ACTIVITY OF TOBACCO AND GARLIC AQUEOUS EXTRACT ON *Duponchelia fovealis* ZELLER (LEPIDOPTERA: CRAMBIDAE)

PRATISSOLI, Dirceu [1]
DAMASCENA, Alixelhe Pacheco [2]
ARAÚJO JÚNIOR, Luis Moreira de [3]
BUENO, Regiane Cristina Oliveira de Freitas [4]
CARVALHO, José Romário de [5]
PETRUCCI, Kharen Priscilla de Oliveira Salomão [6]

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SUMMARY: The *Duponchelia fovealis* caterpillar was recently found in commercial planting of strawberry and it is a problem creator to the culture. Because it is a recent pest, there is no evidence of products for its control. This study aimed to evaluate the efficacy of tobacco and garlic aqueous extracts looking to adopt them as alternative methods for the control of *D. fovealis.* Sixty insects /treatments were used to perform the bioassays for each larval phase and the respective aqueous extracts were applied at a concentration of 10% (m/v), totalizing 4 treatments/extract. The chemical product Pirate® (Chlorfenapyr), was used as a negative witness, and positive witness was pulverized with sterile distilled water (SDW), all treatments delivered with help from a Potter tower. The experiment was conducted in air-conditioned chamber (25 ± 1 ºC, RH 70 ± 10% and photophase of 12h) for seven days, with daily assessments to determine insecticide activity of each treatment. It was estimated the lethal concentration (CL_{50}) of the aqueous extract from tobacco since it presented mortality of over 85% in susceptibility test, analyzing first and second development phases. It is concluded that the tobacco extract can be a viable alternative in phytossanitary management of *D. fovealis*.

Keywords: Insecticide plants; Phytossanitary pest management; Strawberry.

ATIVIDADE DE TABACO E EXTRATO AQUOSO DE ALHO SOBRE *Duponchelia fovealis* ZELLER (LEPIDOPTERA: CRAMBIDAE)

RESUMO: A lagarta *Duponchelia fovealis* foi recentemente encontrada em plantios comerciais do morangueiro no estado do Espírito Santo ocasionando grandes problemas à cultura. Por ser uma praga recente, não há registro de produtos para o seu controle. Assim, este trabalho teve como objetivo avaliar a eficiência do uso dos extratos aquosos de alho e fumo visando sua adoção como métodos alternativos de controle de *D. fovealis.* Para execução dos bioensaios foram utilizados 60 insetos/tratamento para cada ínstar larval e os respectivos extratos aquosos foram aplicados na concentração 10% (m/v), totalizando 4 tratamentos/extrato. O produto químico Pirate® (Clorfenapir), foi utilizado como testemunha negativa, e na testemunha positiva foi pulverizada água destilada estéril (ADE), todos os tratamentos foram pulverizados com o auxílio de uma Torre de Potter. Posteriormente, o experimento foi acondicionado em câmara climatizada (25 ± 1 ºC, UR de 70 ± 10% e fotofase de 12h) durante 7 dias, sendo realizada avaliações diárias para determinar a atividade inseticida dos respectivos tratamentos. Estimou-se apenas a concentração letal (CL_{50}) do extrato aquoso de fumo, pois apresentou mortalidade superior a 85% no teste de suscetibilidade, analisando o 1º e 2º ínstar. Conclui-se que o extrato de fumo pode ser uma alternativa viável no manejo fitossanitário de *D. fovealis*.

Palavras-Chave: Plantas inseticidas. Manejo Fitossanitário de Pragas. Morangueiro.

[1] ORCID- ID -https://orcid.org/0000-0003-4485-1491. Universidade Federal do Espírito Santo.
[2] ORCID- ID - https://orcid.org/0000-0003-1374-5119. Universidade Federal do Espírito Santo.
[3] ORCID- ID - https://orcid.org/0000-0003-3354-9465. Universidade Federal do Espírito Santo.
[4] ORCID- ID - https://orcid.org/0000-0002-6210-8231. Universidade Estadual Paulista.
[5] ORCID- ID - https://orcid.org/0000-0003-0757-7817. Universidade Federal do Espírito Santo.
[6] ORCID- ID - https://orcid.org/0000-0002-7054-2257. Universidade Federal do Espírito Santo.

INTRODUCTION

The culture of strawberry is a typically related to smaller properties once it is considered as a family business, presenting itself as important economic and social source of income and fixating in agricultural zones (PIROVANI *et al*, 2015).

Nonetheless, the loss in production of strawberry can bring a number of downfalls, especially since expenses with agricultural input such as insecticides used in the management of many insect-pests. Recently, a new species of insect-pest was found in the Brazilian state of Espirito Santo, *Duponchelia fovealis* (Lepidoptera: Crambidae), which feeds off of all vegetative parts of the strawberry, including its fruits. Because this is a new pest, there is no registry of biological or chemical products to control *D. fovealis* (PIROVANI *et al.*, 2015).

Though the contribution of synthetic pesticides in production of food is known, the indiscriminated use and non-specification in dosage and time have brought negative effects on people and the environment (MAIRESSE; COSTA, 2009). However, the growing search for healthy foods and free from residuals has determined the dimensioning of production systems once the quality of fruit has been a demand from the market, beyond the external aspect, assuring internal quality (MAIRESSE; COSTA, 2009).

Therefore, research and development of alternatives to conventional insecticides are necessary and vital to the importance of the production chain. Among those alternatives is the use of vegetable extracts as a potential to reduce loss to the environment and humans, since the impact is softer on the environment, beyond advantages such as rapid degradation, lower effect on non-target organisms and the environment, more safety for the consumer and the environment (MAIRESSE; COSTA, 2009).

Numerous botanical pesticides have been in the market for the past 10 years (TIILIKKALA *et al.*, 2011). More promising insecticide plants are in Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae, Canellaceae, Myrtaceae and Piperaceae families, but other plants with insecticide effect are highlighted and gained space such as tobacco (*Nicotiana tabacum* L.) (Solanaceae) and garlic (*Allium sativum* L.) (Liliaceae) presenting insecticide activity on mining, sucking, drilling, and or chewing insects (AGUIAR-MENEZES, 2005, JACOB, 2017, HOLTZ *et al*. 2019).

In this context, it is believed that the use of plants with insecticide properties such as garlic (*Allium sativum*) and tobacco (*Nicotiana tabacum* L.) can be promising in the control of *D. fovealis*, allowing lower environmental impact and that are compatible with phytosanitary pest management programs. Therefore, the aim of this study is to assess insecticide activity of aqueous extracts of garlic and tobacco on *D. fovealis*,

MATERIAL AND METHODS

This study was conducted at the Nucleus for Scientific and Technological Development in Phytosanitary Management (NUDEMAFI) at the Agronomic Sciences Center at the Federal University of Espirito Santo, Brazil (CCA-UFES), Alegre-ES, in a climatized adjusted for temperature of 25 ± 1 °C, 60 ± 10% relative humidity and photophase of 12h.

Attainment and breeding of *Duponchelia fovealis* Zeller (Lepidoptera: Crambidae)

Collectiom of *D. fovealis* was conducted in strawberry plantations in the region of Espírito Santo. Caterpillars were collected, isolated, and sent to the Entomology Department at NUDEMAFI for posterior breeding.

The methodology used for insect breeding was developed by NUDEMAFI, where adult *D. fovealis* were kept in PVC tubes (200 mm x 20 cm) covered internally with A4 paper. The superior extremity of the tube was closed with voile. To feed adults, each tube had a container with 5mL aqueous honey solution at 10% covered with a bunch of cotton and paper. The laying was collected daily and kept in plastic containers until caterpillars' outbreak. Then, the newly out broken caterpillars were transferred to clear plastic containers (16 cm diameter x 10 cm height) with perforated lids to allow gas exchanges. The bottom of the containers was covered in wavy paper and over those a piece of screen to avoid contact between the diet and the bottom of the container. Therefore, over the screen thin slices of artificial diet were offered, adapted to a soy bean flour, wheat germ, and sugar for the entire larvae stage. The insects remained in those containers until they reached pupae stage.

Attainement of aqueous extract from tobacco and garlic

In order to obtain the garlic vegetable powder, garlic bulbs were cultivated in the Espirito Santo highlands. The material was peeled and cut into small pieces and spread on aluminum trays covered with aluminum foil and taken to a forced circulation greenhouse at a temperature of 40 °C until constant weight was reached to conserve its chemical characteristics. After drying the material was powdered in a mill and kept in glass containers with twist lids covered in aluminum foil to avoid loss by photo degradation. For vegetable material from tobacco, industrialized rope tobacco was used and the material shredded, dried, mashed, and conserved the same as the garlic extract.

For each extract, the powder from vegetable material (100 g) was transferred to an Erlenmeyer (1L) containing sterile distilled water (SDW) (900 mL) to obtain 1L of initial solution at 10 % (m/v). Then, they were kept under homogenization for 24h in a transversal agitator (240 rpm). After that time, the mixture was filtered with voile cloth and transferred to a volumetric balloon and volume measured for 1L.

Susceptibility of *D. fovealis* to aqueous extracts of tobacco and garlic

For each stage, aqueous extracts of tobacco and garlic at 10% concentration (m/v) were used for susceptibility bioassays as described previously. Each treatment was composed by six repetitions with 10 caterpillars per repetition, a total of 60 insects per treatment. Each repetition was kept in a Petri dish (9.5 cm diameter and 1.5 cm height) covered with filter paper. The caterpillars were transferred to each dish which contained a Tudla strawberry leaf of 4.5 cm diameter, previously pulverized using the Potter Tower with pressure of 15 lb/pol². For each formulate, 12mL of solution were pulverized being 6mL to each side of the leaf. The insecticide-acaricide Pirate® (Clorphenapyr) was used as a negative witness since it presents a harmful effect on *D. fovealis* in preliminary assays at the dosage of 50µL product for 200 mL sterile distilled water (SDW) and positive witness was pulverized with SDW only. The dishes were kept in a climatized chamber (25±1ºC, RH 70±10% and photophase of 12h). The insecticide effect was evaluated daily until the seventh day. The pulverized leaf discs were replaced when necessary.

The experiment was conducted in a random design with parcels subdivided 2 x 4 x 2 (aqueous extracts of tobacco and garlic at 10 % (m/v) x phases (stages) x repetitions), and the data transformed by $\sqrt{(x+1)}$. To confirm the efficiency in the test, it was performed in two different instances. The corrected mortality was calculated according to the witness by the Abbott formula (1925). The data were submitted to variance analyses with means compared by Tukey test ($p \leq 0.05$). The software Assistat was used for these procedures.

Estimated lethal concentration (LC)

For estimation of LC_{50} and LC_{90}, the aqueous extract with better insecticide action on *D. fovealis* and the phase of higher susceptibility was submitted to the bioassay. Each treatment was composed by six repetitions and 10 caterpillars per repetition, a total of 60 insects per treatment. The aqueous extract was pulverized 6mL on each side of the leaf. The pulverizations were conducted on Petri dishes (9.5 diameter x 1.5 cm height) covered with filter paper using Potter Tower with pressure of 15 lb/pol² corresponding to an average volume of 1.62 mg/cm^2. Logarithmic spaced concentrations were used (between the limits of 0.01% to 10%). The LC curves for the aqueous extract of tobacco were composed by 500 and 460 caterpillars respectively at first and second repetition. The witness took sterile distilled water in 0% concentration (v/v). The experiment was conducted in a climatized chamber (25 ± 10 °C, RH 70 ± 10 % and photophase of 12 h) and evaluated daily until the 7º day, to verify the lethal effect on the caterpillars. The lethal concentrations were estimated using Probit analysis. In face of these results the ideal dosage was estimated in the software Polo Pc.

RESULTS AND DISCUSSION

For corrected mortality of *D. fovealis* no significant differences were observed between the phases (parcels), products (subparcels), and experiments (sub-subparcels) (F=0.13; P>0.05) (Table 1). This indicated that the effect of the experiments factor was not consistent in all combinations with the other factors, indicating the extracts/products effect on the insects was similar for both experiments.

Table 1 - Corrected mortality (%) at different larval stages of *Duponchelia fovealis* fed with Tudla strawberry leaves treated with aqueous extract of garlic (*Allium sativum*) and Tobacco (*Nicotina tabacum*) and commercial insecticide Pirate (25±1°C, RH 70±10% and photophase of 12 h) in two repetitions (Experiments).

Extracts/product	Stage	Experiments^{NS}	
		Assessment: 12 hours	Assessment: 7º day
Garlic	1	34.90	35.93
Garlic	2	14.11	17.70
Garlic	3	15.97	13.70
Garlic	4	14.01	17.34
Tobacco	1	100.00	96.49
Tobacco	2	89.04	86.40
Tobacco	3	11.70	14.23
Tobacco	4	10.33	14.60
Pirate	1	100.00	100.00
Pirate	2	100.00	96.61
Pirate	3	86.02	76.19
Pirate	4	28.35	28.07
CV%-a[1]		26.33	
CV%-b[2]		26.45	
CV%-c[3]		22.59	
$F_{int, axbxc}$		0.1271	
GLRc[4]		60	

P	>0.050

NS Non-significant with F-test for analysis of variance within each combination of products and stage (lineage) (p≤0.05);

[1] Coefficient of variation of parcel (Stages);

[2] Coefficient of variation of subparcel (products);

[3] Coefficient of variation of subsubparcel (experiments);

[4] Degrees of freedom of residual subsubparcel.

In the susceptibility test it was found there was significant differences (F=11.77; p<0.001) among all combinations of stages and products (Table 2). Analyzing each extract/product separately we could observe the caterpillars presented higher susceptibility in garlic extract only in the first stage, when compared to other stages. For tobacco extract, the lethal effect in the first two stages was higher than 85%. Chemical product was effective in the three initial stages with mortality higher than 80%.

The insecticide activity presented by the aqueous garlic extract (*A. sativum*) at the first stage may be related to the composition. Mono and sesquiterpenoids, triterpenoids and steroids and various other sulfur compounds such as allicin and thiosulfinates as well as non-sulfur compounds such as saponins and phenolic acids were found by Lins *et al.* (2012). Other active ingredients present in garlic such as joene, free phosphoric acid, volatile oil, sulfur and oxygenated essences, aliina, allyl sulfide, allyl sulfide, aliglucosium, diallyl oxide disulfide, alinase, alithiamine, sulfides, hormones, resins and isoticionic compounds, inulin, nicotinamine and glalantamine. Among garlic components, allicin has to be highlighted for being considered the substance with the higher biological activity. In reality, the biological activity is closely related to the complexation of alinase and aliina, a process that forms allicin, responsible for the typical aroma of garlic and that works as a defense mechanism for the plant against herbivores (LAWSON ; WANG, 2005; LEDEZMA ; APITZ-CASTRO, 2006, TALAMINI ; DENLOYE, 2018). However, the concentration of allicin may interfere in its efficacy as a bioinsecticide since it is a volatile molecule and not soluble in aqueous solutions, also highly unstable converting quickly in to mono-, di-, and trisulfate and other components such as joene (SINGH ; SINGH, 2008).

In the susceptibility assessment for products at each stage, it was possible to verify that the effect of the tobacco extract and the insecticide were statistically superior to the garlic extract at the 2nd stage with mortality over 85%. The higher percentage of mortality were for those treated with tobacco extract and the chemical product Pirate®. For the tobacco extract and the product (Pirate®) mortality was higher than 98.24% at stage 1. At stage 2, mortality of 87.72%

for tobacco extract and 98.3% for the product (Pirate®). At stage 3, the percentage of mortality for the chemical product Pirate® was statistically superior to that of the extracts, keeping efficiency higher than 80%. When the susceptibility was evaluated at the last stage (fourth), the aqueous garlic extract was statistically similar to the values presented for the tobacco extract and the insecticide.

Table 2 - Susceptibility (percent of mortality) for different larval stages of *Duponchelia fovealis* fed with Tudla strawberry leaves with aqueous garlic extract (*Allium sativum*) and tobacco (*Nicotina tabacum*) and commercial insecticide Pirate (25 ± 1 °C, RH 70 ± 10 % and photophase of 12 h) in two repetitions (experiments).

Stages	**Extracts/Product**		
	Garlic	**Tobacco**	**Pirate**
1	35.42 bA[1]	98.24 aA	100.00 aA
2	15.86 bB	87.72 aA	98.30 aA
3	14.81 bB	12.93 bB	81.03 aA
4	15.63 abA	12.38 bA	28.21 aA
$F_{int, axb}$		11.7681	
GLRb²		45	
P		<0.001	

¹ Means followed by the same letter, lowercase in line and uppercase in column, were not statistically different by Tukey test (p≤0.05);

² Degrees of freedom of residual of subparcel.

The available information on toxicology, mode of action, characterization, and the effects in the ecosystem for most botanical insecticides are scarce, though most are used for over a decade. In the last years, research aimed at presenting the potential for plants extracts and essential oils as bio insecticides and it has shown important results, beyond the fact they present no toxicity to humans and animals and are biodegradable (JACOB *et al*., 2017; MAMOON-UR-RASHID *et al*., 2018; HASSAN ; HAYAT ZADA, 2018).

The efficiency of tobacco extract for *D. Fovealis* in the first stages can be attributed to the presence of substances such as nornicotine, anabasine and nicotine present in higher concentrations. Nicotine is a toxin that acts in the central nervous system of the insect, acting to limit the bond of acetylcholine, competing for its receptors in the postsynaptic membrane in the neuromuscular junction, generating new impulses that cause contractions, spasms, convulsions, and, finally, death (WIESBROOK, 2004; AGUIAR-MENEZES, 2005; CELIS *et al*., 2008). Moreover, there have been studies reporting the efficacy of aqueous extract of *Nicotiana tabacum* L. (tobacco powder) (10% m/v) on *Ascia monuste orseis* (Lepidoptera: Pieridae) and *Plutella xylostella* caterpillars (BOIÇA JÚNIOR *et al*, 2005). Other studies verified that newly hatched

Spodoptera frugiperda caterpillars ate tobacco leaves and presented only 4% survival, not making to pupae phase (SÁ *et al.*, 2009). Overall, independent from the concentration of aqueous tobacco extract the efficiency is intimately related to the exposure time of the plague to the extract (MAMOON-UR-RASHID *et al.*, 2018).

In the market, insecticides known as neonicotinoids, for example Imidacloprid, Tiacloprida, Nitempiram, Acetamiprida, Tiametoxam among others have the active principle synthetic copies ou those due to nicotine structure (CELIS *et al.*, 2008). Some commercialized products present in their liquid concentrated formulation about 40% of nicotine sulfate, diluted and pulverized (WIESBROOK, 2004, AGUIAR-MENEZES, 2005).

The estimated lethal concentration (LC_{50}) was only performed for the aqueous tobacco extract showing mortality over 90% at stage 1 of development. The evaluations were adequated to the Probit model, presenting a significant chi-squared (χ^2 non-significant, $p > 0.05$) (Table 3). For the aqueous garlic extract it was not possible to estimate LC_{50} due to the low mortality observed in the bioassay, not reaching the minimum requirements of the Probit model. The curve inclinations for concentration-mortality present similarities in two of the experiment repetitions (0.82 and 0.81), with no differences being observed in the confidence interval analysis. The lethal concentration required to reach 50% mortality of the *D. fovealis* population varied from 1.66 and 2.18 % m/v.

Table 3 - Estimation of the LC_{50} of Aqueous Tobacoo Extract in *Duponchelia fovealis* caterpillars at stage 1 in development (25 ± 1 °C, RH 70 ± 10% and photophase of 12 h) in two repetitions (Experiments), fed with Tudla strawberry leaves.

Prod[1]	**E[2]**	**N[3]**	**Inclination ± SE[4]**	**LC_{50}[5] (CI[6] 95%)**	**χ^{2} (7)**	**DF[8]**	**P[9]**
Tobacco	1	500	0.82±0.08	1.66 (0.68 – 7.40)	28.51	7	0.202
Tobacco	2	460	0.81±0.08	2.18 (0.99 – 7.86)	16.51	7	0.137

1 Prod: Aqueous tobacco extract;

2 E: experiments through time;

3 N: number of observations;

4 Inclination ± SE: curve inclination ± standard erro;

5 CL: Lethal concentration

6 IC: Confidence intervals;

7 X^2: Chi-squared;

8 DF: degrees of freedom;

9 P: probability.

CONCLUSION

The tobacco extract can be a viable alternative to phytossanitary management of *D. Fovealis* considering its use in properties that adopt agroecological management since the extract is easy to obtain and low cost to farmers planting strawberry.

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