Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

JEZS Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2023; 11(3): 14-18

Received: 16-02-2023
Accepted: 26-03-2023

Johnatan Jair de Paula Marchiori
Federal University Rural of Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil

Anderson Mathias Holtz
Campus Itapina, Federal Institute of Espírito Santo, Colatina, Espírito Santo, Brazil

Ana Beatriz Mamedes Piffer
Campus Itapina, Federal Institute of Espírito Santo, Colatina, Espírito Santo, Brazil

Ronilda Lana Aguiar
Campus Itapina, Federal Institute of Espírito Santo, Colatina, Espírito Santo, Brazil

Jéssica Mayara Coffler Botti
Department of Entomology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

Mayara Loss Franzin
Department of Agriculture, Nestlé Brazil, Várzea de Baixo, São Paulo, Brazil

Vinicius de Souza Oliveira
Federal University of Espírito Santo - Center for Agricultural Sciences and Engineering, Alegre, Espírito Santo, Brazil.

Patrícia Soares Furno Fontes
Campus Itapina, Federal Institute of Espírito Santo, Colatina, Espírito Santo, Brazil

Bruna de Oliveira Magnani
Campus Itapina, Federal Institute of Espírito Santo, Colatina, Espírito Santo, Brazil

Matheus de Paula Gomes
Campus Itapina, Federal Institute of Espírito Santo, Colatina, Espírito Santo, Brazil

Corresponding Author:
Johnatan Jair de Paula Marchiori
Federal University Rural of Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil

Aqueous extract of chili pepper in the management of the pink hibiscus mealybug (Hemiptera: Pseudococcidae)

Johnatan Jair de Paula Marchiori, Anderson Mathias Holtz, Ana Beatriz Mamedes Piffer, Ronilda Lana Aguiar, Jéssica Mayara Coffler Botti, Mayara Loss Franzin, Vinicius de Souza Oliveira, Patrícia Soares Furno Fontes, Bruna de Oliveira Magnani and Matheus de Paula Gomes

DOI: https://doi.org/10.22271/j.ento.2023.v11.i3a.9193

Abstract
Maconellicoccus hirsutus (Hemiptera: Pseudococcidae) is a polyphagous pest, attacks several crops of economic importance, including cocoa and coffee. Due to its rapid dissemination, studies are needed to develop management programs to combat this pest, as few studies seek the effectiveness of alternative products such as plant extracts, which can be promising in its control. Therefore, this work aimed to evaluate the efficiency of the aqueous extract of the fruits of chili pepper Capsicum frutescens (Solanaceae) in the management of the pink hibiscus mealybug. The tests were carried out in acclimatized chambers regulated at a temperature of 25 ± 1 ºC, relative humidity of 70 ± 10%, and a 12-hour photophase. The treatments consisted of five extract concentrations: 0.0, 2.5, 5.0, 7.5, and 10% (weight/volume). Coffee leaves were submerged in the aqueous solution of pepper extract and mounted in Petri dishes with agar solution to transfer the mealybugs. Each treatment consisted of 10 repetitions with 10 young mealybugs each. The experiment was evaluated 24, 48, and 72 h after the procedure. Data were submitted for analysis of variance and regression test ($p \leq 0.05$). Lethal concentration (LC50) was estimated using Probit analysis. The mortality of mealybugs increased with the increase in extract concentrations, with mortalities greater than 70% from the lowest concentration. The data fit the Probit model, with χ^2 of 1.0478 ($p > 0.05$) and a curve slope of 1.7799. The LC50 was estimated at 0.96%. Thus, the aqueous extract of *Capsicum frutescens* fruits is promising for the management of *M. hirsutus*.

Keywords: *Maconellicoccus hirsutus*, *Capsicum frutescens*, alternative pest control, integrated pest management

Introduction
The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), is a species originally from South Asia that quickly spread throughout the world [1, 2]. Worldwide, *M. hirsutus* has been found attacking more than 350 species, including fruit, vegetables, ornamentals, coffee, and cotton [3, 4, 5]. The species was first reported in Brazil in 2010 in the State of Roraima, and since then it has spread to other Brazilian states bringing economic losses to the affected regions. In 2013, the pest was detected in the cocoa crop in the regions of Bahia and Espírito Santo in the municipality of Linhares - ES [5].

The pink hibiscus mealybug is a polyphagous pest, it can cause severe damage and in high populations, it can lead the plant to death. This is because, when feeding, *M. hirsutus* injects toxic saliva into plants. The content injected by the pink hibiscus mealybug saliva can cause the malformation of leaves and fruits, promote the wilting of the apical branches and cause infested flowers and fruits to fall [6, 7].

Although established in Brazil, few control methods are available for the pink hibiscus mealybug. Currently, only the microbiological product *Cryptolaemus montrouzieri* Mulsant (Hemiptera: Coccinellidae) is registered in the information bank on agrochemicals and related products registered with the Ministry of Agriculture for the control of *M. hirsutus* [8]. Due to the scarcity of products recommended for controlling this pest species, many producers end up applying synthetic chemicals not recommended for the crop to alleviate the damage caused by the organism. However, the indiscriminate use of these products can promote the emergence of new resistant populations, cause mortality of natural enemies present in the environment, the intoxication of the surrounding fauna, and pollution of water bodies [9].

As an alternative to synthetic chemical control, research related to the use of extracts and substances obtained from plants has demonstrated satisfactory effectiveness in pest control [10, 9, 11]. Among the plants with acaricidal effects, peppers, belonging to the Solanaceae family, have a variety of alkaloid compounds and saturated or unsaturated fatty acids. These substances are produced in glands located in the placenta of the fruits, where the seeds are inserted and have an insecticidal effect [12, 13]. Thus, this work aimed to evaluate the insecticidal effect of the aqueous extract of *Capsicum frutescens* fruits on *M. hirsutus*.

Materials and Methods

Local Site

The experiment was carried out at the Agricultural Entomology and Acarology Laboratory of the Federal Institute of Education, Science, and Technology of Espírito Santo - Campus Itapina (IFES-Campus Itapina), located in the municipality of Colatina, with coordinates of 19°29'52.7" S 40 °45'38.5" W (Fig. 1).

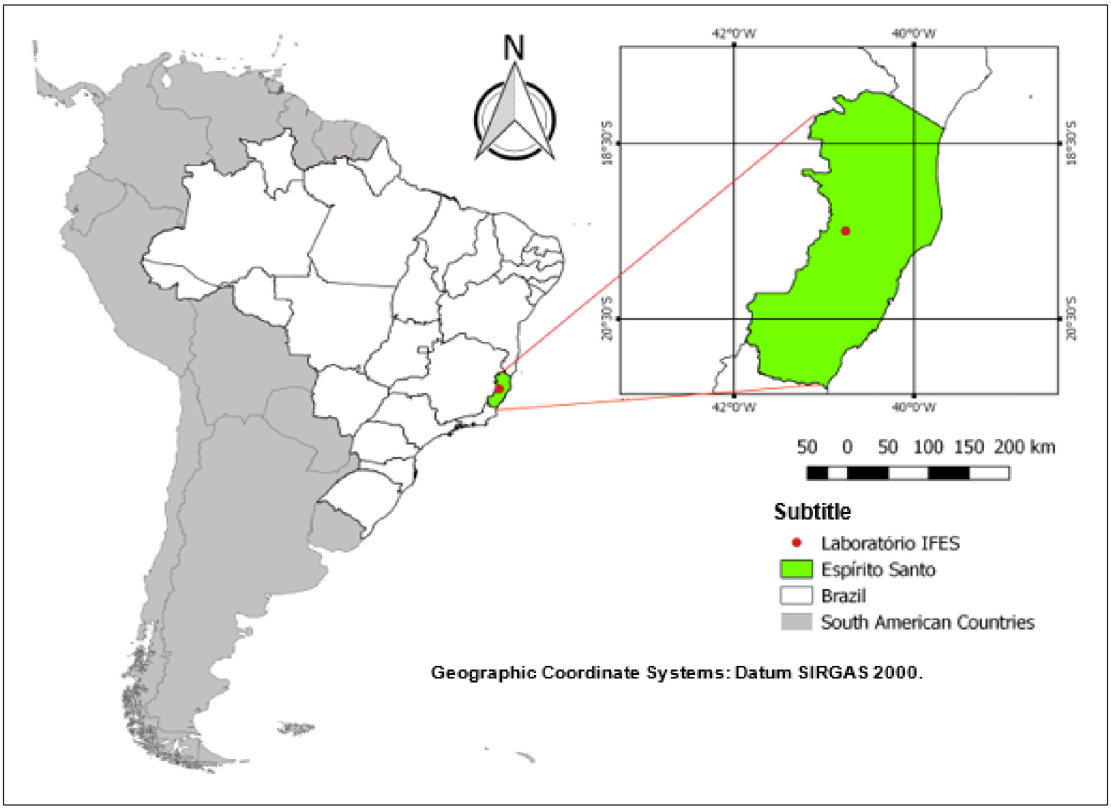

Fig 1: Map of the place where the experiments were carried out

Breeding of the pink hibiscus mealybug

Individuals of *M. hirsutus* were collected in cocoa plantations infested with this insect, in a rural property located in the district of Colatina, ES. Some specimens were sent for identification at the Center for Scientific and Technological Development in Phytosanitary Management (Nudemafi), at the Federal University of Espírito Santo (UFES), in Alegre, ES.

The breeding technique adopted was the same used by Sanches & Carvalho [14]. In the initial infestation of pumpkins, individuals collected in the field on infested host plants were used. After establishing the initial colony, the mealybug multiplication process began. When it was necessary to replace the pumpkins with new ones, they were removed from the institute's experimental area, where chemical products are not used to grow the crop. They were placed in contact with the infested ones for approximately two hours. The proximity of the fruits favors the transfer of newly hatched pink hibiscus mealybug nymphs to the new fruit due to their mobility at this stage.

Obtaining Aqueous Extracts of Chili Pepper Fruits

For the preparation of the extracts, chili pepper fruits were collected in their reproductive phase, in the Experimental Area of the IFES-Campus Itapina, in the Horticulture sector. After this procedure, the fruits were placed to dry in an oven with forced air circulation at a temperature of 40°C for 72 hours. After drying, they were ground using a knife mill to obtain a fine powder.

Bioassays

To obtain the aqueous extract of *C. frutescens*, the crushed plant material powder (10 g) was transferred to an Erlenmeyer flask (100 mL) containing distilled water and Tween® 80 adhesive spreader (0.05%), to obtain 100 mL of the 10% (w/v) starting solution. This solution was maintained under homogenization in a transverse shaker (240 rpm) for a period of 24 hours. After this period, the mixture was filtered with voile fabric and transferred to a volumetric flask, and the volume was checked to 100 mL.

The experimental units were composed of Petri dishes (10.0 x 1.2 cm) on coffee leaf disks of approximately 4 cm in diameter, as described by Holtz *et al.* [15]. The discs were immersed in the solution for 30 seconds, at different concentrations corresponding to the different treatments (0, 2.5, 5.0, 7.5, and 10.0%). The leaf disks were fixed to the

Petri dish, with a layer of 0.5 cm of agar-agar solution and solid Vaseline around the disk to prevent the insects from escaping, and the plates were sealed with transparent PVC film. Each treatment consisted of 10 repetitions with 10 mealybugs in the young stage each, totaling 100 insects per treatment. Subsequently, they were stored in a B.O.D (Biochemical Oxygen Demand), to preserve and maintain a constant ideal temperature, where they were removed and evaluated daily, for three days. Distilled water and adhesive spreader Tween® 80 (0.05) were used as a control. The insecticidal effect was evaluated 24, 48, and 72 hours after immersion.

The experiment was conducted in a completely randomized design. Data were corrected using the Abbott formula [16] and subsequently submitted to analysis of variance and regression test ($p \leq 0.05$), using the SISVAR software. To estimate the lethal concentration (LC50), Probit regression analysis was performed using the Polo Pc program.

Results and Discussion

According to the linear function of the regression test, there was an increase in the mortality of the pink hibiscus mealybug with the increase in the concentration of the aqueous extract of chili pepper (Fig. 2). The data fit the Probit model, showing a chi-square of 1.0478. The slope of the concentration-mortality curve was 1.7799%. The LC50 was estimated at 0.96%, that is, 0.96 g/100 mL would cause mortality in at least 50% of the population (Table 1).

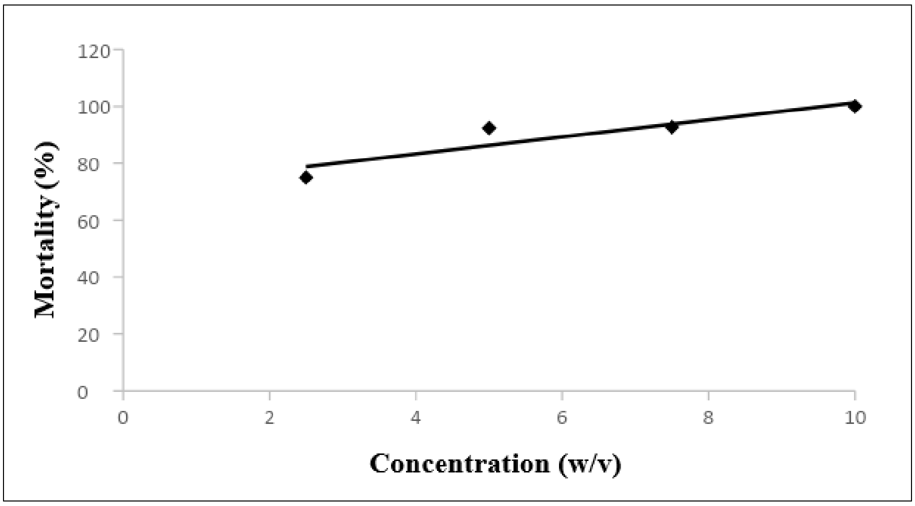

Fig. 2: Mortality (%) of *Maconellicoccus hirsutus* in different concentrations of *Capsicum frutescens* fruit aqueous extract.

Table 1: Concentration-response of chili pepper fruit extract on the pink hibiscus mealybug *Maconellicoccus hirsutus*. Temp.: 25 ± 1° C, RH 70 ± 10% and 12 h of photophase.

N[1]	Slope± EP[2]	LC_{50}[3] (IC[4] a 95%) (g/100ml)	DF[5]	$\chi^{2(6)}$	p[7]
303	1,7799±0,4786	0,96 (0,19 – 1,63)	2	1,0478	0,306

[1]Number of insects used in the test; [2]Curve slope ± standard error; [3]Lethal concentration; [4]Confidence interval at 95% probability; [5]degrees of freedom; [6]Chi-square test; and [7]P value.

The values found demonstrated a high insecticidal potential of the aqueous extract of *C. frutescens* in the control of *M. hirsutus* because even at low concentrations of the extract, mortalities were higher than 70%.

This mortality rate can be attributed to the secondary compounds present in the *C. frutescens* extract. Several studies indicate the presence of alkaloids, tannins, esters, glycosins, saponins, flavonoids, phenols, and other components in fruits of the genus Capsicum. A significant presence of capsaicin is also observed, representing between 50 and 70% of the total capsaicinoids, the substance responsible for its pungency in pepper fruits [17, 18, 19].

Compounds such as alkaloids, phenols, and esters can act in multiple locations and express biocidal, developmental retardant of insects, repellent, and anti-food activity in pest organisms [20, 21]. In this way, GAUTAM *et al*. [22] isolated purified phenolic compounds from *Acacia nilotica* against the caterpillar *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) and observed inhibition of egg emergence, prolongation of the period of larval, pupal and total insect development, as well as observed mortality rates above 60% for adult individuals.

Other compounds such as tannins and saponins can reduce organisms' growth rate, affect feed efficiency and protein digestibility, and degrade their intestinal cells of them [23]. In a study carried out by Farahat *et al*. [24], a delay in the larval development of *Culex pipiens* L. mosquitoes (Diptera: Culicidae) subjected to treatment with tannic acids was observed, which suggests the effectiveness of these compounds in controlling insect pests.

In addition to the secondary compounds of chili pepper extract, other factors can act on the performance of insect pests, such as the extract extraction methodology, the extracted plant part, and the vegetative stage of the plant. In a study conducted by Izah [19], the crude, ketone, and ethanoic extracts of *C. frutescens* var. *minima* were evaluated in larvae of *Anopheles gambiae* (Diptera: Culicidae). The results showed that the ethanolic extract resulted in higher rates of larval mortality, suggesting that this extraction method may allow the extraction of greater amounts of secondary compounds from pepper fruits. This information is important for developing more effective and sustainable pest control strategies.

Also, different parts of the plant may contain varying amounts of specific compounds. In a study carried out by Faleiro *et al*. [25], the mortality of *Atta laevigata* ants (Hymenoptera: Formicidae) was evaluated using ethanol extracts from different plant parts of *Eugenia dysenterica* (Mart.) (Myrtaceae). Results indicated significant mortality rates for all extracts of different plant parts, however, the highest mortality rate was observed for the extract of flowers and leaves. It is possible that the higher concentration of metabolites in these parts of the plant contributed to the mortality of the organisms. This information is important for the development of more effective pest control strategies using plant extracts.

In the present study, as fruits and seeds were used, probably there were higher concentrations of secondary compounds since they are part of the plant responsible for the reproduction and permanence of the species, therefore it needs greater protection of the plant's defenses. The degree of maturation of the fruits is another factor that can influence together with the part of the plant used from the pepper plants in the composition of the extracts and cause the mortality of mealybugs [23]. For example, the physiological changes in metabolites that occur during plant development affect their nutritional and health-promoting properties during vegetative development [26].

Finally, as the coffee leaf discs were submerged in the pepper extract (indirect application of the extracts), mealybugs are likely intoxicated through the digestive tract. Piffer *et al.* [27] studied the effect of the interaction between castor oil (*Ricinus communis*) and physic nut oil (*Jatropha curcas*) on the aphid *M. persicae* in direct (spraying) and indirect (immersion) application forms, insect mortality rates of up to 84% were observed in the study when they were exposed to the indirect treatment method.

Despite the promising results, it is necessary to identify more precisely the substances present in the fruit with insecticidal effects, to evaluate their sublethal effects, in addition to carrying out tests in fields with different concentrations to evaluate the viability of the method.

Conclusion

The aqueous extract of the fruit of *C. frutescens* has insecticidal potential to control *M. hirsutus* at all concentrations tested in the laboratory.

Acknowledgments

This project was made possible by the Federal Institute of Espírito Santo (IFES), the Research Support Foundation of the State of Espírito Santo (FAPES, and the National Council for Scientific and Technological Development (CNPQ).

References

1. Williams, DJ. A brief account of the hibiscus mealybug *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae), a pest of agriculture and horticulture, with descriptions of two related species from southern Asia. Bulletin of Entomological Research. 1996;86(5):617-28. https://doi.org/10.1017/S0007485300039420
2. Ganjisaffar F, Andreason SA, Perring TM. Lethal and sub-lethal effects of insecticides on the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae). Insects. 2019;10(1):31. https://doi.org/10.3390/insects10010031
3. Osborne LS, Cuda JP. Release of exotic natural enemies for biological control: case of damned if we do and damned if we donít? Journal of Land Use and Environmental Law. 2003;18(2):399-407.
4. Zhang A. Sex potion ensnares mealy bugs. Agricultural Research. 2005;53(4):18.
5. Martins DS, Fornazier MJ, Peronti ALBG, Culik MP, Souza CAS, Taques RC, *et al*. *Maconellicoccus hirsutus* (Hemiptera: Pseudococcidae) in Brazil: recent spread, natural enemies, and new hosts. Florida Entomologist. 2019;102(2):438-443. https://doi.org/10.1653/024.102.0225
6. Francis-Ellis D. Paper on background and status of mealybug *Maconellicoccus hirsutus* in Grenada. Ministry of Agriculture, Grenada; c1995.
7. Reddy KN, Ramasamy GG, Agrawal A, Srivastava S, Pathak J, Venkatesan T, *et al*. Reference genes selection for expression studies in *Maconellicoccus hirsutus* (Green) (Pseudococcidae: Hemiptera) under specific experimental conditions. Molecular Biology Reports. 2023;50(2):1221-1230. https://doi.org/10.1007/s11033-022-08120-7
8. AGROFIT - Sistema de Agrotóxicos Fitossanitários. Disponível em: <http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons>. Accessed on: Feb. 28, 2023. Portuguese.
9. Bajwa MS, Tariq M, Gulzar A, Saeed H, Mashwani ZUR. Toxicity of green silver nanoparticles of plant extracts against citrus mealybug *Planococcus citri*. Plant Protection. 2020;4(1):01-10. https://doi.org/10.33804/pp.004.01.3214
10. Holtz AM, Carvalho JR, Gomes MS, Borghi Neto B, Piffer ABM, Aguiar RL. Toxicity Aqueous Extract of Castor Bean (*Ricinus communis*) to *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae). Journal of Experimental Agriculture International. 2019;38(4):1-6. https://doi.org/10.9734/jeai/2019/v38i130291
11. Alloui-Griza R, Cherif A, Attia S, Francis F, Lognay GC, Grissa-Lebdi K. Lethal Toxicity of *Thymus capitatus* Essential Oil Against *Planococcus citri* (Hemiptera: Pseudococcidae) and its Coccinellid Predator *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae). Journal of Entomological Science. 2022;57(3):425-435. https://doi.org/10.18474/JES21-81
12. Pandhair V, Sharma S. Accumulation of capsaicin in seed, pericarp, and placenta of *Capsicum annuum* L fruit. Journal of Plant Biochemistry and Biotechnology. 2008;17(1):23-27.
13. Kishore V, Loach N, Srivastava CN, Mohan L. Toxicity evaluation and chemical composition of *Capsicum frutescens* for natural control of Asian blue tick, *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae). The Journal of Basic and Applied Zoology. 2021;82(1):1-10. https://doi.org/10.1186/s41936-021-00249-4
14. Sanches NF, Carvalho RS. Procedimentos para manejo da criação e multiplicação do predador exótico *Cryptolaemus montrouzieri*. Cruz das Almas: Embrapa Mandioca e Fruticultura; c2010. p. 5. (Embrapa Mandioca e Fruticultura. Circular técnica, 99). Available in: http://ainfo.cnptia.embrapa.br/digital/bitstream/item/29720/1/circular-99ID27552.pdf Accessed in: June, 12, 2016. Portuguese.
15. Holtz AM, Paula Marchiori JJ, Franzin ML, Paulo HH, Botti JMC, Coffler T. Potencial de manejo de *Myzus persicae* com óleo de pinhão manso armazenado em diferentes embalagens. Revista Agrogeoambiental. 2016;8(3):41-50. https://doi.org/10.18406/2316-1817v8n32016835
16. Abbott WS. A method of computing the effectiveness of an insecticide. Journal Economic Entomology. 1925;18(2):265-67.
17. Bello I, Boboye BE, Akinyosoye FA. Phytochemical screening and antibacterial properties of selected Nigerian long pepper (*Capsicum frutescens*) fruits.

African Journal of Microbiology Research. 2015;9(38):2067-78. https://doi.org/10.5897/AJMR2014.7286
18. Sen N, Paul D, Sinha SN. *In vitro* antibacterial potential and phytochemical analysis of three species of chili plant. Journal of Chemical and Pharmaceutical Research. 2016;8(2):443-447.
19. Izah SC, Etim NG, Ilerhunmwuwa IA, Silas G. Evaluation of crude and ethanolic extracts of *Capsicum frutescens* var. *minima* fruit against some common bacterial pathogens. International Journal of Complementary & Alternative Medicine. 2019;12(3):105-108. https://doi.org/10.15406/ijcam.2019.12.00457
20. Sombra KE, Aguiar CV, Oliveira SJ, Barbosa MG, Zocolo GJ, Pastori PL. Potential pesticide of three essential oils against *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). Chilean Journal of Agricultural Research. 2020;80(4):617-28. http://dx.doi.org/10.4067/S0718-58392020000400617
21. Sombra KES, Pastori PL, Aguiar CVSD, André TPP, Oliveira SJD, Barbosa MG, *et al*. Selectivity of essential oils to the egg parasitoid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). Revista Ciência Agronômica. 2022;53:1-11. https://doi.org/10.5935/1806-6690.20220022
22. Gautam S, Chimni SS, Arora S, Sohal SK. Toxic effects of purified phenolic compounds from *Acacia nilotica* against common cutworm. Toxicon. 2021;203:22-29. https://doi.org/10.1016/j.toxicon.2021.09.017
23. Holtz AM, Assis CHB, Piffer ABM, Carvalho JR, Aguiar RL, Pratissoli D. Toxicity of *Moringa oleifera* Lam. seed extract at different stages of maturation on *Tetranychus urticae* Koch (Acari: Tetranychidae). Journal of Pharmacognosy and Phytochemistry. 2020;9(3):01-04.
24. Farahat NM, Khaled AS, Hussein MA, Zyaan OH, *et al*. Biological and Histological Alterations in The Larvae of *Culex pipiens* L. (Diptera: Culicidae) Induced by Imidcaloproid and Tannic Acid. Egyptian Academic Journal of Biological Sciences. 2021;14(1):243-254. https://doi.org/10.21608/eajbsa.2021.160456
25. Faleiros MV, Faleiros JH, Oliveira RC, Rocha EC, Luz JMQ, and Silva Arruda, A. Atividade inseticida do extrato etanólico de *Eugenia dysenterica* (Myrtaceae) sobre formigas cortadeiras. Scientia Plena. 2022;18(10):1-9. https://doi.org/10.14808/sci.plena.2022.107201
26. Önder S, Tonguç M, Erbaş S, Önder D, Mutlucan M. Investigation of phenological, primary and secondary metabolites changes during flower developmental of *Rosa damascena*. Plant Physiology and Biochemistry. 2022;192:20-34. https://doi.org/10.1016/j.plaphy.2022.09.032
27. Piffer ABM, Holtz AM, Botti JMC, Carvalho JR, Aguiar RL, Alves AG, *et al.* Interaction between Castor Bean Oil and Jatropha Oil to Control the Brassica Aphids. International Journal of Plant & Soil Science. 2023:35(8):26-33. https://doi.org/10.9734/IJPSS/2023/v35i82879