AGROCIENCIA URUGUAY

Agrociencia Uruguay 2026 | Volume 30 | Article e2017
DOI: 10.31285/AGRO.30.2017
ISSN 2730-5066

Non-Target Behavioral Effects of Plant-Based Repellents from Two *Allium* Species on Predatory Social Wasps

Barbosa, B. C. [1]; Silva, P. V. A. [2]; Maciel, T. T. [1]; Smagghe, G. [3, 4]; Oliveira, M. L. [2]

[1] *Universidade Federal de Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brasil*

[2] *Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brasil*

[3] *Guizhou University, Institute of Entomology, Guiyang, China*

[4] *Vrije Universiteit Brussel, Department of Biology, Brussels, Belgium*

Abstract

Plant-based repellents are increasingly considered for pest management, but their effects on beneficial non-target insects remain insufficiently understood. This study evaluated the repellent and behavioral responses of three predatory social wasps, *Polybia minarum*, *Polybia sericea*, and *Polistes canadensis*, to 20% (w/v) aqueous extracts of onion (*Allium cepa*) and garlic (*Allium sativum*). Adult females were tested in free-movement arenas containing treated and untreated compartments, and repellency was quantified using an evasion index. Qualitative behavioral responses were also recorded during the assays. Onion extract did not produce significant repellency in any species. Garlic extract induced significant repellency only in *P. canadensis*. No mortality occurred during the assays. Exposure to garlic was associated with agonistic interactions, agitation, increased movement, apparent escape attempts, and self-grooming, while onion caused no conspicuous behavioral alterations. These findings indicate that aqueous extracts from closely related *Allium* species can produce contrasting and species-specific effects on predatory social wasps. Non-target behavioral assays should therefore be included when screening plant-based products for integrated pest management, particularly in systems where social wasps contribute to biological control.

Keywords: agroecology, beneficial insects, biological control, botanical bioinputs, integrated pest management

Editor

Valentina Mujica
Instituto Nacional de Investigación Agropecuaria (INIA), Canelones, Uruguay

Received *8 Jun 2026*
Accepted *30 Sep 2026*
Published *22 Oct 2026*

Correspondence

Bruno Corrêa Barbosa
barbosa.bc@outlook.com

CC BY

Barbosa, B. C., Silva, P. V. A., Maciel, T. T., Smagghe, G., & Oliveira, M. L. (2026). Non-target behavioral effects of plant-based repellents from two *Allium* species on predatory social wasps. *Agrociencia Uruguay, 30*, Article e2017. https://doi.org/10.31285/AGRO.30.2017

Efectos comportamentales no objetivo de repelentes de origen vegetal derivados de dos especies de *Allium* sobre avispas sociales depredadoras

Resumen

Los repelentes de origen vegetal se consideran cada vez más para el manejo de plagas, pero sus efectos sobre insectos benéficos no objetivo aún no se comprenden suficientemente. Este estudio evaluó las respuestas repelentes y comportamentales de tres especies de avispas sociales depredadoras, *Polybia minarum*, *Polybia sericea* y *Polistes canadensis*, a extractos acuosos al 20 % (p/v) de cebolla (*Allium cepa*) y ajo (*Allium sativum*). Las hembras adultas fueron evaluadas en arenas de libre movimiento que contenían compartimentos tratado y no tratado, y la repelencia se cuantificó mediante un índice de evasión. También se registraron respuestas comportamentales cualitativas durante los ensayos. El extracto de cebolla no produjo repelencia significativa en ninguna de las especies. El extracto de ajo indujo repelencia significativa únicamente en *P. canadensis*. No se registró mortalidad durante los ensayos. La exposición al ajo estuvo asociada con interacciones agonísticas, agitación, aumento del movimiento, aparentes intentos de escape y autoacicalamiento, mientras que la cebolla no causó alteraciones comportamentales evidentes. Estos resultados indican que los extractos acuosos de especies filogenéticamente próximas de *Allium* pueden producir efectos contrastantes y específicos de cada especie sobre las avispas sociales depredadoras. Por lo tanto, los ensayos comportamentales con organismos no objetivo deberían incluirse en la evaluación de productos de origen vegetal destinados al manejo integrado de plagas, particularmente en sistemas en los que las avispas sociales contribuyen al control biológico.

Palabras clave: agroecología, insectos benéficos, control biológico, bioinsumos botánicos, manejo integrado de plagas

Efeitos comportamentais não alvo de repelentes de origem vegetal derivados de duas espécies de *Allium* sobre vespas sociais predadoras

Resumo

Os repelentes de origem vegetal são cada vez mais considerados para o manejo de pragas, mas seus efeitos sobre insetos benéficos não alvo ainda são pouco compreendidos. Este estudo avaliou as respostas de repelência e comportamentais de três espécies de vespas sociais predadoras, *Polybia minarum*, *Polybia sericea* e *Polistes canadensis*, a extratos aquosos a 20% (m/v) de cebola (*Allium cepa*) e alho (*Allium sativum*). Fêmeas adultas foram testadas em arenas de livre movimentação contendo compartimentos tratado e não tratado, e a repelência foi quantificada por meio de um índice de evasão. Respostas comportamentais qualitativas também foram registradas durante os ensaios. O extrato de cebola não produziu repelência significativa em nenhuma das espécies. O extrato de alho induziu repelência significativa apenas em *P. canadensis*. Nenhuma mortalidade ocorreu durante os ensaios. A exposição ao alho foi associada a interações agonísticas, agitação, aumento da movimentação, aparentes tentativas de fuga e autolimpeza, enquanto a cebola não causou alterações comportamentais conspícuas. Esses resultados indicam que extratos aquosos de espécies filogeneticamente próximas de *Allium* podem produzir efeitos contrastantes e espécie-específicos sobre vespas sociais predadoras. Portanto, ensaios comportamentais com organismos não alvo devem ser incluídos na avaliação de produtos de origem vegetal destinados ao manejo integrado de pragas, particularmente em sistemas nos quais as vespas sociais contribuem para o controle biológico.

Palavras-chave: agroecologia, insetos benéficos, controle biológico, bioinsumos botânicos, manejo integrado de pragas

1. Introduction

Agricultural pest management relies heavily on synthetic insecticides, although the recurrent use of these products is associated with environmental impacts and negative effects on non-target organisms. Broad-spectrum insecticides may affect not only pest species, but also natural enemies, pollinators, and other beneficial arthropods that contribute to agroecosystem functioning (Casida & Durkin, 2013; Desneux et al., 2007; Jactel et al., 2019). This concern has stimulated the search for more selective alternatives that are compatible with integrated pest management strategies.

Among these alternatives, plant-derived products have received increasing attention because they contain bioactive compounds that may act as repellents, feeding deterrents, growth regulators, or insecticides. These products may be obtained through different methods, including aqueous and ethanolic extraction, extraction with organic solvents, and essential-oil distillation, which can influence their chemical composition and biological activity (Tavares et al., 2021). Plant extracts and essential oils have been proposed as promising tools to reduce dependence on synthetic insecticides, especially in agricultural systems seeking more sustainable practices (Isman, 2020; Souto et al., 2021; Tavares et al., 2021). However, the plant origin of a product does not guarantee ecological safety. Its effects may vary according to the plant species, preparation method, concentration, exposure route, and exposed organism (Costa et al., 2025; Giunti et al., 2022; Soares et al., 2026).

Aqueous extracts are particularly relevant because, compared with extraction methods that require organic solvents such as ethanol, methanol, acetone, or hexane, they can be prepared through simpler procedures using water and widely available, low-cost plant materials. Some organic solvents may themselves raise toxicological and environmental concerns, while other extraction approaches may require more sophisticated techniques and specialized equipment, potentially limiting their accessibility to small-scale farmers and low-input agricultural systems (Souto et al., 2021; Tavares et al., 2021). However, before these extracts can be recommended for pest management, their effects on beneficial organisms need to be assessed.

Onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) are widely used vegetables known for the presence of sulfur-containing and volatile compounds associated with different biological activities. Garlic-derived compounds, especially allicin and related molecules, have been associated with repellent or biocidal effects in different arthropods (Dancewicz & Gabrys, 2008; Mobki et al., 2014; Silva & Pelli, 2021). Onion-derived organosulfur compounds have also been related to bioactive activities, including insecticidal or repellent effects in some organisms (Falcón-Piñeiro et al., 2023). However, most studies on the bioactivity of *Allium* extracts focus on pest organisms, whereas their behavioral effects on beneficial insects remain poorly explored (Giunti et al., 2022; Lisi et al., 2025; Numa Vergel et al., 2011).

Social wasps are widely distributed across natural, agricultural, and urban environments in tropical and subtropical regions (Barbosa et al., 2016). As generalist predators, they capture a wide range of prey, including lepidopteran larvae and other herbivorous insects associated with crops (Prezoto et al., 2019; Southon et al., 2019). For example, colonies of *Polistes simillimus* Zikán, 1951 transferred to corn fields reduced populations of *Spodoptera frugiperda* by 77.16% and increased crop productivity by 15.94% (Prezoto & Machado, 1999a, 1999b, 1999c), whereas *Polybia ignobilis* (Haliday, 1836) was the main predator of *Ascia monuste* caterpillars in *Brassica* crops, causing mortality of up to 65.5% in fourth- and fifth-instar larvae (Picanço et al., 2010). In eucalyptus plantations, *Polistes versicolor* (Olivier, 1791) preyed on defoliating caterpillars and was successfully managed through the relocation of colonies to artificial shelters (Elisei et al., 2010, 2012). Some species also visit flowers and may participate in plant-insect interactions in natural and managed environments (Clemente et al., 2012; Santos et al., 2007; Schmack & Egerer, 2023). Therefore, understanding how plant-derived bioinsecticides affect social wasp behavior is important for evaluating the compatibility of these products with the conservation of natural enemies.

This gap is particularly relevant because behavioral responses may vary among social wasp species. Social wasps rely on chemical cues for nestmate recognition, colony organization, foraging, and interactions with the environment (van Zweden & d'Ettorre, 2010). Because plant-derived volatile compounds can modify the olfactory environment perceived by insects, they may interfere with the perception or interpretation of chemical cues and redirect behavioral responses. Such interference may be expressed as changes in orientation, foraging, movement, avoidance, or grooming, even in the absence of acute mortality (Giunti et al., 2022; Pavela et al., 2026). Differences in social organization, chemical ecology, chemosensory sensitivity, or colony-founding

strategy may influence how different species perceive and respond to external chemical stimuli. Thus, testing a single species may be insufficient to infer the compatibility of plant extracts with social wasps as a functional group.

Onion and garlic were selected based on previous reports of bioactivity and information provided by local farmers, rather than on a known natural association between the evaluated wasps and *Allium* crops. We evaluated the repellent effect of aqueous extracts of onion and garlic on three predatory social wasp species: *Polybia minarum* Ducke, 1906, *Polybia sericea* (Olivier, 1791), and *Polistes canadensis* (Linnaeus, 1758). Specifically, we tested whether these extracts induce evasion under laboratory conditions and recorded behavioral responses associated with exposure.

2. Materials and Methods

2.1 Model Species of Social Wasps

For the repellency tests, three social wasp species commonly found in agricultural environments were selected. *Polistes canadensis* was collected in the Ariaú Reserve, Iranduba, Brazil (3º15'16.207" S, 60º13'35.202" W), in September and October 2024. *Polybia minarum* was collected at the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil (3°05'45.8" S, 59°59'22.0" W), during the same period. *Polybia sericea* was collected at two locations: Manaus Air Base (BAMN) (3°08'43.5" S, 59°59'32.8" W), in March 2024, and INPA, in September 2024.

For *P. canadensis*, adult females were collected from dozens of nest combs to obtain a sufficient number of individuals, considering the independent-founding biology and relatively small colony size of this species. For *P. minarum* and *P. sericea*, adult females were obtained from three colonies of each species using an entomological net. Adult wasps of all species were maintained in plastic cages (30 × 15 × 12 cm) in the laboratory under controlled conditions of 28 °C, 60-70% relative humidity, and a 12 h photoperiod. Individuals were supplied ad libitum with water and sugar solution. The maintenance procedure followed Souza et al. (2021), with adaptations.

2.2 Preparation of Aqueous Extracts

The aqueous-extract preparation protocol was developed specifically for this study. The aqueous extracts were prepared at a concentration of 20% (w/v), calculated based on the fresh weight of onion and garlic tissues. For each extract, the plant material was ground with potable water using an electric hand blender until a homogeneous mixture was obtained, following a maceration procedure. The solutions were placed in glass bottles, kept under refrigeration for 24 h, and subsequently filtered through filter paper. A new set of onion and garlic extracts was prepared for each species on the day preceding its assay, and each species was evaluated on a different experimental day. The extracts were used after the 24-h refrigeration period and filtration. The resulting filtrates were used as aqueous extracts in the treatments applied to the filter paper discs.

2.3 Experimental Assays

The bioassays were conducted in a completely randomized design using a free-movement test (Figure 1). The free-movement bioassay was developed specifically for this study. The arenas consisted of two 300-mL plastic containers connected by a 20 cm tube, allowing free movement of the wasps between compartments. The arenas were reused between assays. After each assay, they were washed with unscented neutral soap, cleaned with 70% ethanol, and allowed to dry completely before reuse to prevent residual odors or compounds from influencing subsequent assays.

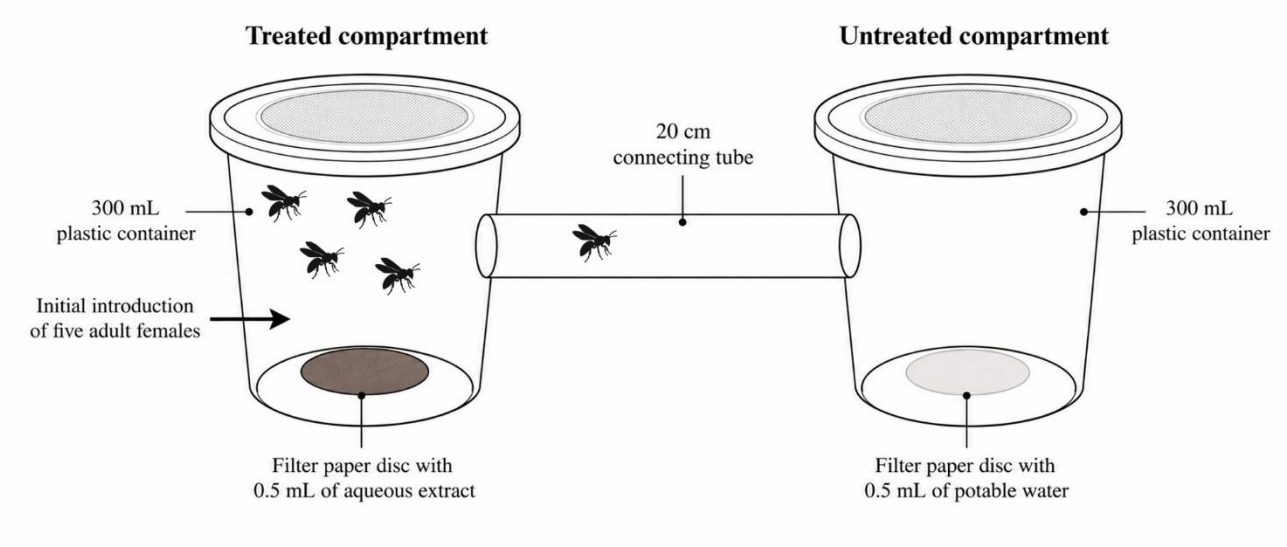


Figure 1. Schematic representation of the free-movement arena used in the repellency assays

The arena consisted of two 300 mL plastic containers connected by a 20 cm tube. One compartment contained a filter paper disc treated with 0.5 mL of aqueous extract, whereas the other contained a filter paper disc treated with 0.5 mL of potable water. Five adult females were initially introduced into the treated compartment.

Each container had a filter paper disc at the bottom. In each experimental arena, one container received 0.5 mL of the aqueous extract on the filter paper disc, whereas the other received 0.5 mL of potable water. In the control treatment, both containers received only potable water. Five adult females of each tested species were initially introduced into the container containing the aqueous extract or, in the case of the control, into one of the containers containing only water. The wasps were initially introduced into the extract compartment to simulate their arrival on a previously treated surface and to evaluate whether they remained in the treated compartment or moved toward the untreated compartment. Each arena containing five females was considered one experimental unit.

For each species and treatment, nine experimental units were established, in addition to three control units per species. The control arenas were included as procedural controls to verify whether the experimental apparatus produced a positional bias when both compartments contained only water and whether handling and confinement in the arenas, in the absence of extract, caused mortality or conspicuous behavioral alterations. Because these controls were intended only as procedural checks and were not included as a comparative treatment in the inferential analyses, a reduced number of three control units per species was used. This smaller sample size limits quantitative inference regarding the control condition. The number of wasps in the treated and untreated containers was recorded every 30 min for five consecutive hours, totaling 10 observations per experimental unit. Qualitative behavioral observations, including aggressive interactions, agitation, mobility, and self-grooming, were made ad libitum throughout each five-hour assay. These observations were descriptive and were not based on a previously established ethogram. The same behavioral categories and observation procedure were applied to all experimental units. The bioassays were performed under controlled laboratory conditions of 28 °C, 60-70% relative humidity, and a 12 h photoperiod.

2.4 Data Analysis

The primary response variable was the mean evasion index obtained for each experimental unit. At each of the 10 observation times, the evasion index was calculated as follows: Evasion index = (N extract – N water) / (N extract + N water), where N extract corresponds to the number of individuals present in the container containing the aqueous extract, and N water corresponds to the number of individuals present in the container containing water. The index ranges from −1 to +1. Values equal to zero indicate equal occupation of both compartments,

whereas positive values indicate greater occupation of the compartment containing the extract. In both cases, the results were interpreted as “No detectable repellency”. Negative values indicate greater occupation of the compartment containing water and were interpreted as “Repellency”.

For each experimental unit, the 10 evasion-index values were averaged, resulting in a single mean value per arena. This averaging procedure provided one summary measure of overall repellency for each arena while avoiding the treatment of repeated observations from the same experimental unit as independent replicates. These arena-level mean values were used as the sampling units in the inferential analyses of overall repellency. The normality of the mean evasion-index values was assessed using the Shapiro-Wilk test. For each species-extract combination, the mean evasion-index values were tested against zero using a one-tailed Student’s t-test, with the alternative hypothesis that the mean index was lower than zero.

Temporal variation in compartment occupation was analyzed using a binomial generalized linear mixed model (GLMM), fitted with the *glmer* function from the *lme4* package, using a binomial error distribution and a logit link. The numbers of wasps in the untreated and extract-treated compartments were included as the binomial response. Time, species, extract, and their interactions were included as fixed effects, while arena was included as a random intercept to account for repeated observations of the same experimental unit. Time was treated as a continuous predictor. Species- and extract-specific temporal slopes (β) were estimated using the *emtrends* function from the *emmeans* package. Positive β values indicated an increase in the occupation of the untreated compartment over time.

The control units, in which both containers contained only water, were used as procedural controls to verify the basal behavior of wasps in the arena and possible occupation biases between containers in the absence of extract. These units did not constitute the main comparative group for the repellency tests, which were conducted based on the mean indices obtained for each extract treatment. All analyses used R software version 4.2.0.

3. Results

3.1 Repellent Effect of Aqueous Extracts

Considering the evasion index, the response to the aqueous extracts varied among the tested social wasp species (Table 1; Figure 2). No significant deviations from normality were detected in any treatment ($P > 0.05$). For the onion extract, no significant repellent effect was detected in any of the tested species: *P. minarum* (EI = 0.69), *P. sericea* (EI = 0.14), and *P. canadensis* (EI = −0.05). Although the mean evasion index for *P. canadensis* exposed to onion was slightly negative, this response was not statistically significant.

For the garlic extract, a significant repellent effect was observed only in *P. canadensis* (EI = −0.20) (Table 1). In contrast, *P. minarum* (EI = 0.34) and *P. sericea* (EI = 0.02) did not show a significant repellent response to the extract. Across the three control units established for each species, no consistent positional bias between compartments, mortality, or conspicuous behavioral alterations were observed. No mortality was recorded in any of the extract treatments during the five-hour assays.

3.2 Temporal Variation in the Evasion Response

Temporal variation in compartment occupation differed among species and extracts (Figure 3). In *P. minarum*, the occupation of the untreated compartment increased significantly over time for both onion ($\beta = 0.211$, $P < 0.001$) and garlic extracts ($\beta = 0.098$, $P = 0.001$). However, the evasion-index values remained positive throughout the assay, indicating no detectable repellency.

Table 1. Evasion index and one-tailed t-tests against zero for social wasps exposed to aqueous extracts of onion and garlic

Social wasp	Treatment	Evasion index	One-tailed t-test against zero	Effect	Shapiro-Wilk W (P)
Polybia minarum Ducke, 1906	*Allium cepa* L. (onion)	0.69	t = 7.13 (P > 0.999)	No detectable repellency	0.865 (0.108)
Polybia sericea (Olivier, 1791)	*Allium cepa* L. (onion)	0.14	t = 1.67 (P = 0.933)	No detectable repellency	0.849 (0.073)
Polistes canadensis (Linnaeus, 1758)	*Allium cepa* L. (onion)	−0.05	t = −0.70 (P = 0.251)	No detectable repellency	0.933 (0.515)
Polybia minarum Ducke, 1906	*Allium sativum* L. (garlic)	0.34	t = 4.38 (P = 0.999)	No detectable repellency	0.888 (0.188)
Polybia sericea (Olivier, 1791)	*Allium sativum* L. (garlic)	0.02	t = 0.02 (P = 0.509)	No detectable repellency	0.950 (0.686)
Polistes canadensis (Linnaeus, 1758)	*Allium sativum* L. (garlic)	−0.20	t = −3.05 (P = 0.008)	Repellency	0.878 (0.150)

Negative values indicate avoidance of the treated area, whereas positive values indicate no detectable repellency. Shapiro-Wilk tests did not indicate significant deviations from normality in any treatment (all P > 0.05), supporting the use of the one-sample Student's t-test.

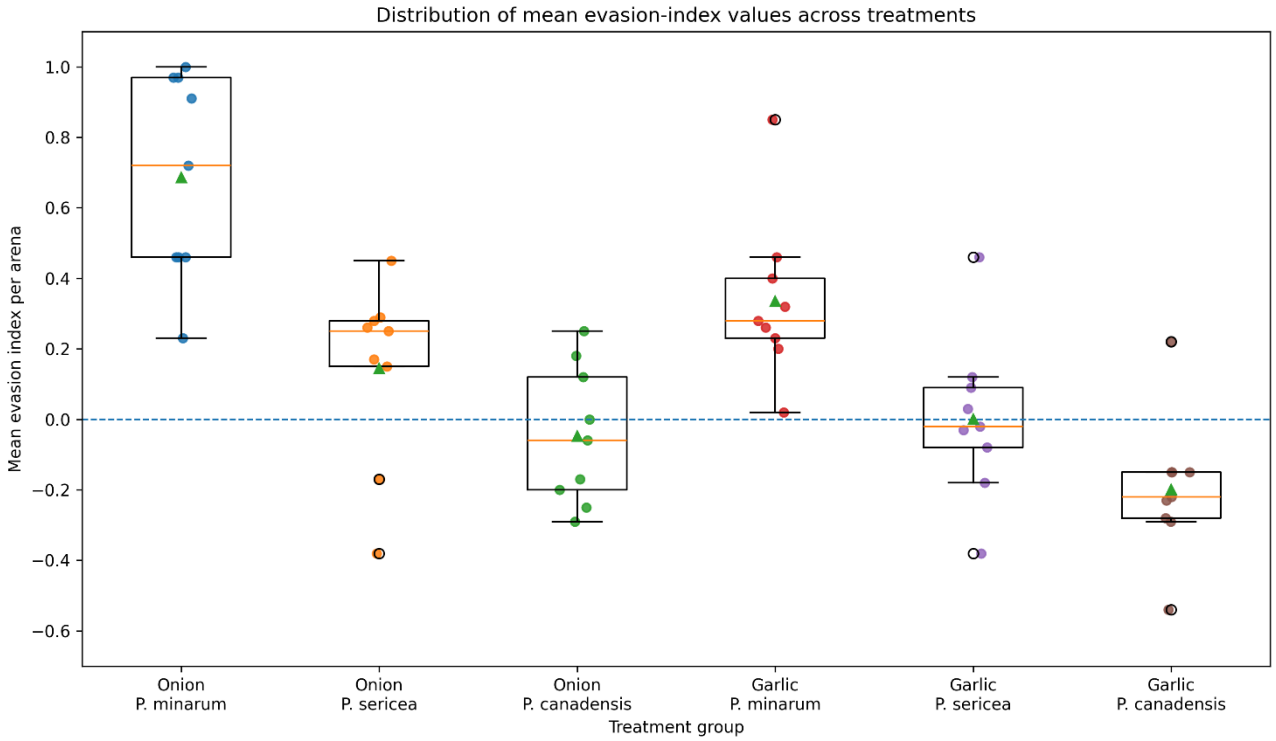


Figure 2. Distribution of mean evasion-index values per experimental unit for three social wasp species exposed to aqueous extracts of onion and garlic

Boxes represent the interquartile range, horizontal lines inside boxes represent the median, triangles indicate the mean, whiskers represent the range of non-outlier values, and points correspond to individual experimental units. The dashed horizontal line indicates EI = 0.

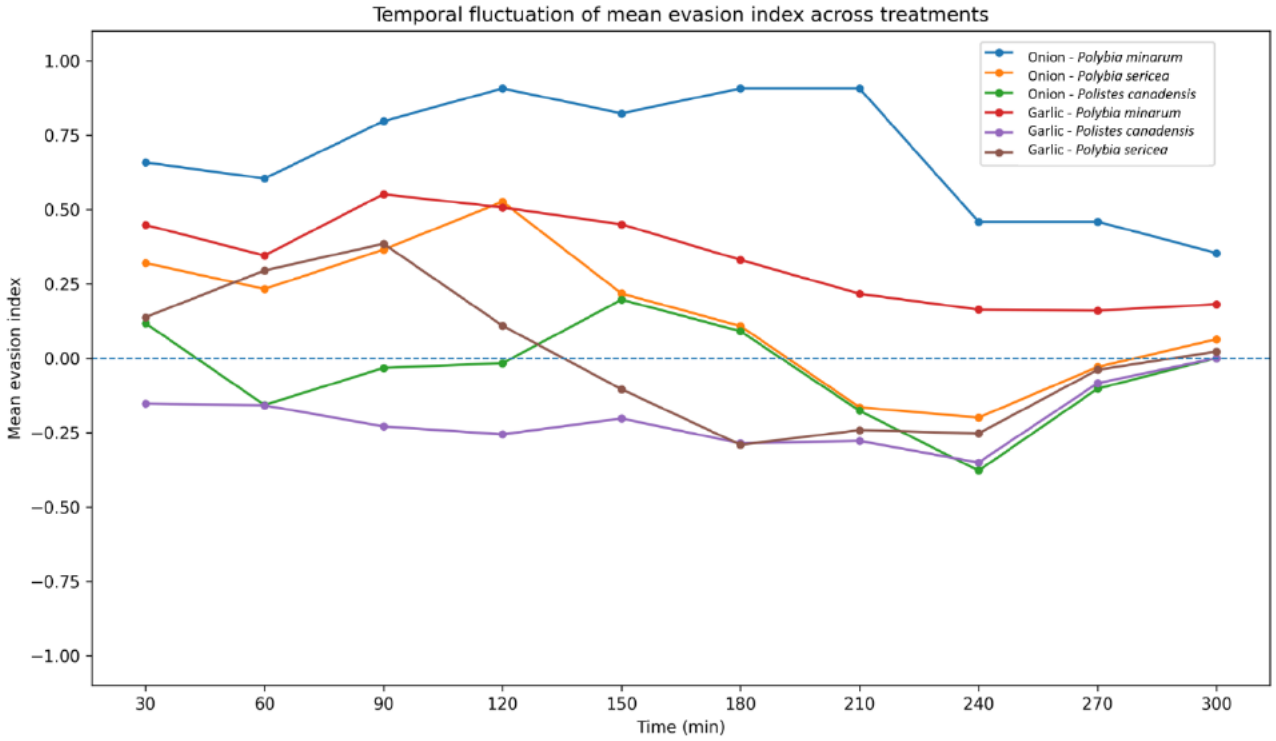


Figure 3. Temporal variation in the mean evasion index of three social wasp species exposed to aqueous onion and garlic extracts

In *P. sericea*, the evasion index decreased significantly over time for both onion ($\beta = 0.226$, $P < 0.001$) and garlic extracts ($\beta = 0.245$, $P < 0.001$), reaching negative values during intermediate and later observations. Nevertheless, the overall response to both extracts was not significant for repellency.

In *P. canadensis*, the evasion index decreased significantly over time in response to onion extract ($\beta = 0.136$, $P = 0.001$), although the overall effect remained non-significant. In contrast, no significant temporal change was detected for garlic extract ($\beta = -0.005$, $P = 0.905$), and the evasion-index values remained predominantly negative throughout the assay, indicating a relatively stable repellent response.

3.3 Behavioral Observations during Exposure

In the bioassays with garlic aqueous extract, agonistic behaviors were observed, characterized by aggressive interactions between individuals, especially in *P. canadensis*. In *P. sericea* and *P. minarum*, mild signs of agitation were also observed after exposure to the garlic extract, including increased movement and apparent escape attempts. Self-grooming behavior was recorded in all three species exposed to garlic. In contrast, no conspicuous qualitative behavioral changes, such as aggression, intense agitation, or self-grooming, were observed in individuals exposed to the onion extract.

4. Discussion

Although onion and garlic belong to the genus *Allium* and share the presence of sulfur-containing compounds, the results indicate that their aqueous extracts should not be treated as functionally equivalent in relation to social wasps. The observed response reinforces that the bioactivity of plant-derived products may vary according to the plant species, preparation method, and exposed organism, even when the extracts derive from closely related plants that are widely recognized for their bioactive compounds (Costa et al., 2025; Giunti et al., 2022;

Soares et al., 2026; Tavares et al., 2021). Because the preparation involved tissue disruption followed by aqueous maceration, the protocol was expected to recover at least part of the water-extractable bioactive fraction. However, the extracts were not chemically characterized, preventing the identification of the compounds responsible for the observed responses.

The absence of a detectable repellent effect of onion extract indicates that the aqueous preparation used in this study, at 20% (w/v), did not induce consistent evasion in the tested social wasps under laboratory conditions. Although organosulfur compounds from *A. cepa*, such as thiosulfinates and thiosulfonates, have been associated with biological activity in other organisms, these effects may depend on extract composition, concentration, exposure route, and the organism evaluated (Falcón-Piñeiro et al., 2023; Tavares et al., 2021). Therefore, the present result should be interpreted specifically for the aqueous extract and experimental conditions evaluated here and should not be extrapolated to other onion-derived preparations or non-target beneficial insects. The slightly negative but non-significant evasion index observed in *P. canadensis* does not demonstrate repellency, although it may justify further evaluation under different concentrations or exposure conditions.

The repellent response observed in *P. canadensis* exposed to garlic extract may be associated with sulfur-containing compounds present in this plant, especially allicin and related molecules. Allicin is formed when garlic tissue is crushed or chopped, a process that activates the enzyme alliinase and converts alliin into allicin. This compound is largely responsible for the characteristic odor of garlic and has been associated with different biological activities (Dausch & Nixon, 1990; Schneider, 1986). In addition, the repellent and biocidal activities of garlic have already been demonstrated under laboratory conditions against different pest organisms, including insects, mites, and nematodes (Dancewicz & Gabrys, 2008; Mobki et al., 2014; Nchu et al., 2016; Numa Vergel et al., 2011; Palermo et al., 2021; Ricupero et al., 2022; Silva & Pelli, 2021). These studies support the plausibility that garlic-derived compounds affect arthropod behavior, although their effects may vary according to the organism, extract type, concentration, and exposure route.

The difference among the tested species suggests that the response to *Allium* extracts may depend on species-specific traits related to chemical and sensory biology. Because social wasps use chemical information for nestmate recognition, colony organization, and interactions with the environment, interspecific variation in chemosensory sensitivity may influence how these insects perceive and respond to external stimuli (van Zweden & d'Ettorre, 2010). Variation in odorant receptor repertoires among *Polistes* species, together with evidence that social wasps can detect and respond to plant volatiles, supports the possibility that interspecific differences in chemosensory biology contribute to distinct responses to external chemical stimuli (Brodmann et al., 2008; Legan et al., 2021). Therefore, the more evident response of *P. canadensis* to garlic may reflect species-specific differences in chemical ecology or sensory sensitivity, although this hypothesis still requires direct testing.

The initial placement of the wasps in the treated compartment was intended to simulate their arrival on a previously treated surface. Although exploratory movement between compartments was expected, in the absence of repellency this movement should result in a more balanced occupation of the arena. In *P. minarum*, temporal fluctuations in the evasion index remained positive for both extracts, indicating that movement between compartments did not result in a repellent response and was more consistent with exploration of the environment. In *P. sericea*, the reduction in the evasion index and the occurrence of negative values after longer exposure periods indicate that the response to the extracts may depend on exposure duration, although this pattern did not result in a consistent overall repellent effect. The tendency of the evasion index to return toward zero during the final observations in several treatments may indicate reduced responsiveness after prolonged exposure, a pattern compatible with possible habituation; however, because this late nonlinear change was not explicitly tested by the linear temporal model, this interpretation remains tentative.

Sublethal behavioral responses to plant-derived products may vary with exposure duration and occur even in the absence of mortality or a significant overall effect (Giunti et al., 2022; Passos et al., 2022; Teixeira et al., 2022). In contrast, the predominantly negative evasion-index values observed in *P. canadensis* exposed to garlic, together with the absence of a significant temporal change, reinforce that movement toward the untreated compartment was not merely a consequence of random exploration, but rather represented a persistent aversive response.

The behavioral observations reinforce that the absence of statistically detectable repellency does not necessarily mean absence of sublethal effects. Garlic extract was associated with agonistic interactions, agitation, increased movement, apparent escape attempts, and self-grooming, with stronger evidence in *P. canadensis*, but also with signs observed in *P. sericea* and *P. minarum*. These behaviors indicate that garlic extract was perceived by the wasps and may have caused behavioral disturbances even when the evasion response was not statistically significant. Thus, the evaluation of plant extracts in beneficial organisms should consider not only evasion, but also sublethal responses that may affect activity, orientation, social interaction, or predatory performance (Passos et al., 2022; Souza et al., 2026; Xavier et al., 2015).

The absence of mortality throughout the experiment indicates that the aqueous extracts of onion and garlic did not produce apparent acute lethal effects under the tested conditions. Therefore, the response observed for garlic in *P. canadensis* appears to be mainly related to behavioral effects rather than mortality. However, this absence of mortality should not be interpreted as a general absence of impact on non-target organisms, since sublethal effects may occur without resulting in death during the observation period (Passos et al., 2022; Souza et al., 2026; Xavier et al., 2015).

5. Conclusions

The contrasting responses to onion and garlic demonstrate that closely related botanical sources cannot be assumed to have equivalent effects on beneficial arthropods. Under the tested conditions, onion appeared less disruptive to the evaluated social wasps and may represent a more compatible option for further evaluation in pest management programs, whereas garlic produced species-specific behavioral effects. These findings support the inclusion of non-target behavioral assays when screening plant-based products for integrated pest management, particularly in systems where social wasps contribute to biological control. Field studies are needed to determine whether these laboratory responses persist under realistic exposure conditions and affect wasp activity or predatory services.

Acknowledgements

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM), and the Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT).

Transparency of Data

Data not available: The data set that supports the results of this study is not publicly available.

Author Contribution Statement

	BC Barbosa	PVA Silva	TT Maciel	G Smagghe	ML Oliveira
Conceptualization	■		■		
Data curation	■	■	■		
Formal analysis	■	■	■		
Funding acquisition	■				
Investigation	■	■	■		
Methodology	■	■	■		
Supervision	■				
Validation	■	■	■	■	■
Writing – original draft	■	■	■	■	■
Writing – review and editing	■	■	■	■	■

References

Barbosa, B. C., Detoni, M., Maciel, T. T., & Prezoto, F. (2016). Studies of social wasp diversity in Brazil: Over 30 years of research, advancements and priorities. *Sociobiology, 63*(3), 858-880. https://doi.org/10.13102/sociobiology.v63i3.1031

Brodmann, J., Twele, R., Francke, W., Hölzler, G., Zhang, Q.-H., & Ayasse, M. (2008). Orchids mimic green-leaf volatiles to attract prey-hunting wasps for pollination. *Current Biology, 18*(10), 740-744. https://doi.org/10.1016/j.cub.2008.04.040

Casida, J. E., & Durkin, K. A. (2013). Neuroactive insecticides: Targets, selectivity, resistance, and secondary effects. *Annual Review of Entomology, 58*, 99-117. https://doi.org/10.1146/annurev-ento-120811-153645

Clemente, M. A., Lange, D., Del-Claro, K., Prezoto, F., Campos, N. R., & Barbosa, B. C. (2012). Flower-visiting social wasps and plants interaction: Network pattern and environmental complexity. *Psyche: A Journal of Entomology, 2012*, Article 478431. https://doi.org/10.1155/2012/478431

Costa, L. M. T., Smagghe, G., Viteri Jumbo, L. O., Santos, G. R., Aguiar, R. W. S., & Oliveira, E. E. (2025). Selective actions of plant-based biorational pesticides: Molecular mechanisms and reduced risks to non-target organisms. *Current Opinion in Environmental Science & Health, 44*, Article 100601. https://doi.org/10.1016/j.coesh.2025.100601

Dancewicz, K., & Gabrys, B. (2008). Effect of extracts of garlic (*Allium sativum* L.), wormwood (*Artemisia absinthium* L.) and tansy (*Tanaceum vulgare* L.) on the behaviour of the peach potato aphid *Myzus persicae* (Sulz.) during the settling on plants. *Pestycydy, 3*, 93-99.

Dausch, J. G., & Nixon, D. W. (1990). Garlic: A review of its relationship to malignant disease. *Preventive Medicine, 19*(3), 346-361. https://doi.org/10.1016/0091-7435(90)90034-h

Desneux, N., Decourtye, A., & Delpuech, J. M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology, 52*, 81-106. https://doi.org/10.1146/annurev.ento.52.110405.091440

Elisei, T., Nunes, J. V., Ribeiro Junior, C., Fernandes Junior, A. J., & Prezoto, F. (2010). Uso da vespa social *Polistes versicolor* no controle de desfolhadores de eucalipto. *Pesquisa Agropecuária Brasileira, 45*(9), 958-964. https://doi.org/10.1590/S0100-204X2010000900004

Elisei, T., Ribeiro Junior, C., Fernandes Junior, A. J., Nunes, J. V., Souza, A. R. de, & Prezoto, F. (2012). Management of social wasp colonies in eucalyptus plantations (Hymenoptera: Vespidae). *Sociobiology, 59*(4), 1167-1174. https://doi.org/10.13102/sociobiology.v59i4.497

Falcón-Piñeiro, A., García-López, D., Gil-Martínez, L., Torre, J. M. de la, Carmona-Yañez, M. D., Katalayi-Muleli, A., Guillamón, E., Barrero-Domínguez, B., López-Feria, S., Garrido, D., & Baños, A. (2023). PTS and PTSO, two organosulfur compounds from onion by-products as a novel solution for plant disease and pest management. *Chemical and Biological Technologies in Agriculture, 10*, Article 76. https://doi.org/10.1186/s40538-023-00452-1

Giunti, G., Benelli, G., Palmeri, V., Laudani, F., Ricupero, M., Ricciardi, R., Maggi, F., Lucchi, A., Guedes, R. N. C., Desneux, N., & Campolo, O. (2022). Non-target effects of essential oil-based biopesticides for crop protection: Impact on natural enemies, pollinators, and soil invertebrates. *Biological Control, 176*, Article 105071. https://doi.org/10.1016/j.biocontrol.2022.105071

Isman, M. B. (2020). Botanical insecticides in the twenty-first century: Fulfilling their promise? *Annual Review of Entomology, 65*, 233-249. https://doi.org/10.1146/annurev-ento-011019-025010

Jactel, H., Verheggen, F., Thiéry, D., Escobar-Gutiérrez, A. J., Gachet, E., Desneux, N., & Neonicotinoids Working Group. (2019). Alternatives to neonicotinoids. *Environment International, 129*, 423-429. https://doi.org/10.1016/j.envint.2019.04.045

Legan, A. W., Jernigan, C. M., Miller, S. E., Fuchs, M. F., & Sheehan, M. J. (2021). Expansion and accelerated evolution of 9-exon odorant receptors in *Polistes* paper wasps. *Molecular Biology and Evolution, 38*(9), 3832-3846. https://doi.org/10.1093/molbev/msab023

Lisi, F., Siscaro, G., Biondi, A., Zappalà, L., & Ricupero, M. (2025). Non-target effects of bioinsecticides on natural enemies of arthropod pests. *Current Opinion in Environmental Science & Health, 45*, Article 100624. https://doi.org/10.1016/j.coesh.2025.100624

Mobki, M., Safavi, S. A., Safaralizadeh, M. H., & Panahi, O. (2014). Toxicity and repellency of garlic (*Allium sativum* L.) extract grown in Iran against *Tribolium castaneum* (Herbst) larvae and adults. *Archives of Phytopathology and Plant Protection, 47*(1), 59-68. https://doi.org/10.1080/03235408.2013.802896

Nchu, F., Eloff, J. N., & Magano, S. R. (2016). Repellent activities of dichloromethane extract of *Allium sativum* (garlic) (Liliaceae) against *Hyalomma rufipes* (Acari). *Journal of the South African Veterinary Association, 87*(1), Article a1356. https://doi.org/10.4102/jsava.v87i1.1356

Numa Vergel, S. J., Bustos, R. A., Rodríguez, C. D., & Cantor, R. F. (2011). Laboratory and greenhouse evaluation of the entomopathogenic fungi and garlic-pepper extract on the predatory mites, *Phytoseiulus persimilis* and *Neoseiulus californicus* and their effect on the spider mite *Tetranychus urticae*. *Biological Control, 57*(2), 143-149. https://doi.org/10.1016/j.biocontrol.2011.02.007

Palermo, D., Giunti, G., Laudani, F., Palmeri, V., & Campolo, O. (2021). Essential oil-based nano-biopesticides: Formulation and bioactivity against the confused flour beetle *Tribolium confusum*. *Sustainability, 13*(17), Article 9746. https://doi.org/10.3390/su13179746

Passos, L. C., Ricupero, M., Gugliuzzo, A., Soares, M. A., Desneux, N., Campolo, O., Carvalho, G. A., Biondi, A., & Zappalà, L. (2022). Sublethal effects of plant essential oils toward the zoophytophagous mirid *Nesidiocoris tenuis*. *Journal of Pest Science, 95*, 1609-1619. https://doi.org/10.1007/s10340-022-01548-7

Pavela, R., Maggi, F., Campolo, O., Giunti, G., & Spinozzi, E. (2026). Essential oils: Promising active ingredients of botanical insecticides or hidden risks for non-target organisms? *Entomologia Generalis, 46*(2), 327-329. https://doi.org/10.1127/entomologia/4462

Picanço, M. C., Oliveira, I. R., Rosado, J. F., Silva, F. M., Gontijo, P. C., & Silva, R. S. (2010). Natural biological control of *Ascia monuste* by the social wasp *Polybia ignobilis* (Hymenoptera: Vespidae). *Sociobiology, 56*(1), 67-76.

Prezoto, F., & Machado, V. L. L. (1999a). Ação de *Polistes (Aphanilopterus) simillimus* Zikán (Hymenoptera: Vespidae) na produtividade de lavoura de milho infestada com *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae). *Revista Brasileira de Zoociências, 1*(1), 19-30.

Prezoto, F., & Machado, V. L. L. (1999b). Ação de *Polistes (Aphanilopterus) simillimus* Zikán (Hymenoptera: Vespidae) no controle de *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae). *Revista Brasileira de Zoologia, 16*(3), 841-850. https://doi.org/10.1590/S0101-81751999000300021

Prezoto, F., & Machado, V. L. L. (1999c). Transferência de colônias de vespas (*Polistes simillimus* Zikán, 1951) (Hymenoptera: Vespidae) para abrigos artificiais e sua manutenção em uma cultura de *Zea mays* L. *Revista Brasileira de Entomologia, 43*(3-4), 239-241.

Prezoto, F., Maciel, T. T., Detoni, M., Mayorquin, A. Z., & Barbosa, B. C. (2019). Pest control potential of social wasps in small farms and urban gardens. *Insects, 10*(7), Article 192. https://doi.org/10.3390/insects10070192

Ricupero, M., Biondi, A., Cincotta, F., Condurso, C., Palmeri, V., Verzera, A., Zappalà, L., & Campolo, O. (2022). Bioactivity and physico-chemistry of garlic essential oil nanoemulsion in tomato. *Entomologia Generalis, 42*(6), 921-930. https://doi.org/10.1127/entomologia/2022/1553

Santos, G. M. M., Bichara-Filho, C. C., Resende, J. J., Cruz, J. D., & Marques, O. M. (2007). Flower-visiting social wasps (Hymenoptera: Vespidae) from a Brazilian cerrado ecosystem. *Neotropical Entomology, 36*(6), 834-838. https://doi.org/10.1590/S1519-566X2007000600007

Schmack, J. M., & Egerer, M. (2023). Floral richness and seasonality influences bee and non-bee flower interactions in urban community gardens. *Urban Ecosystems, 26*, 1099-1112. https://doi.org/10.1007/s11252-023-01353-9

Schneider, E. (1986). *A cura e a saúde pelos alimentos*. Casa Publicadora Brasileira.

Silva, A. C. B., & Pelli, A. (2021). Avaliação da repelência de *Allium sativum* L. (alho) visando o controle de *Nauphoeta cinerea* (Olivier, 1789). *Acta Ambiental Catarinense, 19*, 1-7. https://doi.org/10.24021/raac.v19i1.6225

Soares, A. P., Zocolo, G. J., & Bueno, A. F. (2026). The overlooked impact of botanical pesticides on non-target organisms. *Plants, 15*(6), Article 917. https://doi.org/10.3390/plants15060917

Southon, R. J., Fernandes, O. A., Nascimento, F. S., & Sumner, S. (2019). Social wasps are effective biocontrol agents of key lepidopteran crop pests. *Proceedings of the Royal Society B, 286*(1914), Article 20191676. https://doi.org/10.1098/rspb.2019.1676

Souto, A. L., Sylvestre, M., Tölke, E. D., Tavares, J. F., Barbosa-Filho, J. M., & Cebrián-Torrejón, G. (2021). Plant-derived pesticides as an alternative to pest management and sustainable agricultural production: Prospects, applications and challenges. *Molecules, 26*(16), Article 4835. https://doi.org/10.3390/molecules26164835

Souza, A. R. de, Baptista, C. F., Teixeira, G. V. M., & Lima, M. A. P. (2021). Artificial environments for studying eusocial wasps. In F. Prezoto, F. S. Nascimento, B. C. Barbosa, & A. Somavilla (Eds.), *Neotropical social wasps* (pp. 449-465). Springer. https://doi.org/10.1007/978-3-030-53510-0_23

Souza, A. R. de, Diniz, R., Santos, S. A. dos, Lima, L. D., Santos, E. F., Polo, J. L. L., Nascimento, F. S. do, & Lima, M. A. P. (2026). Imidacloprid ingestion alters the behavior of the natural enemy *Mischocyttarus cassununga* (Hymenoptera: Vespidae). *Environmental Toxicology and Chemistry, 45*(3), 710-718. https://doi.org/10.1093/etojnl/vgaf300

Tavares, W. R., Barreto, M. C., & Seca, A. M. (2021). Aqueous and ethanolic plant extracts as bio-insecticides: Establishing a bridge between raw scientific data and practical reality. *Plants, 10*(5), Article 920. https://doi.org/10.3390/plants10050920

Teixeira, G. V. M., Souza, A. R. de, Barbosa, W. F., Bernardes, R. C., & Lima, M. A. P. (2022). Chronic exposure to a common biopesticide is detrimental to individuals and colonies of the paper wasp *Polistes versicolor*. *Science of The Total Environment, 810*, Article 152108. https://doi.org/10.1016/j.scitotenv.2021.152108

van Zweden, J. S., & d'Ettorre, P. (2010). Nestmate recognition in social insects and the role of hydrocarbons. In G. J. Blomquist & A. G. Bagnères (Eds.), *Insect hydrocarbons: Biology, biochemistry, and chemical ecology* (pp. 222-243). Cambridge University Press.

Xavier, V. M., Picanço, M. C., Chediak, M., Santana Júnior, P. A., Ramos, R. S., & Martins, J. C. (2015). Acute toxicity and sublethal effects of botanical insecticides to honey bees. *Journal of Insect Science, 15*(1), Article 137. https://doi.org/10.1093/jisesa/iev110