EFFECTIVENESS OF AQUEOUS COFFEE EXTRACT AND CAFFEINE IN CONTROLLING PHYTOPHAGOUS HETEROPTERAN SPECIES

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Abstract. Heteroptera species are a growing problem in the cultivation of horticultural crops, and intensive chemical control of these pests is not sustainable. Therefore, natural products are used in sustainable pest management. We investigated the potential of aqueous coffee and caffeine extracts in the control of three heteropteran species *Nezara viridula* (L.), *Halyomorpha halys* (Stål), and *Corythucha ciliata* (Say). The Soxhlet extraction method was used to prepare the aqueous coffee extracts. Contact efficacy was evaluated by spraying the pests with the aqueous extract of coffee and the aqueous solution of pure caffeine. Significant efficacy of coffee and caffeine standard was found for control of adult *H. halys* (97% coffee; 83% caffeine) and adult *C. ciliata* (47% coffee; 70% caffeine). Low efficacy was observed for control of *N. viridula* nymphs (33% coffee; 28% caffeine), while no insecticidal efficacy was observed for adult *N. viridula*. There was no statistically significant difference in efficacy between coffee extract and caffeine standard, suggesting that these caffeine levels are sufficient for pest control, while the influence of other various compounds in coffee extract (e.g., polyphenols) could not be detected. This study opens a perspective for the production of natural plant extracts using only water as the extraction medium for pest control.
Keywords: *alternative insecticides, aqueous extract, brown marmorated stink bug, green stink bug, sycamore lace bug*

Introduction

The phytophagous heteropteran species: the southern green stink bug *Nezara viridula* (L.) (Hemiptera: Pentatomidae) and the brown marmorated stink bug *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) are extremely polyphagous pests. Their host range includes over 100 different horticultural species, many of which are economically important, including vegetables, field crops, fruits, and ornamentals (Kiritani et al., 1965; Leskey and Nielsen, 2018; EPPO 2019; Pajač Živković et al., 2021.). *H. halys* has spread across most of the United States and Canada and become serious agricultural pest in some European countries (Britt et al., 2019; Zovko et al., 2019; Schumm et al., 2020). The first report of *H. halys* in Croatia is from 2017 (Šerić Jelaska and Šapina, 2018). *H. halys* is considered an important horticultural pest, particularly attacking fruits and various trees (Hamilton and Shearer, 2003; Musolin et al., 2018). When it feeds on fruit, it causes the formation of necrotic spots and eventually destruction of the

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flesh (Bariselli et al., 2016; Ciceoi et al., 2017). Recently, economic damages in agricultural production have been recorded in Hungary, Slovenia, and Italy (Bariselli et al., 2016; Vétek and Korányi, 2017; Rot et al., 2018). This pest did not establish itself in Australia, but it has been intercepted at the border, therefore future incursions and establishment are very likely (Horwood et al., 2019; Caron et al., 2021). Several things have helped this pest to become so successful in spreading across the world, including climate change, lack of natural predators, and global trade (Schumm et al., 2020). *N. viridula* is an economic pest of tomato and other vegetables, whose pods are deformed and not fully developed (Brier and Rogers, 1991; Todd, 1989). It is cosmopolitan pest and is widely distributed in America, Asia, Australia, and Europe (Todd, 1989; Panizzi and Lucini, 2016; Kment et al., 2021). *N. Viridula* feeding results in plant damage, reduced seed germination as well as the spread of plant pathogens (Leskey et al., 2012). In addition, the sycamore lace bug *Corythucha ciliata* (Say) (Hemiptera: Tingidae) is a pest on ornamentals in urban areas, especially on various *Platanus* species (Hufnagel et al., 2006). In Europe, the preferred host plants are *Platanus X acerifolia* (Willd.) (Proteales: Platanaceae) and *P. orientalis* (L.) (Proteales: Platanaceae), but *C. ciliata* may also feed on *Fraxinus sp.* (Lamiales: Oleaceae), *Betula sp*. (Fagales: Betulaceae) and *Morus sp.* (Rosales: Moraceae) (Tzanakakis, 1988). This pest causes dechlorophyllization, premature leaf desiccation and defoliation as early as summer, which affects the aesthetic value of ornamental trees (Maceljski, 2002; Halbert and Meeker, 2004).

All of these heteropteran species, especially invasive species in Europe such as *H. halys* and *C. ciliata*, have great dispersal ability (Kiritani et al., 1965; Leskey and Nielsen, 2018; Rabitsch, 2008). *C. ciliata* is usually spread passively by wind drifts, by trade-in horticultural plants, or by human-mediated translocation via clothing, automobiles, etc. (Rabitsch, 2008). Originally, *N. viridula* is an Ethiopian species but has been introduced to tropical and subtropical regions on all continents, mostly through the transport of goods. This cosmopolitan pest is also a good flyer and uses the wind for its wide dispersal (Todd, 1989). *H. halys* which is native to Asia (Hamilton and Shearer, 2003), is capable of flying long distances, especially in summer (Wiman et al., 2015). This pest can also spread on foot and by moving frequently between crops during the growing season (Lee et al., 2014).

Chemical control is the most commonly used strategy to control the above horticultural pests (Halbert and Meeker, 2004; Rice et al., 2014; Knight and Gurr, 2007). In the last decade effectiveness of many active ingredients in the management of heteropteran pests has been studied (Lee et al., 2014; Rice et al., 2014; Lee et al., 2013; Leskey et al., 2012). The best effect has been established for several pyrethroids, neonicotinoids, carbamates, some organophosphates, and organochlorines (Leskey et al., 2012). However, chemical substances often used for management of heteropteran pests can harm beneficial arthropods and can cause increasing pest outbreaks. For example, relatively nonselective insecticides, such as deltamethrin or endosulfan have been used to control *N. viridula*. Such insecticides are harmful to the environment and can lead to outbreaks of various secondary pests (Knight and Gurr, 2007). Although there is still no recorded resistance of *N. viridula* on insecticides, new compounds with distinct mode of actions are needed to prevent potential resistance mechanisms (Riga et al., 2020.). In addition, control of *H. halys* in crops is mainly based on repeated insecticide sprays with pyrethroids and neonicotinoids (Lee et al., 2013). The used insecticides are usually nonselective, so they can be very harmful to natural enemies

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and can interfere with pest management programs (Leskey et al., 2012). Sparks et al. (2020) predict the emergence of resistance based on genes from *H. halys*. Recently, much of the research has focused on the egg parasitoid *Trissolcus* species, which have shown promise as candidates for biocontrol of *N. viridula* and *H. halys* (Moraglio et al., 2020; Scaccini et al., 2020; Tillman et al., 2020; Caron et al., 2021; Zapponi et al., 2021; Yonow et al., 2021; Ivezić et al., 2022; Scala et al., 2022).

A number of insecticides and their application methods (e.g., foliar sprays, trunk injections, soil treatments) are available for the control of *C. ciliata*. These methods are costly and inefficient, and there are also problems associated with large-scale insecticide use in urban areas (Halbert and Meeker, 2004). However, the most suitable method for trunk injections is a botanical insecticide based on azadirachtin. Only a single application by injection at the beginning of the growing season can protect sycamore throughout the year (Pavela et al., 2013). In the meantime, it remains important to find more effective crop protection technologies that both protect the environment and control the pest (Ju et al., 2009).

Polyphenolic compounds are highly effective as repellents and insecticides (Fraenkel, 1959; Singh et al., 2021; Scalbert and Haslam, 1987; Haslam, 1988; Kadir and Hassan, 2020; Morisawa et al., 2002; Abdelkhalek et al., 2020; Di Ilio and Cristofaro, 2020). The effect of natural polyphenols on plant-insect interactions was first studied by Fraenkel et al. (1959), and this research later laid the foundation for many other studies on related topics. Polyphenols are secondary metabolites in plants that exert insecticidal activity and protect most plant species from many harmful insects (Singh et al., 2021). For example, the relationship between changes in phenolic content of leaves of English oak (*Quercus robur* (L.) (Fagaceae: Quercus)) and insect infestation has been established (Scalbert and Haslam, 1987; Haslam, 1988). Previous studies have shown that the chemical compounds in the extracts of various long-lived wood species consist of phenolic compounds (Kadir and Hassan, 2020; Morisawa et al., 2002). The bark extract of *Eucalyptus camaldulensis* (Dehnh.) (Myrtales: Myrtaceae) also showed high insecticidal activities and contained a potent inhibitor against tobacco mosaic virus (TMV) infection, so this polyphenolic extract can be considered to have potential for wood protection (Abdelkhalek et al., 2020). Hernandez-Trejo et al., 2021. established insecticidal activity of the polyphenolic compounds found in neem on *S. frugiperda* larvae. In addition, the polyphenolic compounds of olive tree show strong bioactive properties as insecticides and growth regulators. Olive mill effluent is an important source of these compounds, so the polyphenolic fractions extracted from olive mill effluent can be used as a potent natural insecticide and can be considered as ovicidal agents (Di Ilio and Cristofaro, 2020). Salamatullah (2022) suggests that *Withania adpressa* derivatives have promising antioxidant and insecticidal properties, and consequently may be used as natural insecticides.

Caffeine, (1,3,7-trimethylxanthine), is a bitter white crystalline alkaloid. It is a natural secondary metabolite of many plant species, such as coffee, tea, kola nuts, and guarana (Araque et al., 2007). Its physiological function in the plant life cycle has not been fully explored, but two major chemical functions have been proposed (Ashihara and Crozier, 2001). The first function is to control the growth of competing plant species (Chou and Waller, 1980) and the other is an effective chemical defense for the young plant against various invasive herbivorous insects and pathogens (Kim et al., 2006). The efficacy of caffeine as an insecticide or repellent depends on the concentration and application method. For example, tobacco hornworm (*Manduca sexta*

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(L.) (Sphingidae: Manduca)) larvae have been shown to be suppressed by caffeine at concentrations comparable to those in coffee seeds (0.8-1.8 wt.%) (Araque et al., 2007). In addition, aqueous caffeine solutions (2.0 wt.%) have shown insecticidal activity against snails (*Veronicella cubensis* (L. Pfeiffer) (Veronicelloidea: Veronicellidae)) and orchid snails (*Zonitoides arboreous* (Say) (Stylommatophora: Gastrodontidae)) (Hollingsworth et al., 2002). Moreover, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae), a known invasive pest of coffee plantations, is virtually insensitive to caffeine and other alkaloid compounds found in coffee beans (Frischknecht et al., 1986). Its resistance to the toxic effects of caffeine is partly an evolutionary adaptation but may also be related to the application method used for the bioassay, namely the use of aqueous solutions instead of a caffeine-based emulsion (Araque et al., 2007). In addition, many other works demonstrate the efficacy of caffeine in controlling pest populations, for example; small fruit flies (*Drosophila* spp. (Diptera: Drosophilidae)) (Laranja et al., 2003) bed bugs (*Cimex lectularius* L. (Hemiptera: Cimicidae)) (Shripat and Kamble, 2015), mosquitoes (*Aedes aegypti* (L.) (Diptera: Culicidae)) (Laranja et al., 2003), tobacco cutworms (*Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae)) (Kim et al., 2006), etc. Šimůnková et al. (2021) recommend caffeine as an environmentally friendly alternative for wood protection against termites and brown rot fungi. It is also important to mention that caffeine in combination with other chemical compounds present in coffee (chlorogenic acid) could affect insects by increasing their movement speed (Shripat and Kamble, 2015; Magalhães et al., 2008). We hypothesized that natural plant-based extracts can act as a pesticide and that is possible to use only water as the extraction medium. In this study, we tested a new perspective for the application of a natural plant-based aqueous coffee extract rich in polyphenols and caffeine obtained by the simple Soxhlet method in controlling several important horticultural pests.

Materials and methods

All chemicals used for the experimental procedures were of analytical grade. Alginic acid sodium salt (CAS number: 58-08-2, molecular weight 194.19 g mol^{-1}) was purchased from Sigma-Aldrich.

Preparation of coffee extracts and determination of total polyphenols and caffeine

Coffee beans were comminuted using a FOSS 2094 homogenizer (Hillerod, Denmark) and sieved through a mesh size of 450 μm. Grounded coffee beans were immediately used for the preparation of coffee extracts. Soxhlet method (AOAC 963.15) was used in the preparation of coffee extracts. Coffee concentration (25 and 45 g/L (w/v)) and extraction time (2 to 12 h) were varied. Distilled water was used as a solvent. The average Soxhlet cycle time was 60 min, and the temperature was adjusted to maintain a stable cycle frequency. All extractions were performed in three experimental replicates. Total polyphenol content and caffeine concentration were measured in the obtained coffee extracts. An aqueous solution of pure caffeine (positive control) was prepared as adjusted to the caffeine concentration in the coffee extracts.

The modified Folin-Ciocalteu method using gallic acid as a standard was used to determine the total polyphenol content (TPC) (Singleton et al., 1999). A mixture of 0.1 mL of extract with 7.9 mL of distilled water and 0.5 mL of Folin Ciocalteu's reagent

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(diluted with distilled water in a 1:2 ratio) and 1.5 mL of 20% sodium carbonate (w/v) was initially mixed and allowed to stand for 2 h to complete the reaction. Optical absorbance at 765 nm using a spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan) was measured against water, and data are expressed as mg gallic acid equivalents (GAE) per L of extract.

The wavelength at which caffeine absorbs maximally in water was determined by scanning the range 150-400 nm. The wavelength at which caffeine absorbs maximally was found at 275 nm (Aranda and Morlock, 2006) and selected for further analysis. The calibration plot was recorded for the determination of caffeine concentration in coffee extract.

Insects in experiment

Three phytophagous heteropteran species with different developmental stages were obtained for the research. *H. halys* and *N. viridula* were collected in a soybean field in the vicinity of Šašinovec (Zagreb County, Croatia, 45°50’13.9”N 16°11’38.9”E). *C. ciliata* adults were collected on sycamore trees in district Dubrava (Zagreb, Croatia, 45°48’55.4364”N 15°57’59.6448”E). Collected insects were kept in entomological cages to recover overnight before testing, without additional feeding and previous contact with insecticides. A laboratory trial was set up with adults of all three species and third and fourth nymph stages of *H. halys* and *N. viridula.*

The list of insects and developmental stages tested in the study is presented in *Table 1.* The experiments were conducted at a temperature of 25 °C and 40% RH in the zoological laboratory of the Zagreb Faculty of Agriculture.

Table 1. *Description of the investigated insect pest species*

Species	Developmental stage tested	Tested ingredients	Investigated action	No. of tested individuals
H. halys	Adults Nymph 4th stage	Coffee, caffein, water coffee, caffein, water	Contact	120 120
N. viridula	Adults Nymph 3rd stage Nymph 4th stage	Coffee, caffein, water Coffee, caffein, water Coffee, caffein, water	Contact	120 120 120

Estimation of efficacy on phytophagous heteropteran species

For all treatments, ten adults or nymphs (depending on the variant) of Heteroptera species were placed in a Petri dish (radius (r) = 90 mm). Contact toxicity was evaluated by applying the ingredients to the bugs in the Petri dishes (r = 90 mm) by spraying the pure aqueous coffee extracts/aqueous solution of pure caffeine with a laboratory sprayer at a volume of 3 mL per Petri dish. One Petri dish (surface = 565,2 mm^2) represented one replicate. The untreated control for all experiments included a treatment in which the bugs were placed in Petri dishes treated with distilled water. Each application and investigated effect of the tested ingredients (coffee extract with 2053.11 mg of total polyphenolic compounds/L and 2388.89 mg of caffeine/L vs. caffeine standard in equal concentration) occurred in four replicates. Each replicate contained ten individuals of a particular life stage of the insects tested. In total, 18 different variants were tested on 720 individuals.

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Data analysis

The number of dead heteropteran specimens in each Petri dish was determined every 24 h for three days. Based on the number of dead individuals found in the treatment and the untreated control, the efficacy (%) of the ingredients was determined according to Abbott's formula as follows:

$$\text{Corrected \%} = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}}\right) x100$$

where: n = insect population, T = treated, Co = control.

Statistical data analysis (ANOVA, Tukey's HSD test, post-hoc t-test with Bonferroni adjustment) was performed using ARM 2019® GDM software: Gylling Data Management.

Results

Soxhlet extraction and determination of total polyphenol content and caffeine in coffee extracts

To determine the extent of the effect of coffee extracts on insects, the effect of caffeine had to be separated from the effect of polyphenols and other substances present in coffee, as well as from possible synergistic effects. To achieve this, pure caffeine solutions were prepared with the same concentration as in the aqueous coffee extract. This made it possible to separate the effect of caffeine from other chemical substances in coffee. The Soxhlet extraction conditions and the obtained concentrations of total polyphenolic compounds and caffeine are shown in *Tables 2* and *3*, respectively.

Table 2. *Total polyphenols content in Soxhlet aqueous coffee extracts*

Extraction time (h)	Grounded coffee concentration (g/L)	Total polyphenols (mg GAE/L)	Total polyphenols (mg GAE/g of grounded coffee beans)
2	45	1314.22 ± 11.97 c	52.57 ± 0.44 a
2	25	789.78 ± 1.11 e	17.55 ± 0.34 d
6	45	2053.11 ± 16.67 b	49.46 ± 0.52 b
6	25	1236.41 ± 12.46 d	45.65 ± 0.57 c
12	45	2180.89 ± 14.65 a	54.12 ± 0.66 a
12	25	1353.81 ± 16.90 c	48.46 ± 0.28 b

Values superscripted with the same letter within a column are not significantly different according to the post-hoc t-test with Bonferroni adjustment ($p < 0.05$). *GAE: gallic acid equivalents*

Table 3. *Caffeine concentration in Soxhlet aqueous coffee extracts*

Extraction time (h)	Grounded coffee concentration (g/L)	mg Caffeine/L	mg Caffeine/g of grounded coffee beans
2	45	1536.33 ± 14.96 d	61.45 ± 0.60 b
2	25	986.13 ± 16.03 e	21.91 ± 0.36 d
6	45	2388.89 ± 14.96 b	53.09 ± 0.33 c
6	25	1436.97 ± 41.69 d	57.48 ± 1.67 b
12	45	2612.18 ± 48.09 a	58.56 ± 1.07 b
12	25	1744.66 ± 5.34 c	69.79 ± 0.21 a

Values superscripted with the same letter within a column are not significantly different according to the post-hoc t-test with Bonferroni adjustment ($p < 0.05$)

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Considering the consumption of the active ingredient (coffee), energy, and time required to obtain the extracts, optimal extraction conditions were chosen (6 h, 45 g/L). The obtained extract had 2053.11 mg of total polyphenolic compounds/L and 2388.89 mg of caffeine/L. Standard caffeine solution (positive control) was prepared by dissolving exactly 2388.89 mg of caffeine in 1000 mL of distilled water.

Efficacy on phytophagous heteropteran species

No mortality of tested heteropteran pests on control variants treated with water has been recorded during the experiment. The results presented in *Tables 4* and *5* show that coffee has high efficacy, in the form of insect mortality, against *H. halys*, especially on adults, and that the main efficacy is reached three days after treatment (97%), while in nymphs the highest efficacy of 73% was observed at same checking period. ANOVA revealed significant differences in caffein efficacy ($F_{2,23} = 15.44$; $P < 0.0001$) and coffee efficacy ($F_{2,23} = 13.57$; $P < 0.0001$) on *H. halys* control based on developmental stage. Very good efficacy of caffeine treatment (adults: 83%; nymphs: 63%) was also observed three days after treatment. The results of the study indicate an intermediate efficacy (three days after treatment) of caffeine on adult *C. ciliata*; about 70% mortality and about 47% mortality with coffee treatment. ANOVA revealed significant differences in *C. ciliata* control based on ingredient type ($F_{2,23} = 19.05$; $P < 0.0001$). Coffee and caffeine have low efficacy against nymphs and adults of *N. viridula*. In the first three days after treatment, caffeine had no significant effect on nymphs and adults, but some efficacy was observed in third instar nymphs (max E ~28%). According to the results presented in *Tables 4* and *5*, on day three no significant difference was also observed with the coffee treatment, but again some efficacy was observed on the third stage nymphs (max. E ~33%). ANOVA revealed no significant differences in caffein efficacy ($F_{2,35} = 1.35$; $P = 0.273$) and coffee efficacy ($F_{2,35} = 1.13$; $P = 0.336$) on *N. viridula* control in all developmental stages tested.

Discussion

Polyphenols are secondary metabolites in plants that have insecticidal activity and protect most plant species from numerous harmful insects. Treatment of phytophagic heteropteran species with polyphenolic compounds has demonstrated their insecticidal activity. However, the commercialization of such pesticides is hindered by expensive, inefficient, and time-consuming extraction protocols. Research is focused on developing cheap and simple extraction methods that utilize plant processing by-products (Scalbert and Haslam, 1987).

When considering extraction time and initial coffee beans concentration, significant differences are noted for total polyphenolic compounds extractability (*Table 2*). Remarkable 56.22% (6 h, 45 g/L relative to 2 h, 45 g/L) and 56.55% (6 h, 25 g/L relative to 2h, 25 g/L) higher total polyphenolic compound values were detected when considering initial concentration and extraction time. A significant but much lower increase was observed at 12 h of extraction when compared to 6-h extraction time. Soxhlet extraction is robust and is performed at high temperatures (100 °C). Polyphenolic compounds are prone to degradation at higher temperatures thus relatively low increase was observed after 6 h of extraction time (compared to the 12 h extraction time). This might partially be due to the degradation of extracted polyphenols, and the addition of polyphenols which are further extracted. Results are in correlation with

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previously published data on polyphenolic compounds' degradation under higher extraction temperatures (Pajač Živković et al., 2020). Opposite of that, caffeine is extractable at higher temperatures. From *Table 3* we can observe that higher temperature and longer extraction time with higher initial concentration (coffee beans) correspond to a higher concentration of caffeine in the extract. The obtained results are in agreement with other studies in which, for example, Várady et al. (2020) determined a total content of polyphenols of 28.6-46.8 mg GAE/g in coffee beverages, which varied depending on the degree of roasting of the coffee beans, before the extraction process. The obtained caffeine levels in the extracts are also in agreement with the previously published results of a similar coffee blend, where 1876 mg caffeine/L was determined (Olechno et al., 2021).

Table 4. *Efficacy (%) of coffee and caffeine on adult heteropterans in the experiment*

Treatment	**Insect**	**Developmental stage**	**Efficacy**		
			1 day	**2 days**	**3 days**
Caffeine	*H. halys*	Adults	11.8 ± 0.4 ab*	**86.7 ± 9.0 a**	**83.3 ± 9.0 a**
	N. viridula	Adults	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 c
	C. ciliata	Adults	6.8 ± 0.3 ab	46.7 ± 13.6 b	**70.0 ± 9.1 a**
Coffee	*H. halys*	Adults	**30.0 ± 0.0 a**	**93.3 ± 6.1 a**	**96.7 ± 6.1 a**
	N. viridula	Adults	0.0 ± 0.0 b	0.0 ± 0.0 d	0.0 ± 0.0 c
	C. ciliata	Adults	6.1 ± 0.3 ab	30.0 ± 4.1 bc	46.7 ± 2.5 b
HSD = 0.05			28.7	32.2	22.4

*Mean values of the same column followed by the same letter are not significantly different (p ≥ 0.05; HSD test). **HSD was determined by comparing the ingredient efficiency between the different insect species for each checking time (days after treatments)

Table 5. *Efficacy (%) of coffee and caffeine on larval stages of studies heteropterans*

Treatment	**Insect**	**Developmental stage**	**Efficacy**		
			1 day	**2 days**	**3 days**
Caffeine	*H. halys*	Nymphs 4th stage	0.0 ± 0.0 b	**48.8 ± 12.2 a**	**62.5 ± 10.2 a**
	N. viridula	Nymphs 3th stage	0.0 ± 0.0 b	20.1 ± 13.1 ab	23.3 ± 16.9 b
		Nymphs 4th stage	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 c
Coffee	*H. halys*	Nymphs 4th stage	**22.4 ± 0.0 a**	**58.0 ± 11.9 a**	**64.6 ± 11.6 a**
	N. viridula	Nymphs 3th stage	0.0 ± 0.0 b	12.0 ± 11.5 ab	14.8 ± 10.2 b
		Nymphs 4th stage	0.0 ± 0.0 b	0.0 ± 0.0 d	0.0 ± 0.0 c
HSD = 0.05			4.36	55.1	59.1

*Mean values of the same column followed by the same letter are not significantly different (p ≥ 0.05; HSD test); **HSD was determined by comparing the ingredient efficiency between the different insect species for each checking time (days after treatments)

Generally, a higher initial concentration in the suspension resulted in higher values of observed compounds (total polyphenolic compounds and caffeine) in the final extracts. Obtained data can serve for further research and a more depth extraction analysis of both polyphenolic compounds and caffeine from grounded coffee beans using simple extraction methods and water as a solvent.

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This study confirmed the efficacy of aqueous coffee extracts and caffeine in controlling Heteropteran pests. Coffee showed the highest efficacy against *H. halys*, E = 97% in adults and E = 73% in nymphs, paving the way for further research on the effects of coffee on this important invasive species. Moreover, a particularly good effect of caffeine treatment (adults: E = 83%; larvae: E = 63%) was observed, indicating that *H. halys* is the most sensitive to the influence of the natural polyphenols used among all the insects studied. Coffee extracts and caffeine showed intermediate efficacy on adult *C. ciliata*; approximately 70% efficacy for caffeine and approximately 47% efficacy for coffee treatment. These results suggest that coffee extract has the potential to control this species but should probably be used in a different formulation that enhances its insecticidal activity. Therefore, the effect of other natural polyphenols on this ornamental pest should be investigated in future research. Coffee and caffeine did not show satisfactory efficacy against either nymphs or adults of *N. viridula* in this study (max E = 33% on 3rd nymph stage), indicating resistance of older developmental stages and adults to these polyphenols. These results indicate that it would be advisable to investigate in future studies the efficacy of the same (or other polyphenols) to control this pest but considering the younger developmental stages of *N. viridula* or other formulations.

It is known that horticultural plants produce endogenous substances (such is caffeine) that can prevent insects from feeding (Singh et al., 2021). The first evidence of the insecticidal action of caffeine was obtained by studying the insecticidal action of coffee by its effect on *Manduca sexta* (L.). The larvae died within 24 h, establishing that caffeine is an effective insecticide on contact to control this pest. The results of this study also suggest that naturally occurring methylxanthines (a group to which caffeine belongs) may also act as systemic insecticides, which would be of interest for future research on species from this study (Nathanson, 1984). The insecticidal effects of caffeine have been noted in numerous other insect species. For example, in two biological models, *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) and *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae). Caffeine was formulated as an oil emulsion and an aqueous solution. Aqueous solutions of caffeine showed no insecticidal activity, while oil emulsions had high activity on both pests (Araque et al., 2007). Sarajlić et al. (2022) also used an aqueous extract, but of *Ambrosia artemisiifolia*, with *H. halys* showing higher sensitivity to the aqueous extract of *A. artemisiifolia* than *N. viridula*. In the study by Bhuvaneswari et al. (2007), the effect of caffeine on *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), the major pest of various cereals in warehouses, was determined. Recently, it was also found that tea leaf extract, whose active insecticidal component is caffeine, can be considered as a potential plant insecticide for the control of aphids, especially *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (Khoshraftar et al., 2019).

In addition to the use of caffeine as a botanical insecticide, the possibility of its use to create transgenic plants or plants that endogenously produce caffeine has also been noted (Kim et al., 2006, 2011; Uefuji et al., 2005). In the study by Kim et al. (2006) and Uefuji et al. (2005), tobacco plants (*Nicotiana tabacum* (L.)) (Solanales: Solanaceae) were used as experimental plants for caffeine production and resulted in a reduction in biotic stress of the experimental plants and reduced disease and pest infestation. In the studies by Kim et al. (2011), the same method was applied to *Chrysanthemum* (Chrysantemum sp. (Asterales: Asteraceae)) plants, which also showed resistance to various diseases and pests. In these studies, resistance of transgenic tobacco plants to

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moth caterpillars, to the species *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidea) and *Pieris rapae* (L.) (Lepidoptera: Pieridae) (Kim et al., 2006; Uefuji et al., 2005), furthermore, resistance of transgenic chrysanthemum plants to *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidea), but also to a very important pest in agriculture, the aphid *Aphis gossypii* (Glover) (Hemiptera: Aphididae) (Kim et al., 2011) was found.

In addition to caffeine, coffee has a lethal effect on various insects due to its chemical composition. For example, a study on the effect of coffee on mortality and reduced growth of Egyptian mosquito larvae (*Aedes aegypti* (L.)) was conducted by Aditama and Zulfikar (2019). In this study, coffee grounds were used as an insecticide, and its use was found to increase mortality and inhibit growth of mosquito larvae. Significant effects of different doses of filtered coffee on larval mortality were reported, suggesting that coffee grounds from *Coffea arabica* L. can be used to control Egyptian mosquitoes, reducing pollution and the incidence of insecticide resistance in this species (Aditama and Zulfikar, 2019). In addition, drying coffee grounds yielded a biological oil that was found to be highly effective in controlling two species of bacteria and the insect *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae) (Bedmutha et al., 2011).

Furthermore, the efficacy of a polyphenol-rich pericarp extract in controlling the growth and development of *Manduca sexta* (L.) (Lepidoptera: Sphingidae) and increasing mortality of 1st larval of *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) has been demonstrated (Tayal et al., 2020., Saad et al., 2021), suggesting the possibility of using other simpler and more natural polyphenol sources for pest control in addition to the natural polyphenol variants from this study. Tlak Gajger and Dar (2021) described the role of plant-derived biochemicals with insecticidal activity and concluded that certain plant species offer numerous promising opportunities for the discovery of novel and environmentally friendly methods to control numerous insect pests.

Based on previous research and the results of this work, it is evident that *H. halys* and *C. ciliata* are sensitive to natural insecticides and that there are several environmentally friendly alternatives to control these pests.

Although this study established the efficacy of caffeine-based preparations, the results show that there is no statistically significant difference between coffee extract and caffeine standard, suggesting that these levels of caffeine are sufficient to control the pests, while the influence of other different compounds in the coffee extract (e.g., polyphenols) was not established. Therefore, coffee and caffeine preparations equally controlled the tested phytophagous heteropteran species, suggesting their insecticidal efficacy.

Conclusions

This study confirms the effectiveness of the tested preparations as alternative insecticides in controlling important pests in horticulture. It opens the possibility of producing natural plant-based extracts that use only water as the extraction medium to control pests. Significant efficacy of coffee and caffeine was observed in the control of *H. halys* and *C. ciliata*. Low but present efficacy was observed for control of *N. viridula*. No difference was observed between coffee extract and caffeine standard, suggesting that these levels of caffeine are sufficient to control the pests.

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