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Bioinsecticides based on neem (*Azadirachta indica*) and rue (*Ruta graveolens*) against *Agrotis ipsilon*

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Abstract

Agrotis ipsilon is an important pest of several crops, where it causes damage to various parts of the plant such as stems, roots, leaves and tubers. Due to the growing demand for controlling these pests, there is a constant need for new products as the organisms acquire resistance after continuous contact. And this also generates a demand for bioinsecticides, which normally pose a lower health risk and are less harmful to the environment. Among the plants commonly used as insecticides are neem and rue, which have already been studied for several pests, although never together. This work aimed to determine the insecticidal activity of a natural insecticide derived from a mixture of neem and rue, commonly used in the region of Senhor do Bonfim-BA against *A. ipsilon*. Four different solutions were prepared, containing a) 20 neem leaves, 10 rue leaves and 5 *A. ipsilon caterpillars*, b) 20 neem leaves and 10 rue leaves, c) 20 neem leaves and d) 10 rue leaves. Water was tested as control. In the tests carried out, both the mixture popularly used in the region, containing neem, rue, and *A. ipsilon* caterpillars, as well as the solution containing only neem and rue had good results, killing all caterpillars in 72 hours. Isolated plant extracts did not obtain good results, only delaying the formation of pupae for neem extracts. Therefore, the mixture of Neem and Rue showed good insecticidal potential against *A. ipsilon*, with much higher activity than its isolated components.

Keywords: *Agrotis ipsilon,* botanical pesticides, insecticidal activity, lettuce

Introduction

The effects of the usage of pesticides are seen both in the rural population, mainly through the environmental contamination of water and soil, and in the general population, mainly through residues of pesticides within the food (Brito et al., 2021). There are numerous compounds on the market to control pests, insects, and other organisms. Since those organisms acquire resistance after continuous contact with these compounds, there is an increasing demand for new products (Braibante & Zappe, 2012). Therefore, the application of bioinsecticides becomes feasible, as it resorts to organisms from the environment itself to control pests (Oliveira-Filho, 2008).

A wide variety of plants have insecticidal effects that, if studied, can replace pesticides for pest control (Badalamenti et al., 2021; Devrnja et al., 2022; Faleiros et al., 2022; Demirak & Canpolat, 2022; Tavares et al., 2021; Zhang et al., 2020). Neem, *Azadirachta indica*, was used to control the pests *Diabrotica speciosa* and *Empoasca kraemeri* (Kinhama et al., 2022), the aphids *Brevicoryne brassicae* and *Myzus persicae* (Carvalho et al., 2008), the fall armyworm *Spodoptera frugiperda* (Santos & Barbian, 2022), fungi that cause powdery mildew (Carneiro et al., 2007) disease, the leaf miner fly *Liriomyza sativae* (Costa et al., 2016), among other uses.

Another well-studied plant, *Ruta graveolens*, known as rue (Wang et al., 2022), was used to control the pests *Diabrotica speciosa* (Barbosa et al., 2009), *Alphitobius diaperinus* in combination with mastruz, cinnamon and neem (Marcomini et al., 2009), *Ephestia kuehniella* (Barbosa et al., 2011), *Acanthoscelides obtectus* and corn weevil *Sitophilus zeamais* (Almeida & Silveira, 2019).

One of the several pests of plantations is the *Agrotis ipsilon* (Rings et al., 1975): its larva causes damage

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to various parts of the plant, such as stem, root, and tuber (Sherrod et al., 1979). Its most striking characteristics are its cylindrical shape, smooth appearance, grayish color, and the behavior of curling up when touched, which is why they are popularly known as thread caterpillar (Fernandes et al., 2013). Studies have shown that this caterpillar is a pest of corn (Cruz et al., 1982), coffee (Fernandes et al., 2013), tobacco (Matioli, 1986), beans (Costa & Link, 1984), among others.

The use of insecticides for the chemical control of *A. ipsilon* requires a variety of products and methods for effective treatment. Currently, a wide variety of insecticides are used to control this caterpillar, especially pyrethroids, carbamates, organophosphates, and neonicotinoids. However, in case of indiscriminate use, these compounds can cause serious damage to the environment and human health (Lustosa et al., 2023; Joshi et al., 2020).

In the present study, the aim was to analyze the effectiveness of natural insecticides based on neem and rue for *A. ipsilon* pest control.

Material and Methods

The caterpillars used in the tests were collected at Serra Verde settlement, in Senhor do Bonfim - BA and brought to laboratory. Lettuce leaves (*Lactuca sativa var crispa*) used in the test were collected from those that were free of infestation from the plantation in the Serra Verde settlement. Neem and rue leaves used for the mixture preparation were collected also in Serra Verde settlement.

Extracts preparation

For the preparation of the extracts, the method popularly used in the region was followed, in which 250 mL of water, 20 neem leaves, 10 rue leaves and five *A. ipsilon* caterpillars are placed in PET bottles. Thus, to evaluate the activity of each component of the mixture, the following solutions were prepared in duplicate:

Treatment 1: 250 mL of water, 20 neem leaves, 10 rue leaves and 5 *A. ipsilon* caterpillars.
Treatment 2: 250 mL of water, 20 neem leaves and 10 rue leaves.
Treatment 3: 250 mL of water and 20 neem leaves.
Treatment 4: 250 mL of water and 10 rue leaves.
Treatment 5 (control): 250 mL of water

All mixtures were prepared one week before application, following the method popularly used in the region and filtered.

Bioassays

Lettuce leaves were placed in plastic pots and 30 mL of the mixture was sprayed to the leaves. After that ten 6th instar caterpillars were placed in each pot. All treatments were realized in triplicate. The pots were closed with voil and mortality was observed every 24 hours for six days.

Statistics

Data of mortality and pupae formation were tabulated and analyzed using one-way ANOVA. Differences between treatments were determined by Tukey's multiple range tests and were considered statistically significant at $P < 0.05$.

Results and Discussion

All pots were analyzed every 24h to observe how many of the caterpillars were still alive and for how many there was formation of pupae. All results are shown in **Table 1.**

Treatment 1 had an insecticidal action on the caterpillars: after 48 hours all caterpillars were dead. It is important to highlight that, after using the extract, the mixture may have caused anatomical abnormalities in the larvae, as some of them seemed to melt and darken after death. Substances tested in other works, as basil, gautheria and eucalyptus essential oils, showed to cause deformation in *A. ipsilon* larvae in higher concentrations (Jeyasankar, 2012; Shadia et al., 2007). Thus, it may be necessary to conduct a more detailed study to verify the true effect of the neem, rue and caterpillar mixture. There is no information in the literature about this method of extract preparation, especially about the presence of caterpillars in it, and therefore this is an alternative method that needs to be explored.

In treatment 2, insecticidal action was also verified: after 72 hours all caterpillars were eliminated. This treatment took a day longer to kill all caterpillars then treatment 1, but also showed excellent mortality. In this treatment, only neem and rue leaves were used to prepare the mixture, without caterpillars. There is also a gap in the literature regarding studies that deal with the use of the two plants in a single defensive, although both plants were known for their insecticidal activity (Almeida & Silveira, 2019; E. M. Costa et al., 2016; Kinhama et al., 2022; Santos & Barbian, 2022; Wang et al., 2022). There is no literature data on how adding caterpillars to the mixture can change its mortality activity. We can only assume that some compounds produced by the dying caterpillar may increase the toxicity of the mixture.

In treatment 3, in which green neem leaves

Table 1: Alive caterpillars and pupae formed after treatments.

Treatment	Alive caterpillars/pupae formed					
	24h	48h	72h	96h	120h	144h
1	16 a/0 A	0 a/0 A	0 a/0 A	0 a/0 A	0 a/0 A	0 a/0 A
2	26 b/0 A	12 b/0 A	0 a/0 A	0 a/0 A	0 a/0 A	0 a/0 A
3	30 c/4 B	30 c/7 B	30 b/17 B	30 b/24 B	30 b/28 B	30 b/30 B
4	30 c/18 C	30 c/30 C	30 b/30 C	30 b/30 C	30 b/30 C	30 b/30 B
5	30 c/16 C	30 c/29 C	30 b/30 C	30 b/30 C	30 b/30 C	30 b/30 B

(1) Values followed by equal small case or equal capital letters in the column are not significantly different by Tuckey test at $P < 0.05$.

were crushed with 250 mL of water, the pupae formation was observed with some delay probably caused by the application of the mixture. According to Viana et al., most neem derivatives studied to control pests are obtained from seeds by grinding or compression to remove the oil (Viana & Ribeiro, 2010). Crushed dry leaves are also used, as can be seen in the work by Rosado et al. and Carvalho et al (Carvalho et al., 2008; Rosado et al., 2011). As for the usage of green leaves in the preparation of the insecticide, it is noted that this type of preparation is still poorly explored. But in a study by Viana and Ribeiro it is possible to observe that the aqueous extract of crushed green neem leaves, applied in three sprays, can effectively control *Spodoptera frugiperda* in maize (Viana & Ribeiro, 2010).

In treatment 4, in which an aqueous extract of rue was used, no changes were noted when compared to control treatment. It is important to highlight that, according to Ayres et al. (Ayres et al., 2020), rue is the subject of several studies that deal with the insecticidal action of the plant. One of these studies evaluated the deterrent effect of plant extracts on *Papilio thoas brasiliensis*, given that they consumed few leaves (Cupertino de Souza & Mara-Mussury, 2010). In the study by Marangoni et al., it is also possible to observe the deterrent effect of rue (Marangoni et al., 2013). However, the plant is still not recognized by Brazilian agencies for its effectiveness. Treatment 4 was only slightly different from 5 (control) in the first day, but in the end in both treatments all caterpillars formed pupae.

Conclusion

It was possible to conclude that the method used showed insecticidal activity on populations of *A. ipsilon* after 48 hours of spraying the extracts containing Neem, Rue and *A. ipisilon* caterpillars. The second treatment, containing Neem and Rue also showed good insecticidal activity, with all larvae dead after 72h. These two treatments demonstrated efficiency in pest control. Being an easy-to-handle natural pesticide, it can be considered an alternative with economic and ecological perspectives, which can cause less damage to the environment and the health of workers and consumers, contributing to sustainable rural development, as well as to the production of healthy foods. Treatment 3, using only crushed green Neem leaves have delayed pupae formation. It is worth highlighting the need for more studies that explore the use of this extract, due to the lack of studies focused on this method used by the settlers.

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References

Almeida, A.D.O., Silveira, J.V.W. 2019. Perspectivas da utilização de arruda (Ruta graveolens) no armazenamento de grãos. *Blucher Chemical Engineering Proceedings* 6: 896–902.

Alves Lustosa, E., Nobrega de Sousa, M.A., Lucena de Oliveira, T., Pereira, G.JS., & Nóbrega, F.V.A. 2023. Toxicidade de inseticida agrícola em bioensaio com Artemia salina. *Scientia Plena* 19.

Ayres, M.I.C., Puente, R.J.A., Fernandes Neto, J.G., Uguen, K., Alfaia, S.S. 2020. Defensivos naturais: manejo alternativo para pragas e doenças. Editora INPA, Manaus, Brasil. 34p.

Badalamenti, N., Ilardi, V., Bruno, M., Pavela, R., Boukouvala, M.C., Kavallieratos, N. G., Maggi, F., Canale, A., Benelli, G. 2021. Chemical Composition and Broad-Spectrum Insecticidal Activity of the Flower Essential Oil from an Ancient Sicilian Food Plant, Ridolfia segetum. *Agriculture* 11: 304.

Barbosa, F.S., Leite, G.L.D., Alves, S.M., Nascimento, A.F., D'Ávila, V.D.A., Costa, C.A. 2011. Insecticide effects of Ruta graveolens, Copaifera langsdorffii and Chenopodium ambrosioides against pests and natural enemies in commercial tomato plantation. Acta Scientiarum. *Agronomy* 33: 37-43.

Barbosa, F.S., Leite, G.L.D., Martins, E R., Guanabens, R.E.M., Silva, F.W.S. 2009. Métodos de extração e concentrações no efeito inseticida de Ruta graveolens L., Artemisia verlotorum Lamotte e Petiveria alliacea L. a Diabrotica speciosa Germar. *Revista Brasileira de Plantas* Medicinais 11: 221–229.

Braibante, M.E F., Zappe, J.A. 2012. A Química dos Agrotóxicos. *Química Nova Na Escola* 34: 10–15.

Brito, L.M., Maracajá, P.B., Medeiros, A.C., Feitosa, A.N.A., Dantas, M.C.A.M., Fernandes Filho, A. 2021. Intoxicações por agrotóxicos: Impactos causados pela utilização indiscriminada em comunidades rurais. Research, Society and Development 10: e56710817418.

Carneiro, S. M. de T. P. G., Pignoni, E., Vasconcellos, M. E. da C., & Gomes, J. C. (2007). Eficácia de extratos de nim para o controle do oídio do feijoeiro. *Summa Phytopathologica* 33: 34–39.

Carneiro, S.M.T.P.G., Pignoni, E., Gomes, J.C. 2008. Efeito do nim (Azadirachta indica A. Juss.) no controle da mancha angular do feijoeiro. *Revista Brasileira de Plantas Medicinais* 10: 6–10.

Carvalho, G.A., Santos, N.M., Pedroso, E.C., Torres, A.F. 2008. Eficiência do óleo de nim (Azadirachta indica A. Juss) no controle de Brevicoryne brassicae (Linnaeus, 1758) e Myzus persicae (Sulzer, 1776) (Hemiptera: Aphididae) em couve-manteiga Brassica oleracea Linnaeus var. Acephala. *Arquivos Do Instituto Biológico*, 75: 181–186.

Costa, E. C., Link, D. 1984. Estimativa de danos e estudo sobre o comportamento de Agrotis ipsilon (Hufnagel). Revista Do Centro de Ciências Rurais, 14: 9-17.

Costa, E.M., Torres, S.B., Ferreira, R.R., Silva, F.G., Araujo, E.L. 2016. Aqueous extract of neem seeds in the control of Liriomyza sativae (Diptera: Agromyzidae) in the melon. *Revista Ciência* Agronômica 47:401-406.

Cruz, I., Santos, J. P., & Waquil, J. M. (1982). Principais pragas da cultura do milho. In Recomendações técnicas para o cultivo de milho). EMBRAPA, Brasília, Brasil. pp. 31–37

Cupertino de Souza, D.M., & Mara-Mussury, R. 2010. Avaliação do efeito deterrente de extratos vegetais sobre Papilio thoas brasiliensis (Lepidoptera: Papilionidae) Rothschild & Jordan, 1906. *Journal of the Selva Andina Research Society* 1: 50–56.

Demirak, M.Ş., Canpolat, E. 2022. Plant-Based Bioinsecticides for Mosquito Control: Impact on Insecticide Resistance and Disease Transmission. *Insects* 13: 162.

Devrnja, N., Milutinović, M., & Savić, J. 2022. When Scent Becomes a Weapon—Plant Essential Oils as Potent Bioinsecticides. *Sustainability* 14: 6847.

Faleiros, M.V., Faleiros, J.H., Oliveira, R.C., Rocha, E.C., Queiroz Luz, J.M., Arruda, A.S. 2022. Atividade inseticida do extrato etanólico de Eugenia dysenterica (Myrtaceae) sobre formigas cortadeiras. *Scientia Plena* 18: 107201

Fernandes, F.L., Diniz, J.F.S., Silva, P R., Mosca, E. 2013. Damage of Agrotis ipsilon (Lepidoptera: Noctuidae) on Coffea arabica in Brazil. *Revista Colombiana de Entomologia* 39: 49–50.

Jeyasankar, A. 2012. Antifeedant, insecticidal and growth inhibitory activities of selected plant oils on black cutworm, Agrotis ipsilon (Hufnagel) (Lepidoptera: Noctuidae). Asian Pacific Journal of Tropical Disease 2: S347–S351.

Joshi, M., Rana, A., Raj, P.V, Kaushal, S., Inamdar, A., Verma, K., Chandel, R. 2020. The potency of chemical insecticides in management of cutworm, Agrotis ipsilon Hufnagel (Noctuidae: Lepidoptera): A review. *Journal of Entomology and Zoology Studies* 8: 307–311.

Kinhama, V., Mampassi, N., Kabutoto, J., Sousa Neto, F. 2022. Avaliação laboratorial do efeito de extratos de Azadirachta indica no controle de insetos-praga. *Meio Ambiente (Brasil)* 4: 69–76.

Marangoni, C., Moura, N.F., Garcia, F.R.M. 2013. Utilização de óleos essenciais e extratos de plantas no controle de insetos. *Revista de Ciências Ambientais* 6: 95-112

Marcomini, A.M., Alves, L.F.A., Bonini, A.K., Mertz, N.R., Santos, J.C. 2009. Atividade inseticida de extratos vegetais e do óleo de nim sobre adultos de Alphitobius diaperinus panzer (Coleoptera, Tenebrionidae). *Arquivos Do Instituto Biológico* 76: 409–416.

Matioli, J.C. 1986. Métodos de aplicação e dosagens de acefato no controle de Agrotis sp.(Lepidoptera: Noctuidae) em fumo. *Pesquisa Agropecuária Brasileira* 21: 823–828.

Oliveira-Filho, E.C. 2008. Avaliação da Periculosidade Ambiental de Bioinseticidas como uma Nova Perspectiva para a Ecotoxicologia no Brasil. *Journal of The Brazilian Society of Ecotoxicology* 3: 1–7.

Rings, R.W., Arnold, F.J., Johnson, B.A. 1975. Host Range of the Black Cutworm on Vegetables: A Bibliography. *Bulletin of the Entomological Society of America* 21: 229–234.

Rosado, L.D.S., Pinto, J.E.B.P., Botrel, P.P., Bertolucci, S.K.V., Niculau, E.S., Alves, P.B. 2011. Influência do processamento da folha e tipo de secagem no teor e composição química do óleo essencial de manjericão cv. Maria Bonita. *Ciência e Agrotecnologia* 35: 291–296.

Santos, L.A.O., Barbian, L.P.J. 2022. Revisão bibliográfica sobre o uso de nim (Azadirachta indica A Juss.) Para o controle de lagartas de Spodoptera frugiperda. In Fitoquímica: potencialidades biológicas dos biomas brasileiros - Editora Científica Digital, São José do Rio Preto, Brasil. pp. 160–169.

Shadia, E., Aziz, A.E.I., Omer, E.A., Sabz, A.S. 2007. Chemical Composition of Ocimum americanum Essential Oil and Its Biological Effects Against, Agrotis ipsilon, (Lepidoptera: Noctuidae). *Research Journal of Agriculture and Biological Sciences* 3: 740–747.

Sherrod, D W., Shaw, J.T., Luckmann, W.H. 1979. Concepts on Black Cutworm Field Biology in Illinois. Environmental Entomology 8: 191–195.

Tavares, W.R., Barreto, M.C., Seca, A.M. L. 2021. Aqueous and Ethanolic Plant Extracts as Bio-Insecticides—Establishing a Bridge between Raw Scientific Data and Practical Reality. *Plants* 10: 920.

Viana, P.A., Ribeiro, P.E.A. 2010. Efeito do extrato aquoso de folhas verdes de nim (Azadirachta indica) e do horário de aplicação sobre o dano e o desenvolvimento larval de Spodoptera frugiperda (J. E. Smith, 1797) (Lepidoptera: noctuidae) na cultura do milho. *Revista Brasileira de Milho e* Sorgo 9: 27–37.

Wang, S., Li, S., Cheng, F., Ren, T., Fan□Li, Mei, D., Gao, K., Song, Q. 2022. Antifungal, Repellency, and Insecticidal Activities of Cymbopogon distans and Ruta graveolens Essential Oils and Their Main Chemical Constituents. *Chemistry & Biodiversity* 19: e202200351

Zhang, P., Qin, D., Chen, J., Zhang, Z. 2020. Plants in the Genus Tephrosia: Valuable Resources for Botanical Insecticides. *Insects* 11: 721.

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