated in the list. The following pesticides were used in treatments: Malathion: an organophosphate insecticide toxic to both fruit flies and parasitoids. Spinosad: a biopesticide derived from a soil bacterium and toxic to fruit flies but not to parasitoids.

The bioassay was carried out in a completely randomized design, with four replicates using *F. arisanus* descendants (F1) of the following treatments: hydrolyzed protein (Biofruit® 3%); sugarcane molasses (7%); commercial bait Success® 0.02 CB and honey (80%) (control), where the mortality rate did not reach more than 50%. In cages, treatments and experimental conditions used were the same as described in the bioassay on the effect of toxic baits on *F. arisanus*.

The variables evaluated were parasitism percentage (P) and the sex ratio (rs). In addition to these biological parameters, to evaluate the sublethal effect of different toxic baits, the initial and final parasitism percentage of *F. arisanus* on *C. capitata* eggs was also compared.

To determine the initial parasitism percentage of *F. arisanus* on *C. capitata* eggs, after the end of the 24-hour exposure period, 50 eggs were transferred to an acrylic plate (4 cm in diameter) containing 2.5% sodium hypochlorite solution (Moretti and Calvitti, 2003). After approximately 2 minutes, the solution was removed with distilled water using a pipette. In this process, the chorion from host eggs was removed. Using a brush, eggs were placed on a plate under binocular stereomicroscope (32x magnification) to observe *F. arisanus* eggs within the host (Wang and Messing, 2003).

Data obtained from the parasitism percentages, and initial parasitism sex ratio were submitted to analysis of variance (ANOVA), and when significant, means were compared using the Tukey test. For the sex ratio, the mean proportions of treatments were compared using the Pearson's chi-square test, which was performed considering the observed frequencies of females in relation to the observed frequency of the entire population (males + females). Analyses were performed using the R statistical software (R Core Team, 2022). The significance level of tests was $\alpha=0.05$.

RESULTS AND DISCUSSION

The results of the sub-lethal effect in the F1 generation of the toxic bait bioassay for the control of fruit flies on *F. arisanus*, showed that the percentage of parasitism of treatments with offsprings did not differ significantly between them. However, the parasitism percentages of *F. arisanus* on *C. capitata* eggs in 80% honey (control) were not significantly different from Biofruit® (3%), sugarcane molasses (7%), and Success® 0 .02 CB treatments (Table 1).

Table 1. Parasitism percentage, initial parasitism, and sex ratio of *Fopius arisanus* in the sublethal effect of different toxic baits for the control of *Ceratitis capitata*.

Treatment	Parasitism (%)1	Initial Parasitism (%)1	Sex ratio
Biofruit®	26.31 ± 3.24 a	37.50 ± 3.31 a	0.51 ± 0.04 ns
Sugarcane Molasses	29.25 ± 3.08 a	38.75 ± 3.01 a	0.49 ± 0.03
Success® 0.02 CB	26.38 ± 3.68 a	34.63 ± 5.09 a	0.47 ± 0.03
Honey	27.00 ± 2.18 a	40.50 ± 3.36 a	0.50 ± 0.02

[1]Means followed by the same letter in the column do not differ by the Tukey test (α=0.05); ns did not differ from each other (in the column) by the Pearson's Chi-square test (α=0.05).

For the initial parasitism percentages, no significant differences were observed between Biofruit®, sugarcane molasses, Success® 0.02 CB and 80% honey (control) (Table 1). For the sex ratio variable, Biofruit® (3%), sugarcane molasses (7%) and Success® 0.02 CB treatments had no significant difference from each other (Table 1).

For the parasitism percentage of *F. arisanus* on the F1 generation, no significant differences were observed among treatments, and honey (control) (27%) and Biofruit® (3%) (26.31%), sugarcane molasses (7%) (29.25%) and Success® 0.02 CB (26.38%) treatments were similar to each other (F= 0.20; GL= 3; P= 0 .05). Thus, *F. arisanus* parasitism for all treatments remained at the same level as control on different days of offering of *C. capitata* eggs without negative impacts on the normal parasitoid development.

The sublethal effect of different pesticides on an organism depends on several factors, such as type of formulation, doses, ways in which the insect was exposed, and development stage. For example, Vanaclocha et al. (2013) studied the lethal effects of freshly applied and laboratory-aged residues of 18 selected pesticides recommended for integrated pest management in citrus on *Aphis melinus* adults. The authors found that the pesticides could be divided into four groups based on their toxicity:

Harmful and persistent: These pesticides caused high mortality to *A. melinus* adults and their residues remained toxic after several days.

Moderately harmful and slightly persistent: These pesticides caused moderate mortality to *A. melinus* adults and their residues remained toxic for a few days.

Slightly harmful and moderately persistent: These pesticides caused slight mortality to *A. melinus* adults and their residues remained toxic for a short period of time.

Harmless: These pesticides did not cause mortality to *A. melinus* adults.

Roubos et al. (2014) studied the relative toxicity and residual activity of insecticides used in blueberry IPM and the mortality of natural enemies. The authors found that broad-spectrum insecticides caused high mortality to all biocontrol agents, but approved products for use in organic agriculture had little effect.

In the present work, the offspring of Biofruit® (3%), sugarcane molasses (7%) and Success 0.02 CB® females of the toxic bait effect test for the control of fruit flies in *F. arisanus* were evaluated and compared to control. The parasitism percentage and the sex ratio showed no significant differences. Van Nieuwenhove et al. (2012) observed that when the ready-to-use toxic bait (GF-120) was offered to the *Diachasmimorpha longicaudata* parasitoid, it did not present negative effects on the progeny, compared to honey, and did not differ significantly in longevity and reproduction.

Using Spinosad-based baits, Castilhos et al. (2019) found that toxic baits composed of attractants added of spinosad and Success® 0.02 CB are harmful to *Trichogramma pretiosum*, with reductions in the parasitism capacity up to 87%.

The trial also evaluated the initial parasitism percentage and compared to 80% honey (control) (40.50%), Biofruit® (3%) (37. 50%), sugarcane molasses (7%) (38.75%), and Success® 0.02 CB treatments (34.63%), it did not show significant difference, but compared to the parasitism percentage in the emergence phase, the reduction was on average 10% for each treatment.

Paranhos et al. (2021)Anastrepha fraterculus (Wiedemann observed that *C. capitata* eggs and larvae when offered to *F. arisanus* had mortality rate that reached 90%, with only 30% of eggs being parasitized by females; therefore, mortality was attributed to trauma performed by parasitoid ovipositors. Montoya et al. (2009) observed that *Anastrepha ludens* eggs (Loew, 1873) (Diptera: Tephritidae) were particularly sensitive to the parasitism action of *F. arisanus*, reducing egg hatch by 46%.

In addition to trauma caused by the action of the ovipositor, the egg hatching reduction or increased host larvae mortality can also occur when there is super-parasite activity, and the host does not offer the necessary resources for the development of more than one individual (Abou El-Ela et al., 2021). The results of the sublethal effect on the F1 generation showed that the parasitism percentage of treatments with offspring did not differ significantly from each other. The Success® 0.02 CB formulation did not show sub-lethal effect on *F. arisanus* adults. The parasitism of *F. arisanus* for all treatments remained at the same level as the control, without negative impacts on the normal parasitoid development.

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