HOLOS
ISSN 1807 - 1600

DELETERIOUS EFFECT OF VICILINS FRACTIONS OF LEGUMINOUS SEEDS *in vitro* AND IN SEMI-FIELD CONDITIONS FOR *Ceratitis capitata*

C. S. MACEDO[1], A. F. J. FRANÇA[2], L. L. P. MACEDO[3], T. M. L. AMORIM[4], R. R. O. ARAÚJO[5], Y. Q. SANTOS[6], G. S. C. CARELLI[7], A. F. UCHOA[8], F. P. MACEDO[9], A. S. OLIVEIRA[10], E. A. SANTOS[11], J. N. ARAUJO[12]
Instituto Federal de Ciência e Tecnologia do Rio Grande do Norte[1], Universidade Federal do Rio Grande do Norte[2, 5, 7, 8, 9, 10, 11, 12], Empresa Brasileira de Pesquisa Agropecuária[3], Universidade Federal do Piauí[4], Instituto Federal do Ceará[6],
ORCID ID: https://orcid.org/0000-0001-8233-7915[1]
cleysyvan.macedo@ifrn.edu.br[1]

Submetido 23/03/2021 - Aceito 10/09/2021

DOI: 10.15628/holos.2021.12213

ABSTRACT

Fruit-bearing food products are controlled by phytosanitary regulatory mechanisms. Among the existing pests of economic interest, *Cetatitis capitata* (medfly) can be highlighted, which can infest from 250 to 400 vegetable varieties. Vicilins, constitutive proteins of chitin-binding seeds, are potentially candidates to fight this pest. Vicillin-rich fractions of *Erythrina veluntina* (mulungu), *Canavalia ensiformis* (pork beans) and *Phaseolus vulgaris* (common beans), isolated by affinity chromatography on a chitin column, were tested on adults of *C. capitata*, in laboratory and semi-laboratory bioassays. field. All were able to bind chitin, have acidic characteristics and association with carbohydrates. *E. velutina* vicilin was the only one to present a high lethality (100 %) in a 15 % diet (w/w). In semi-field it was observed that 10 and 15 % (w/w) of vicilin added to the diet were lethal. For TL_{50}, death of half the population in days were 3.5 (10 %) and 3 (15 %). These results showed that vicilins are potential bioinsecticides in the control of *C. capitata*.

KEYWORDS: Vicilin, Bioinsecticide, Ceratitis capitata.

EFEITO DELETERIO DE FRAÇÕES DE VICILINAS DE SEMENTES DE LEGUMINOSAS *in vitro* E EM CONDIÇÕES DE SEMI-CAMPO PARA *Ceratitis capitata*

RESUMO

Produtos alimentares frutíferos são controlados por mecanismos de regulação fitossanitárias. Dentre as pragas existentes de interesse econômicos, pode-se destacar a *Cetatitis capitata* (mosca da fruta), que é capaz de infestar de 250 a 400 variedades vegetais. Vicilinas, proteínas constitutivas de sementes ligantes a quitina, são potencialmente candidatas a combater essa praga. Frações ricas em vicilinas de *Erythrina veluntina* (mulungu)*, Canavalia ensiformis* (feijão de porco) *and Phaseolus vulgaris* (feijão comum), isoladas em cromatografia de afinidade em coluna de quitina foram testadas em adultos de *C. capitata*, em bioensaios no laboratório e semi-campo. Todas foram capazes de se ligar a quitina, possuem características ácidas e associação com carboidratos. Vicilina de *E. velutina* foi a única apresentar uma letalidade alta (100 %) em dieta em 15 % (p/p). Em semi-campo observou-se que 10 e 15 % (p/p) de vicilina adicionada à dieta foram letais. Para TL_{50}, morte de metade da população em dias, foram 3,5 (10 %) e 3 (15 %). Esses resultados mostraram que vicilinas são potenciais bioinseticida no controle *C. capitata*.

PALAVRAS-CHAVE: Vicilina, *Bioinseticida*, *Ceratitis capitata*.

1. INTRODUCTION

The Food and Agriculture Organization of the United Nations has highlighted that access to a quality food diet is essential to ensure healthy development for all people worldwide (FAO, 2020). Among the available food, fruits are great sources of nutrients that have a direct relationship with the prevention of chronic diseases, such as heart disease, cancer, type 2 diabetes and obesity (Hung et al., 2004; Moazzen et al., 2020). Brazil is the third largest fruit producer in the world, only behind to China and India, with a volume of 40.5 million tonnes and a 4.6 % participation in world fruit production (*FAOSTAT*, 2020; Gerum, 2019).

In this international context, the production of fresh fruits is strongly conditioned by various mechanisms of phytosanitary regulation, mainly associated with absent quarantine pests and the entry of new pests in border regions with other producing or exporting countries (Gerum, 2019). In the case of fresh products, the concern is heightened as an infected batch can eliminate efforts to eradicate pests or diseases that took years and cost millions of dollars (Qin et al., 2015). In Brazil, fruit flies occupy a prominent position among the greatest pests of Brazilian fruit production, preventing a greater number of farmers from exporting their products to large markets (Qin et al., 2015).

Fruit flies (Diptera: Tephritidae) are insect pests that attack a wide variety of plant species and can infest flowers, branches as well as seeds causing one of the greatest economic damage to fruit production (Nishida, 1980). The Tephritidae family corresponds to one of the largest Diptera families with approximately 4000 species and 500 genera (White, I. M., & Elson-Harris, 1992). Today, there are 1500 species of fruit flies, over 250 of which are of economic importance (Zhihong et al., 2013). Tefritides, are found in almost all fruit crops of the world (White, I. M., & Elson-Harris, 1992). One of the species that most threatens world fruit production is *Ceratitis capitata* (medfly) (Stuhl et al., 2011), being cosmopolitan, with the capacity to infest 250 to 400 different vegetable varieties. Recently, it is estimated that this species causes U$ 242 million/year in economic losses in Brazil alone (Oliveira et al., 2013).

An integrated management of this pest from the perspective of interfering in the insect's vital processes, be it genetic, biochemical or morphophysiological, so that such interference has little or no negative influence on other living beings, and at the same time, can serve as potential instrument for controlling this pest. Within this perspective, the digestive system of insects is an important region for their exposure to the environment. Thus, a potential strategic target to interfere in it’s biochemistry and physiology aiming to reduce the nutrients absorption thus, acting as an efficient tool in pest management (Shewry & Lucas, 1997).

Chitin [poly (β-1,4-N-acetyl-D-glucosamine)] comprises a non-branched amino sugar polysaccharide that is part of the peritrophic insect film that surrounds the bolus. In most insects, this film can be a peritrophic gel (PG) or peritrophic membrane (PM) (Terra, 2001). PM and PG are made up of glycoproteins, proteoglycans, proteins and chitin, being acellular and semipermeable. These structures assists in digestive enzymes recycling from the intestine, being a natural barrier against microorganisms as well as preventing abrasion of the food with the absorbent epithelium

while isolates the digesting food present in the intestinal lumen of the epithelial cells of the midgut (Hegedus et al., 2009; Terra, 2001). A probable interaction of the mentioned chitinous peritrophic structures with the constituent protein of the seeds (vicilin) was reported showing to have an important role on development interference and survival of insects (Mota et al., 2003; Paes et al., 2008).

Vicilins (7S), comprise a very well-known class of reserve protein known as globulins, which can make up 70-80 % of the total seed proteins. They are usually proteins with a quaternary structure, being its tertiary structure composed of β-sheets with β-barrel motif, followed by two antiparallel α-helices, with molecular mass ranging from 150 to 190 kDa showing no disulfide bonds due to the absence of cysteine residues and ability to bind to chitin (Cândido et al., 2011; Derbyshire et al., 1976; Sales et al., 1996).

Currently, the association of this protein class with digestive tract structures of insect pests that have a peritrophic chitin in its composition, has become a potential bioinsecticide and can be used as allelochemicals (toxins) in the control of these pests, either as a toxic component of baits or in breeding programs for plants grown using transgenic techniques. With this, we intend to investigate the bioinsecticidal potential of the globulin protein-rich fractions of seeds of the legumes *Erythrina veluntina* (mulungu), *Canavalia ensiformis* (pork beans) and *Phaseolus vulgaris* (common beans) on adult females of the fruit fly *Ceratitis capitata*.

2. METHODS

2.1. Insects and artificial diet

The colony of *C. capitata* used in this work was maintained with insects obtained at the Department of Cell Biology and Genetics, Federal University of Rio Grande do Norte, Brazil. The larvae were raised on artificial diets, the preparation of 500 g is added 52 g (10.4 %) of finely ground cane fibers, 32.5 g (6.5 %) of wheat flour, 60 g (12 %) of crystal sugar , 49.5 g (9.9 %) of yeast extract, 1.5 g (0.3 %) of sodium benzoate, 4.5 g (0.9 %) of HCl, 15g (3 %) of wheat germ and 285 g (57 %) of H_2O (Macedo et al., 2008). Insect colonies were maintained at 28 °C, 70-80 % relative humidity and a 12 h photoperiod.

2.2. Protein and Carbohydrate Quantification

Protein quantification was performed according to the method described by Bradford, (1976) with adaptations for the microplate assay. In order to characterize the glycoprotein nature of the isolated vicilins, carbohydrate dosage was performed using the phenol/sulfuric acid method, adapted by Masuko *et al*., (2005).

2.3. Extraction, fractionation and isolation of proteins in seeds

The seeds of *Erythrina velutina* used in the present study were donated by the seed bank of the National Forest Nísia Floresta, located in the municipality of Nísia Floresta, Rio Grande do Norte, Brazil. The seeds of *Canavalia ensiformis* and *Phaseolus vulgaris* were purchased from the local market. They were pulverized with a refrigerated mill (TE® 631/2) until a low-grain flour was obtained. The low-grain flour were homogenized in 0.05 M sodium tetraborate buffer, pH 7.5, under constant agitation using a magnetic stirrer (Solab® SL-91 / A) for 4 hours at 4 °C. The homogenate was centrifuged (Hettich ® MIKRO 200/200R) at 10000 x g for 30 minutes at 4 °C. The supernatant was called crude extract (EB). EB was fractionated sequentially in two stages, 0 to 70 % (F1) and 70 to 90 % (F2), with ammonium sulfate (w/v) and subsequently centrifuged at 10000 x g for 30 minutes at 4 °C, with fraction F2 selected in all seeds. The sediment was resuspended in distilled water and dialyzed against the same solvent (Yunes et al., 1998).

The plate was read at 595 nm, using the EPOCH microplate reader (Biotek®). The F2 fraction was dissolved in borax buffer and applied to an affinity chromatography on a 5 ml chitin (Poly- (1-4) -β-N-acetyl-D-glucosamine) (Sigma-Aldrich®) column, balanced with 0.05 M borax buffer at pH 7.5. The matrix was washed with the equilibration buffer and the adsorbed proteins were eluted with 100 mM glycine-HCl buffer at pH 2.0 (Macedo et al., 2008).

2.4. Detection of Peptidases Inhibitory Activities

The inhibition of the trypsin enzyme against peptidases extracts from *C. macutatus* larvae were performed on 1 % azocasein substrate at pH 8.0 according to Xavier-Filho *et al*., (1989) with adaptations. 50 µL of EB, F1 or F2 (3 $mg.mL^{-1}$) was incubated with 50 µL of midgut homogenates and 400 µL of 50 mM Tris-HCl and 20 mM $CaCl_2$ in pH 8.0 buffer at 37 °C for 15 min before adding 500 µL of 1 % azocasein, 50 mM Tris-HCl and 20 mM $CaCl_2$ on substrate. After 30 min of incubation, the reaction was stopped by adding 150 µL of 20 % TCA solution. After this step, the samples were centrifuged at 10000 x g for 10 min and the supernatants were made alkaline with 0.2 N NaOH solution. The results were read in absorbance at 440 nm. Inhibitory unit (UI) was defined as the amount of inhibitor activity that decreased the absorbance by 0.01 to 440 nm. The appropriate controls without the samples were prepared under the same conditions as the tests. All tests were performed in triplicate.

2.5. Detection of Hemagglutinating and Hemolytic Activities

The Hemonorte Dalton Cunha, Rio Grande do Norte, Brazil, I give blood bags of the different types of the ABO system, were treated with the commercial enzymes papain and trypsin (both at 0.5 mg/mL) incubated in serial dilutions of BE, F1 or F2 in saline solution (NaCl 0.15 M) in a 96-well plate, in the proportion of 1:1. The plate was incubated for 1 hour (pH 7.4 and 22 °C), and negative control (saline and red blood cells) was done for later comparison. The degree of agglutination was analyzed visually and the titer expressed in hemagglutination unit (H.U.), which is defined as the inverse of the highest dilution where agglutination of red blood cells (RBCs) was observed. The tests were carried out according to Santos *et al*., (2019).

The RBCs were separated from the plasma by sedimentation and washed three times with saline. Then, 100 µL of the red cell suspension was incubated with 100 µL of (EB, F1 or F2) for 60 minutes at 25 °C. Positive control, 100 µL of red cell suspension was incubated with 100 µL of 1 % Triton X - 100, for the negative control, 100 µL of saline solution were incubated with the same volume of red blood cell suspension. After incubation, the mixture was centrifuged (Hettich® MIKRO 200/200R) at 3200 x g for 5 minutes at 25 °C. Aliquots of 100 µL of supernatants were transferred to 96-well plates and analyzed by spectrophotometry with readings at 405 nm (Pharmacia Biotec® Ultrospec 2100 para o). The mean and standard deviation were determined in triplicate (Santos et al., 2019).

2.6. Two-dimensional SDS-PAGE and analysis

Electrophoretic proteic pattern of fractions were observed by SDS-PAGE 12.5 % (kit SDS-PAGE 1615100, Bio-Rad®) according to Laemmli (1970). The protein bands were also visualized by silver staining and the approximate molecular mass was estimated by SDS-PAGE using as reference the molecular weight (Kaleidoscope™, Bio-Rad®) and bovine serum albumin migration pattern (BSA) (code A9418, Sigma -Aldrich®).

Two-dimensional SDS-PAGE was performed in the Ettan IPGphor II system (GE Healthcare) on gel strips 7 cm long and linear gradient of immobilized pH (IPG) ranging from 4-7 (GE Healthcare), 100 µg of proteins were used (Bore et al., 2007) of each sample previously selected. And then in the miniVE SE-260 system at a constant temperature of 4 °C. The gels were stained using Coomassie Brilliant Blue (CBB), with some modifications to the method by Neuhoff et al (Neuhoff et al., 1988) followed by the digitization of the images by the ImageScanner scanner (GE Health care). The images of the 2-D gels were made using the ImageMaster ™ 2D Elite imaging software, according to the manufacturer's description (GE Healthcare®). The molecular weight marker for protein and peptides used was from Cytiva Life Sciences®, which corresponds to the known proteins: 97.0 kDa of Phosphorylase B; 66.0 kDa of Albumin (BSA); 45.0 kDa of Ovoalbumin; 30.0 kDa of Carbonic Anhydrase; 20.1 kDa of Trypsin Inhibitor; and 14.4 kDa of α-lactoalbumin.

2.7. Bioassays with adult *C. capitata* insects under laboratory conditions

To evaluate the effect of protein fractions on the survival of adult insects of *C. capitata*, vicilins were added to the insect diet in the final concentrations of 0; 5, 10 and 15 % (w/v). The standard diet adapted from Macedo et al., (2008). In this bioassay 20 adult females of *C. capitata* were placed in cages made with PET bottles in which the vicilins were added to the standard diet at the concentrations described. Water and a standard diet with BSA (Sigma-Aldrich®, A2153), negative control and lectin from *Triticum vulgaris* (Wheat germ agglutinin - WGA, Sigma-Aldrich®), a chitin-binding protein as a positive control. The bioassay time was 6 days and every 24 h the diets were changed and the number of dead insects was counted. The experiments were carried out in triplicates and the cages were randomly ordered. To evaluate the digestibility of vicilins ingested in the artificial diet, the feces of *C. capitata* were collected, resuspended and homogenized in 0.05

M sodium tetraborate buffer, pH 7.5 and subjected to electrophoresis, along with their respective integral vicilins.

2.8. Semi-field bioassay for adult *C. capitata* insect

To evaluate the insecticidal potential of these vicilins, a semi-field test was carried out with the protein fractions using aluminum cages, measuring 1 m x 0.5 m x 0.5 m, with white plastic mesh faces. In these cages, a guava tree, approximately 0.7 m high, was placed, which was seated on a plastic table. Jackson's traps were placed inside each cage (Sobrinho et al., 2001) specifically for the control of *C. capitata*, without adhesives at the base, attached to the stem of the plant. Within the traps, diets were made available. Vicilin *E. velutina* was added to the diet of adult insects at final concentrations of 0; 5, 10 and 15 % (w/v). In this bioassay, 20 adult females of *C. capitata* were placed in the cages in which the diets were offered. The bioassay time was six days with the diets being changed every 12 hours and the number of insects killed every 24 hours was counted. The experiments were carried out in triplicates and the cages were randomly ordered.

3. RESULTS

3.1. Isolation and Characterization of the vicilins of *E. velutina*, *C. ensiformis* and *P. vulgaris*

Globulinic fractions F2, from precipitation with ammonium sulfate, were subjected to chitin matrix chromatography to isolate the respective proteins of *E. velutina*, *C. ensiformis* and *P. vulgaris* (Figures 1). These fractions were subjected to detection of other defense proteins, existing in plants with the ability to chitin-binding, such as serine proteinase inhibitors, lectins and/or proteins with hemolytic activity. They were not found in the material eluted from the chitin matrix of the chromatographs.

The chromatographic profiles of these fractions, when subjected to reading in a spectrophotometer at a length of 280 nm, revealed protein fractions with affinity to the chitin matrix. The eluted proteins were dialyzed against distilled water and lyophilized~~,~~. The content of carbohydrates present in the analyzed vicilines were 1.62 % - *E. velutina*, 1.21 % - *C. ensiformis* and 1.29 % - *P. vulgaris*. Positive control was lectin from *Triticum vulgaris*.

HOLOS
ISSN 1807 - 1600

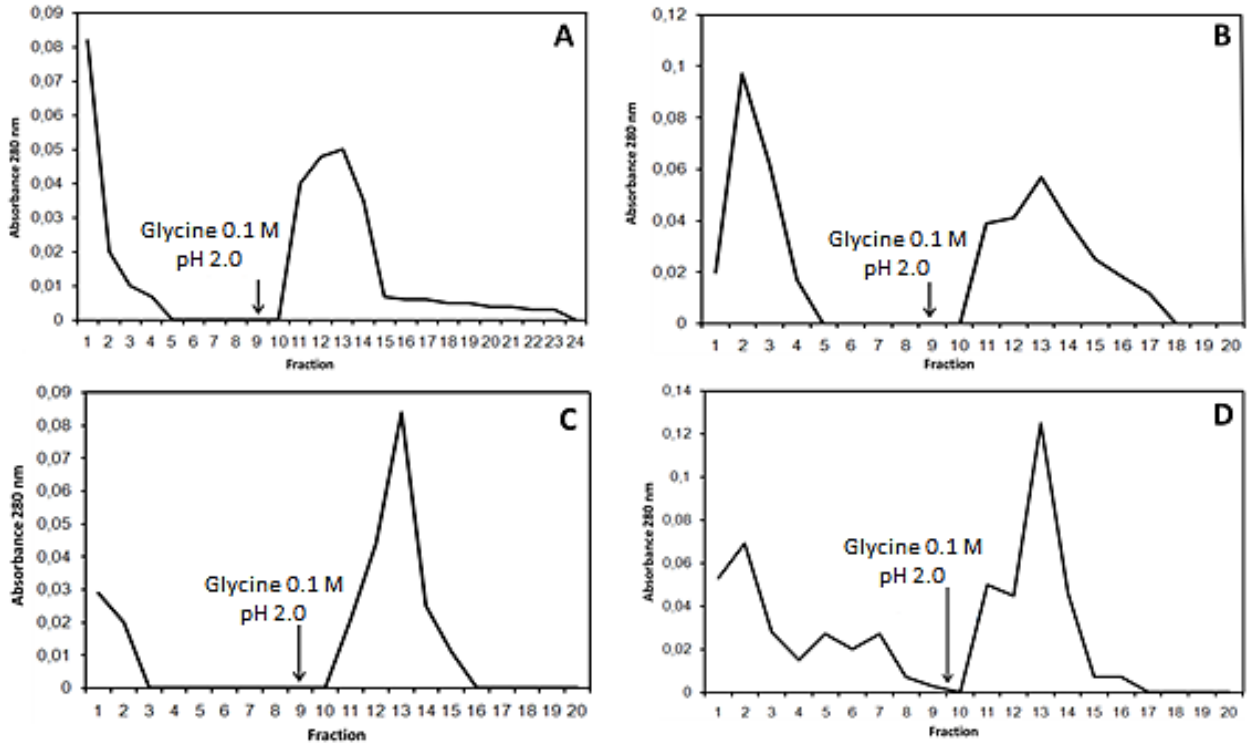

Figure 1. Elution profiles of *E. velutina* Vicilin - EvV (A), *C. ensiformis* Vicilin - CeV (B), *P. vulgaris* Vicilin - PvV (C) and positive control wheat germ agglutinin - WGA (D) in affinity chromatography with chitin matrix. The column was previously equilibrated with 50 mM Tris-HCl buffer, pH 7.5 ≅ 100 mg/mL were applied, the absorbed proteins were eluted with 0.1 M glycine, pH 2.0 in a constant flow of 2 mL/min. Absorbance measured at 280 nm.

Two-dimensional electrophoresis showed that all fractions revealed a different protein banding, either by analyzing the migration as a function of molecular mass, with proteins from high to low mass, or by migration of the isoelectric point, as shown by the arrows (Figure 2), showing that there is a predominance of acidic proteins. Thus these fractions were called *E. velutina* Vicilin – EvV; *C. ensiformis* Vicilin - CeV and *P. vulgaris* Vicilin – PvV as described by the compilation of results carried out by Cândido *et al*., (2011).

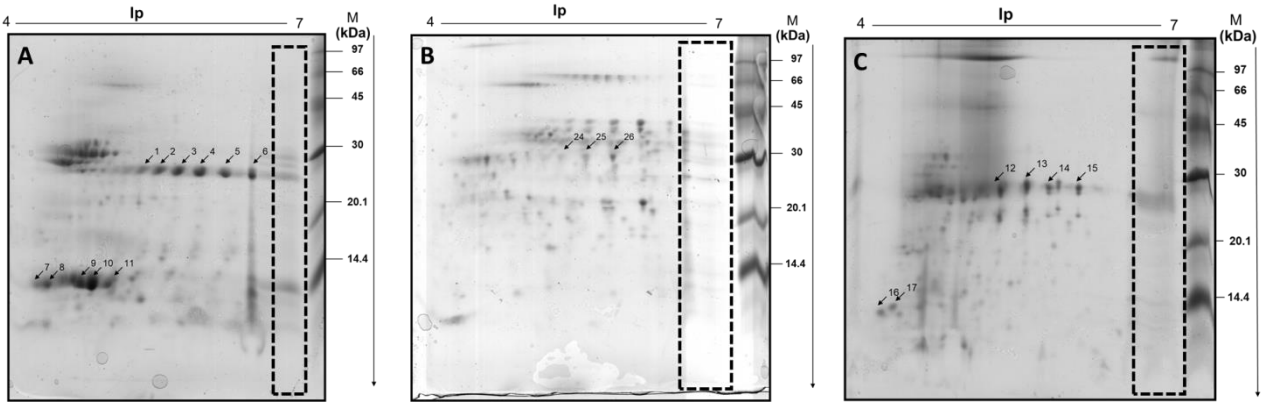

Figure 2. Vicilin electrophoretic analysis of *E. velutina* Vicilin - EvV (A), *C. ensiformis* Vicilin - CeV (B), *P. vulgaris* Vicilin - PvV (C). SDS-PAGE (12.5%) of the fractions, shows a variety of bands (indicated by the dotted box), showing the potential to form subunits of vicilins.

HOLOS
ISSN 1807 - 1600

3.2. Insecticidal activity test by bioassays for adults of *C. capitata* under laboratory conditions

The effects of EvV, CeV and PvV vicilins on adult insect mortality were evaluated in a bioassay system, where increasing concentrations of vicilins were added to the diet. The experimental period established in the bioassay was six days, during which mortality was monitored in the different treatments. During the bioassay, all flies submitted to water treatment, that is, without any food, died in the first 24 hours of the experiment. In treatments in which EvV were added to the diet at concentrations of 5, 10 and 15 %, high mortality was observed, especially at the highest concentration, reaching a mortality rate of 100% at the end of the experiment (Figure 3A). In treatments with CeV (Figure 3B), PvV (Figure 3C) and WGA (Figure 3D), the observed mortality did not differ statistically from the control of the protein-free diet. The control with BSA added to the insect diet, in the same concentrations of 5, 10 and 15 %, also showed a mortality rate similar to that of the diet without protein (Figure 3E).

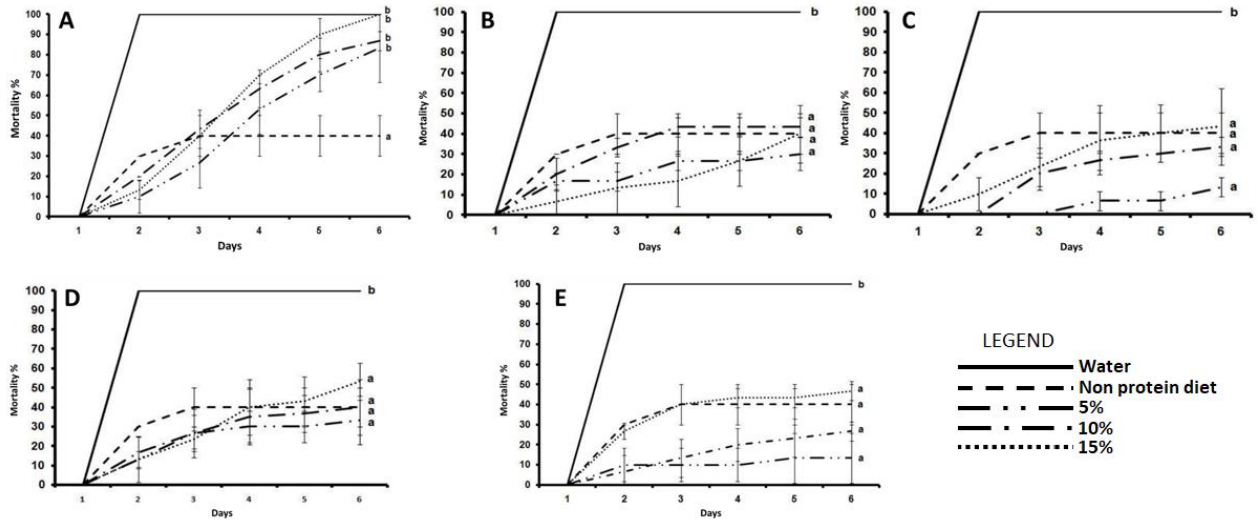

Figure 3. Effect of *E. velutina* Vicilin - EvV (A), *C. ensiformis* Vicilin - CeV (B), *P. vulgaris* Vicilin - PvV (C), Wheat Germ Agglutinin - WGA (D) and Bovine Serum Albumin - BSA (E) on the mortality of adult *C. capitata* insects in the laboratory. For the same day, averages with the same symbols do not differ, using the Tukey test at the 5 % significance level.

The digestibility of vicilins from *E. velutina*, *C. ensiformis* and *P. vulgaris* was evaluated *in vivo* when adult insects were fed diets containing 10 % vicilins (w/v). The proteins extracted from feces were submitted to an SDS-PAGE. The results showed that vicilins from different origins show different patterns of digestion by insects. The vicilin of *E. velutina* was the most resistant to digestion by insects. For the other vicilins, after 3 hours of hydrolysis, band digestion was observed characteristic of vicilins. WGA, a commercial lectin, was the most susceptible to digestion by the enzymes of the insect (Figure 4).

HOLOS
ISSN 1807 - 1600

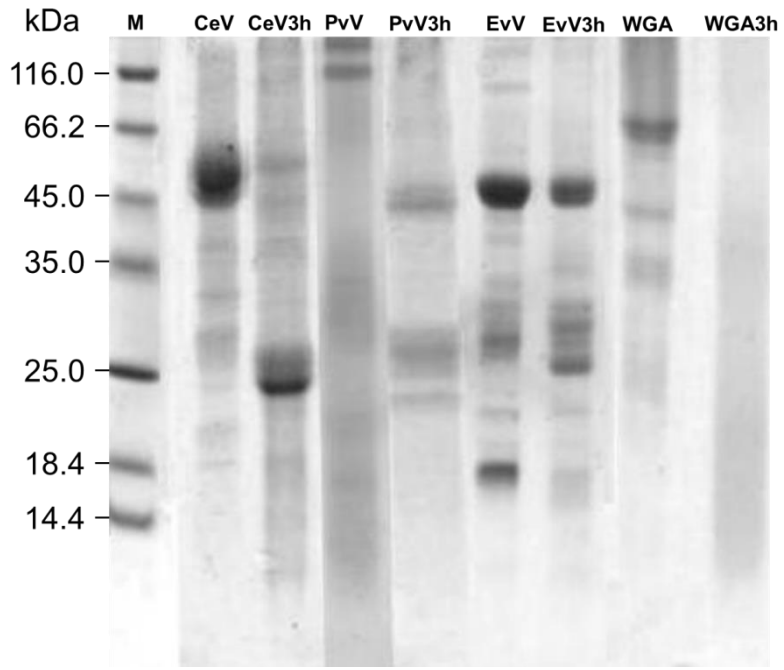

Figure 4. Polyacrylamide electrophoresis (SDS-PAGE) of the in vivo digestibility of CeV vicilins, PvV, EvV and WGA. M - molecular weight marker (β-galactosidase 116.0 kDa; Bovin serum albumin 66.2 kDa bovine; ovalbumin 45.0 kDa; lactate dehydrogenase 35.0 kDa; Bsp98 restriction enzyme 25.0 kDa; β-lactalbumin 18.4kDa, lysozyme 14.4kDa), *C. ensiformis* Vicilin - CeV; CeV3h - *C. ensiformis* Vicilin after 3 h of digestion; *P. vulgaris* Vicilin - PvV; PvV3h - *P. vulgaris* Vicilin after 3 h of digestion, *E. velutina* Vicilin - EvV; EvV3h - *E. velutina* Vicilin after 3h of digestion; Wheat Germ Agglutinin - WGA; WGA - Wheat Germ Agglutinin after 3h of digestion.

3.3. Insecticidal activity test by bioassays in a semi-field system for adult insects of *C. capitata*

Due to its high insecticidal activity in the bioassay under laboratory conditions, only *E. velutina* vicilin was tested in the semi-field system. The effect on adult insects was evaluated at doses of 5, 10 and 15 % (w/v) added to the standard insect diet (Figure 5). The experimental period established in the bioassay was also six days, during which mortality was monitored in the different treatments. Similar to the result of the bioassay in the laboratory, EvV, caused mortality in the three tested concentrations, presenting a better statistically similar bioinsecticidal effect in the concentrations of 10 and 15 % (Figure 5B and C). During the bioassay, flies were subjected to water treatment died in the first days of the experiment.

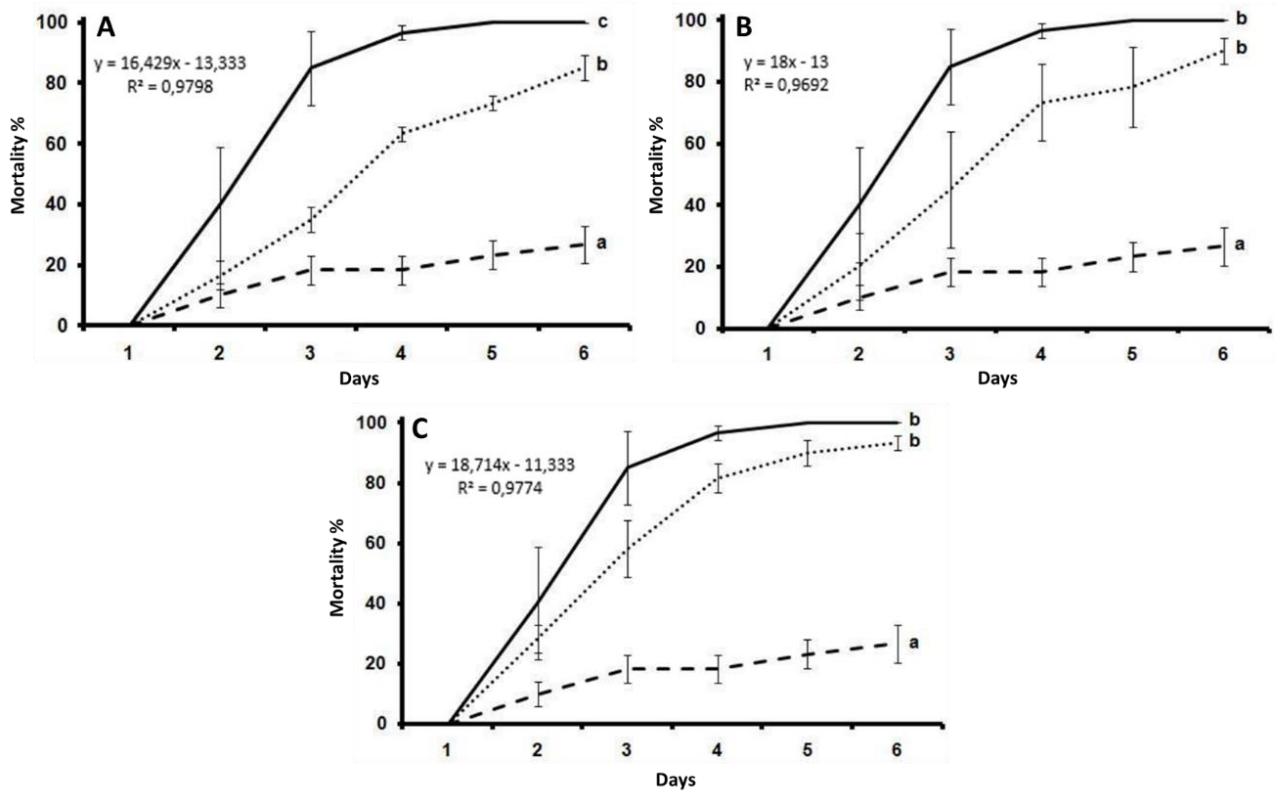

Figure 5. Effect of EvV at 5 % (A), 10% (B) and 15 % (C) (·····) on mortality of adult insects of *C. capitata* in the semi-field system. For the same day, equal symbols do not differ by Tukey's test at the 5 % significance level. Water (——), Standard diet (– – –).

In order to assess which concentration was more efficient in causing the death of 50% of the tested population in a shorter time, the lethality time was calculated. The TL_{50} found was 3.8 days for the concentration of 5% of EvV, 3.5 days for 10% and 3 days for the concentration of 15% of EvV. The group with only water was provided, obtained a TL_{50} of 2.5 days and the group fed with the standard diet was 9.4 days.

4. DISCUSSION

The vicilins have a dual function in leguminous seeds, providing amino acids for seedling germination and growth and acting in defense during the quiescence period (Cândido et al., 2011). Sales *et al*., (1996) were the first to observe the ability to bind chitin *in vitro*, a characteristic that was observed in the three fractions submitted to chitin affinity chromatography. The electrophoresis showed the presence of a single band, in EvV, CeV and PvV, with high molecular mass, the presence of subunits both in the migration as a function of the mass and in that which occurred as a function of the isoelectric point, thus characterizing the three isolates as acidic proteins. These data were observed in other vicilins described by Cândido *et al*., (2011) as well as Yunes *et al*., (1998). In the fractions analyzed, the following concentrations were determined relative to the masses composed of carbohydrates 1.62 % - EvV, 1.21 % - CeV and 1.29 % - PvV. This characterizes the isolates as glycoproteins that present an expected average for other vicilins

already described, staying between 1 and 2 % (Cândido et al., 2011; Derbyshire et al., 1976; Coelho et al., 2010).

Vicilins have been shown to be effective in promoting the death of different pests in their larval stage, *Tenebrio molitor* (Paes et al., 2008), *Callosobruchus maculatus* (França et al., 2021) and *Plodia interpunctella* (Amorim et al., 2008), in their respective agricultural crops. Yunes *et al*., (1998) demonstrated that CeV and PvV over twenty days of larval development of *C. maculatus*, obtained effective ED_{50}, potential to reduce the emergency of adults by 50 %, concentrations of 2.2 and 2.1 respectively. In *C. capitada* larvae Macedo *et al*., (2008) demonstrated the ability of EvV to eliminate it with a lethal dose, LD_{50}, of 0.14 %. Therefore, the bioassays with adults of *C. capitata*, showed that of the analyzed vicilins, they presented effective death of the tested individuals. However, only EvV was effective in causing death, when statistically analyzed in relation to the positive control group (WGA). Miranda et al., (2020) shed light on the low efficiency of CeV and PvV. It was demonstrated, *in silico*, that chemical modifications in the amino acid residues lysine-223 and tryptophan-316, decreasing the binding affinity to chitin and, consequently, attenuating toxicity of vicilin in relation to the insect *C. maculatus*. In tests with adults, from the initial point which was the ingestion and subsequent elimination of the fecal content of *C. capitata*, 3 hours passed. Fecal analysis revealed complete digestion of WGA and partial digestion of CeV and PvV samples. EvV was the one that showed greater resistance to the enzymatic action of the fruit fly with the subunits of vicilin observed practically integral. Due to this resistance, EvV was selected for the semi-field experiments.

Thus, EvV proved to maintain its toxic effect at concentrations of 10 % and 15 % (w/v) when added to the diet of adults of *C. capitata*, with TL_{50} of 3.5 days for 10 % concentration and 3 days for 15 % EvV concentration. This efficiency was also demonstrated by Macedo et al., (2008) when in vitro they showed the ability of FITC-labeled EvV to bind to the peritrophic membrane and gut epithelial cells, proving that EvV obstructs nutrient absorption by *C. capitata* larvae, which is directly associated with low nutritional status and subsequent larval death.

5. CONCLUSION

The protein isolates obtained in *E. velutina*, *C. ensiformes* and *P. vulgares* chitin affinity chromatography are acidic, oligomeric glycoproteins with high molecular mass. The three isolates were harmful to adults of *C. capitata*. EvV being the one that showed the best results in concentrations of 10 and 15 % (w/v) when offered the diet to adults of *C. capitata* in laboratory and semi-field conditions. EvV is, therefore, an excellent candidate to be used as a biodegradable bioinsecticide, non-toxic to humans and animals, easily isolated and highly toxic in the control of the adult insect population of *C. capitata*.

Acknowledgments

In this work, our special thanks to Maurício Pereira de Sales (in memory) and José Xavier Filho (in memory) for their ideas and contributions to the study of vicilins and their mechanisms of

bioinsecticide activities. The authors are grateful for the support of the National Council for Scientific and Technological Development (CNPq) and the Coordination for the Improvement of Higher Education Personnel (CAPES).

6. REFERENCES

Amorim, T. M. L., Macedo, L. L. P., Uchoa, A. F., Oliveira, A. S., Pitanga, J. C. M., Macedo, F. P., Santos, E. A., & De Sales, M. P. (2008). Proteolytic digestive enzymes and peritrophic membranes during the development of Plodia interpunctella (Lepidoptera: Piralidae): Targets for the action of Soybean Trypsin Inhibitor (SBTI) and Chitin-Binding Vicilin (EvV). *Journal of Agricultural and Food Chemistry*, *56*(17), 7738–7745. https://doi.org/10.1021/jf801224d

Bore, E., Hébraud, M., Chafsey, I., Chambon, C., Skjæret, C., Moen, B., Møretrø, T., Langsrud, Ø., Rudi, K., & Langsrud, S. (2007). Adapted tolerance to benzalkonium chloride in Escherichia coli K-12 studied by transcriptome and proteome analyses. *Microbiology*, *153*(4), 935–946. https://doi.org/10.1099/mic.0.29288-0

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(8), 248–254. https://doi.org/10.1088/1751-8113/44/8/085201

Cândido, E. D. S., Pinto, M. F. S., Pelegrini, P. B., Lima, T. B., Silva, O. N., Pogue, R., Grossi-de-Sá, M. F., & Franco, O. L. (2011). Plant storage proteins with antimicrobial activity: novel insights into plant defense mechanisms. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *25*(10), 3290–3305. https://doi.org/10.1096/fj.11-184291

Coelho, M. B., Macedo, M. L. R., Marangoni, S., Da Silva, D. S., Cesarino, I., & Mazzafera, P. (2010). Purification of legumin-like proteins from coffea arabica and coffea racemosa seeds and their insecticidal properties toward cowpea weevil (Callosobruchus maculatus) (Coleoptera: Bruchidae). *Journal of Agricultural and Food Chemistry*, *58*(5), 3050–3055. https://doi.org/10.1021/jf9037216

Derbyshire, E., Wright, D. J., & Boulter, D. (1976). Legumin and vicilin, storage proteins of legume seeds. In *Phytochemistry* (Vol. 15, Issue 1, pp. 3–24). Pergamon. https://doi.org/10.1016/S0031-9422(00)89046-9

FAO. (2020). *FOOD SECURITY AND NUTRITION IN THE WORLD THE STATE OF TRANSFORMING FOOD SYSTEMS FOR AFFORDABLE HEALTHY DIETS*. https://doi.org/10.4060/ca9692en

FAOSTAT. (2020). http://www.fao.org/faostat/en/#data/QC

França, A. F. J., Araújo, J. N., Santos, Y. Q., Carelli, G. S. C., Silva, D. A., Amorim, T. M. L., Migliolo, L., Santos, E. A., Oliveira, A. S., & Uchôa, A. F. (2021). Vicilin from Anadenanthera colubrina Seeds: An alternative tool to combat Callosobruchus maculatus. *Saudi Journal of Biological Sciences*, *xxxx*, 1–9. https://doi.org/10.1016/j.sjbs.2021.05.041

Gerum, Á. F. A. de A. (2019). Fruticultura Tropical: potenciais riscos e seus impactos. *EMBRAPA Mandioca e Fruticultura*, 28. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/197322/1/Documento232-AureaGerum-Ainfo.pdf

Hegedus, D., Erlandson, M., Gillott, C., & Toprak, U. (2009). New Insights into Peritrophic Matrix Synthesis, Architecture, and Function. *Annual Review of Entomology*, *54*(1), 285–302. https://doi.org/10.1146/annurev.ento.54.110807.090559

Hung, H.-C., Joshipura, K. J., Jiang, R., Hu, F. B., Hunter, D., Smith-Warner, S. A., Colditz, G. A., Rosner, B., Spiegelman, D., & Willett, W. C. (2004). Fruit and Vegetable Intake and Risk of Major Chronic Disease. *JNCI Journal of the National Cancer Institute*, *96*(21), 1577–1584. https://doi.org/10.1093/jnci/djh296

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*(5259), 680–685. https://doi.org/10.1038/227680a0

Macedo, L. L. P., Amorim, T. M. L., Uchôa, A. F., Oliveira, A. S., Ribeiro, J. K. C., De MacEdo, F. P., Santos, E. A., & De Sales, M. P. (2008). Larvicidal effects of a chitin-binding vicilin from erythrina velutina seeds on the mediterranean fruit fly ceratitis capitata. *Journal of Agricultural and Food Chemistry*, *56*(3), 802–808. https://doi.org/10.1021/jf072746n

Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S. I., & Lee, Y. C. (2005). Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. *Analytical Biochemistry*, *339*(1), 69–72. https://doi.org/10.1016/j.ab.2004.12.001

Miranda, M. R. A., Uchôa, A. F., Ferreira, S. R., Ventury, K. E., Costa, E. P., Carmo, P. R. L., Machado, O. L. T., Fernandes, K. V. S., & Amancio Oliveira, A. E. (2020). Chemical Modifications of Vicilins Interfere with Chitin-Binding Affinity and Toxicity to Callosobruchus maculatus (Coleoptera: Chrysomelidae) Insect: A Combined in Vitro and in Silico Analysis. *Journal of Agricultural and Food Chemistry*, *68*(20), 5596–5605. https://doi.org/10.1021/acs.jafc.9b08034

Moazzen, S., van der Sloot, K. J. W., Bock, G. H. d., & Alizadeh, B. Z. (2020). Systematic review and meta-analysis of diet quality and colorectal cancer risk: is the evidence of sufficient quality to develop recommendations? In *Critical Reviews in Food Science and Nutrition*. Taylor and Francis Inc. https://doi.org/10.1080/10408398.2020.1786353

Mota, A. C., DaMatta, R. A., Filho, M. L., Silva, C. P., & Xavier-Filho, J. (2003). Cowpea (Vigna unguiculata) vicilins bind to the peritrophic membrane of larval sugarcane stalk borer (Diatraea saccharalis). *Journal of Insect Physiology*, *49*(9), 873–880. https://doi.org/10.1016/S0022-1910(03)00139-2

Neuhoff, V., Arold, N., Taube, D., & Ehrhardt, W. (1988). Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. *ELECTROPHORESIS*, *9*(6), 255–262. https://doi.org/10.1002/elps.1150090603

Nishida, T. (1980). Food System of Tephritid Fruit Flies in Hawaii. *Proc Hawaiian Entomol Soc*, *23*(2),

245–254. http://hdl.handle.net/10125/11110

Oliveira, C. M., Auad, A. M., Mendes, S. M., & Frizzas, M. R. (2013). Economic impact of exotic insect pests in Brazilian agriculture. In *Journal of Applied Entomology* (Vol. 137, Issues 1–2, pp. 1–15). John Wiley & Sons, Ltd. https://doi.org/10.1111/jen.12018

Paes, E. V., Uchôa, A. F., Pinto, M. S. T., Silva, C. P., Fernandes, K. V. S., Oliveira, A. E. A., & Xavier-Filho, J. (2008). Binding of Vigna unguiculata vicilins to the peritrophic membrane of Tenebrio molitor affects larval development. *Entomologia Experimentalis et Applicata*, *129*(1), 11–17. https://doi.org/10.1111/j.1570-7458.2008.00746.x

Qin, Y., Paini, D. R., Wang, C., Fang, Y., & Li, Z. (2015). Global establishment risk of economically important fruit fly species (Tephritidae). *PLoS ONE*, *10*(1), e0116424. https://doi.org/10.1371/journal.pone.0116424

Sales, M. P., Gomes, V. M., Fernandas, K. V. S., & Xavier-Filho, J. (1996). Chitin-binding proteins from cowpea (Vigna unguiculata) seeds. *Brazilian Journal of Medical and Biological Research*, *29*(3), 319–326. http://www.ncbi.nlm.nih.gov/pubmed/8736124

Santos, Y. Q. dos, Carelli, G. S. C., Veras, B. O. de, Batista, V. C., França, A. F. J. de, Silva, M. V. da, & Santos, E. A. dos. (2019). Antitryptical, anticoagulant and hemagglutinating activities of Eucalyptus sp. seeds. *F1000Research*, *8*, 28. https://doi.org/10.12688/F1000RESEARCH.17080.1

Shewry, P. R., & Lucas, J. A. (1997). Plant Proteins that Confer Resistance to Pests and Pathogens. *Advances in Botanical Research*, *26*(C), 135–192. https://doi.org/10.1016/S0065-2296(08)60120-2

Sobrinho, R. B., Malavasi, A., Mesquita, A. L. M., & Ometo, A. C. F. (2001). *Manual operacional para monitoramento e controle de moscas-das-frutas* (1st ed.). Embrapa Agroindústria Tropical. https://www.embrapa.br/agroindustria-tropical/busca-de-publicacoes/-/publicacao/423835/manual-operacional-para-levantamento-deteccao-monitoramento-e-controle-de-moscas-das-frutas

Stuhl, C., Sivinski, J., Teal, P., Paranhos, B., & Aluja, M. (2011). A Compound Produced by Fruigivorous Tephritidae (Diptera) Larvae Promotes Oviposition Behavior by the Biological Control Agent Diachasmimorpha longicaudata (Hymenoptera: Braconidae). *Environmental Entomology*, *40*(3), 727–736. https://doi.org/10.1603/en10198

Terra, W. R. (2001). The origin and functions of the insect peritrophic membrane and peritrophic gel. *Archives of Insect Biochemistry and Physiology*, *47*(2), 47–61. https://doi.org/10.1002/arch.1036

White, I. M., & Elson-Harris, M. M. (1992). Fruit flies of economic significance: their identification and bionomics. In *CAB International, Wallingford, Oxon* (p. 601). https://doi.org/https://doi.org/http://dx.doi.org/10.1093/ee/22.6.1408

Xavier-Filho, J., Campos, F. a. P., Ary, M. B., Silva, C. P., Carvalho, M. M. M., Macedo, M. L. R., Lemos,

F. J. a., & Grant, G. (1989). Poor correlation between the levels of proteinase inhibitors found in seeds of different cultivars of cowpea (Vigna unguiculata) and the resistance/susceptibility to predation by Callosobruchus maculatus. *Journal of Agricultural and Food Chemistry*, *37*(4), 1139–1143. https://doi.org/10.1021/jf00088a071

Yunes, A. N. A., de Andrade, M. T., Sales, M. P., Morais, R. A., Fernandes, K. V. S., Gomes, V. M., & Xavier-Filho, J. (1998). Legume seed vicilins (7S storage proteins) interfere with the development of the cowpea weevil (Callosobruchus maculatus (F)). *Journal of the Science of Food and Agriculture*, *76*(1), 111–116. https://doi.org/10.1002/(sici)1097-0010(199801)76:1<111::aid-jsfa932>3.0.co;2-4

Zhihong, L., Fan, J., Xingli, M., Yan, F., Zhuangzhi, S., Yujia, Q., & Qiaoling, W. (2013). Review on prevention and control techniques of Tephritidae invasion. *Plant Quarantine*, *02*. http://en.cnki.com.cn/Article_en/CJFDTOTAL-ZWJY201302001.htm

COMO CITAR ESTE ARTIGO:

Macedo, C. S., França, A. F. J., Macedo, L. L. P., Amorim, T. M. L., Araújo, R. R. O., Santos, Y. Q., . . . Araujo, J. N. (2021). Deleterious effect of vicilins fractions of leguminous seeds *in vitro* and in semi-field conditions for *ceratitis capitata. Holos*. 37(8), 1-17.

SOBRE OS AUTORES,

C. S. MACEDO
Graduação em Ciências Biológicas e Mestrado pelo programa de Pós-graduação em Bioquímica e Biologia Molecular, ambos pela Universidade Federal do Rio Grande do Norte - UFRN. Atualmente professor no Instituto Federal de Ciência e Tecnologia do Rio Grande do Norte, Campus Caicó – RN, Brasil. E-mail: cleysyvan.macedo@ifrn.edu.br
ORCID ID: https://orcid.org/0000-0001-8233-7915

A. F. J. FRANÇA
Graduação em Ciências Biológicas e Doutorado pelo programa de Pós-graduação em Bioquímica e Biologia Molecular, ambos pela Universidade Federal do Rio Grande do Norte - UFRN. Atualmente professor efetivo da Secretaria de Educação e Cultura: Natal, Rio Grande do Norte, Brasil e professor colaborador voluntário na Escola Multicampi de Ciências Médicas, Universidade Federal do Rio Grande do Norte, Caicó – RN, Brazil. E-mail: anderson.franca.010@ufrn.edu.br
ORCID ID: https://orcid.org/0000-0003-0735-6643

L. L. P. MACEDO
Possui graduação em Ciências Biológicas pela Universidade Federal do Rio Grande do Norte (2005). O curso de Mestrado em Bioquímica foi realizado na Universidade Federal do Rio Grande do Norte (2007). Com doutorado concluído pelo programa de Ciência Genômicas e Biotecnologia da Universidade Católica de Brasília (2012). Tem experiência em Bioquímica e Biologia Molecular. Atuando nos seguintes temas: Clonagem e expressão de proteínas em sistemas heterólogos; Bioprospecção de proteínas com atividade entomotóxicas (vicilinas, lectinas, inibidores de proteinases e toxinas Cry) visando o controle de insetos dípteros, lepidópteros e coleópteros; e desenvolvimento de estratégias de silenciamento gênico via RNA interferente (RNAi) para o controle de insetos-praga. E-mail: leonardo.lima@embrapa.br
ORCID ID: https://orcid.org/0000-0001-6786-0644

T. M. L. AMORIM
Possui graduação em Ciências Biológicas pela Universidade Estadual da Paraíba (2004). Mestrado em Bioquímica pela Universidade Federal do Rio Grande do Norte. Doutorado em Bioquímica, com áreas de concentração em Bioquímica e Biologia Molecular, também pela Universidade Federal do Rio Grande do Norte, realizando pesquisas relacionadas à aplicação de inibidores recombinantes em processos de importância para a saúde humana. Atualmete professora no curso de bacharelado em Medicina- Núcleo de Pesquisa em Ciências Médicas/NPCMed, Universidade Federal do Piauí, Picos - PI, Brasil. E-mail: ticianaamorim@ufpi.edu.br
ORCID ID: https://orcid.org/0000-0003-3408-9544

R. R. O. ARAÚJO
Bacharel em Biomedicina, formado pela Universidade Federal do Rio Grande do Norte. Experiência na área de Bioquímica, com ênfase em Química de Macromoléculas Bioativas. Aluno de mestrado do Programa de Pós-Graduação em Bioquímica e Biologia Celular (PPG/DBq), trabalhando com imunoematologia, inflamação e câncer. E-mail: raphael.russi@yahoo.com.br
ORCID ID: https://orcid.org/0000-0003-4183-0513

Y. Q. SANTOS
Possui graduação em Ciências Biológicas (2014) pela Universidade Federal da Paraíba, mestrado (2016) e doutorado (2020) em Bioquímica e Biologia Molecular pela Universidade Federal do Rio Grande do Norte - UFRN. Atualmente desenvolve pesquisas no Laboratório Química e Função de Proteínas Bioativas (LQFPB), do Depto. de Bioquímica da UFRN e no Laboratório de Microbiologia do Instituto de Medicina Tropical (IMT-UFRN). Possui experiência nas áreas de Bioquímica e Genética de Microrganismos, com especial foco no isolamento e caracterização de enzimas, identificação molecular de microrganismos e determinação da atividade antibacteriana de moléculas bioativas. Possui graduação em andamento no curso de Direito na Universidade Federal do Rio Grande do Norte, atuando na área de Direito Constitucional (Teoria Geral dos Direitos Fundamentais). É professor efetivo do Instituto Federal do Ceará. E-mail: yagoqs@hotmail.com
ORCID ID: https://orcid.org/0000-0002-6453-3661

G. S. C. CARELLI
Mestranda em Bioquímica e Biologia Molecular do programa de pós-graduação em Bioquímica (PPGBIOQ) da Universidade Federal do Rio Grande do Norte, graduada em Ciências Biológicas (2019) pela (UFRN). Possui formação em Técnico de Enfermagem (2015) pela UFRN. Atualmente desenvolve pesquisas no Laboratório de Química e Função de Proteínas Bioativas (LQFPB) no departamento de bioquímica da UFRN e no Laboratório de microbiologia (LMAC) do Instituto de Medicina Tropical (IMT-UFRN). E-mail: gabicarelli@hotmail.com.
ORCID ID: https://orcid.org/0000-0002-4390-9666

A. F. UCHOA
Possui graduação em Ciências Biológicas pela Universidade Federal do Ceará (1997), mestrado em Biociências e Biotecnologia pela Universidade Estadual do Norte Fluminense Darcy Ribeiro (2000) e doutorado em Biociências e Biotecnologia pela Universidade Estadual do Norte Fluminense Darcy Ribeiro (2006). Atualmente é professora associado II do Departamento de Biologia Celular e Genética da Universidade Federal do Rio Grande do Norte. Pesquisadora do Instituto de Medicina Tropical do RN (UFRN). Tem experiência na área de Bioquímica, com ênfase em Química de Proteínas, atuando principalmente nos seguintes temas: Extratos vegetais e Proteínas bioativas, atividade bioinseticida, fungicida e nematicida; inibidores de proteases, proteínas de ligação a quitina do tipo vicilinas, prospecção de atividades biológicas de moléculas bioativas de sementes e proteoma. E-mail: afuchoa2003@yahoo.com.br
ORCID ID: https://orcid.org/0000-0002-3822-6502

F. P. MACEDO
Departamento de Biologia Celular e Genética, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Natal, Rio Grande do Norte, Brasil. E-mail: depepino@cb.ufrn.br
ORCID ID: https://orcid.org/0000-0003-2342-2751

A. S. OLIVEIRA
Possui graduação em Ciências Biológicas pela Universidade Federal do Rio Grande do Norte(1993), especialização em Bioquímica da Nutrição pela Universidade Federal do Rio Grande do Norte(1994), mestrado em Bioquímica pela Universidade Federal do Rio Grande do Norte(2000), doutorado em Bioquímica pela Universidade Federal do Ceará(2007) e pós-doutorado pela Universidade Federal do Rio Grande do Norte(2013). Atualmente é Revisor de periódico da Comitê Local de Publicações da Embrapa Algodão. Tem experiência na área de Bioquímica, com ênfase em Química de Macromoléculas. Atuando principalmente nos seguintes temas: inibidor de tripsina, Pithecellobium dumosum, Insetos pragas. E-mail: cisteana@yahoo.com.br
ORCID ID: https://orcid.org/0000-0003-1012-9687

E. A. SANTOS
Professor Titular da Universidade Federal do Rio Grande do Norte. Graduado em Ciências Biológicas pela Universidade Federal do Rio Grande do Norte (1987), Mestre em Ciências Biológicas (Biologia Molecular) pela Universidade Federal de São Paulo (1990) e Doutor em Ciências Biológicas (Biologia Molecular) pela Universidade Federal de São Paulo (1997). Tem experiência na área de Bioquímica, com ênfase em Química de Proteínas e Glicobiologia (Lectinas e Glicosaminoglicanos), atuando principalmente nos seguintes temas: lectinas, inibidores proteicos de proteinases, enzimas, heparina, glicosaminoglicanos, organismos invertebrados, mollusca, porifera, fungos, bactérias e leguminosas. É orientador de mestrado e doutorado no Programa de Pós-Graduação em Bioquímica da UFRN. Atua, ainda, em atividades pedagógicas de formação do Ensino Superior (presencial e a distância) e na formação de professores do Ensino Básico. Atual vice-coordenador do curso de Ciências Biológicas a distância (SEDIS-UFRN). Cargos já exercidos na UFRN: Chefia do Departamento de Bioquímica; Coordenador do curso de Ciências Biológicas; membro do Conselho Superior de Ensino, Pesquisa e Extensão; Coordenador do Programa de Pós-Graduação em Bioquímica (mestrado e doutorado). E-mail: elizeu.ufrn@gmail.com
ORCID ID: https://orcid.org/0000-0003-4372-2161

J. N. ARAUJO
Possuo graduação em Ciências Biológicas pela Universidade Federal do Rio Grande do Norte, mestrado em Bioquímica e Biologia Molecular pela Universidade Federal do Rio Grande do Norte - UFRN. E-mail: jonalsonaraujo@gmail.com
ORCID ID: https://orcid.org/0000-0003-2133-6403

Editor(a) Responsável: Leandro Costa

Pareceristas *Ad Hoc*: Luciana Rabêlo e Rafael Camara