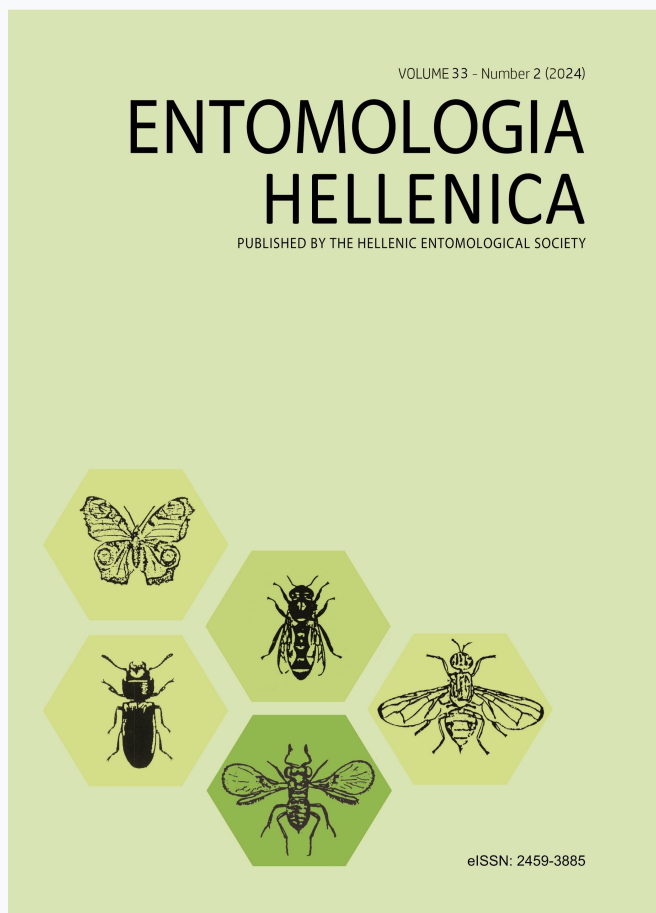


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Insecticidal activity of garden waste compost tea against *Aphis craccivora* (Koch) aphids: *In vitro* and *in silico* study

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ABSTRACT

Aphids are important pests of many crops. However, the use of chemical insecticides has provoked ecological and health problems, thus the valuation of natural products becomes an interesting alternative. Compost teas are organic products that are viewed as substitutes for common pesticides. The present study aims to screen the potential insecticidal effect of garden waste compost tea, through *in vitro* and *in silico* approaches. Five concentrations of a compost tea (20, 40, 60, 80 and 100%) were tested against *Aphis craccivora*, in comparison to negative and positive controls. The repellency and toxicity tests were conducted under laboratory conditions. Moreover, an evaluation of the inhibitor capacity of some compost compounds against acetylcholinesterase was carried out using molecular docking. Results revealed that compost had a very weak insecticidal effect against *A. craccivora* (where the corrected mortality did not exceed 24%) compared to the tested chemical pesticide. Furthermore, the repellency test showed that the compost had some repellency effect in comparison to the tested chemical pesticide which had an attractant effect. Concerning the results of the molecular docking, pirimicarb (active molecule of pesticides) recorded a better S score than the three compost compounds.

KEY WORDS: Insect pests, repellency, toxicity, bioinsecticides, molecular docking.

Introduction

Biotic stress in plants caused by insect pests is one of the most significant problems, leading to yield losses (Tlak Gajger & Dar, 2021). These insect pests belong to different groups, including Aphididae. Among its important species, *Aphis craccivora* attacks about 50 crops in 19 different plant families (Blackman & Eastop, 2007). It causes damage by direct feeding as well as by transmission of plant viruses causing diseases such as broad bean yellow mosaic and bean leafroll (Weigand & Bishara, 1991).

Synthetic pesticides are being widely used for the management of pests to avoid losses both in field and post-harvest storage (Kumar et al., 2022). The estimated 30% losses from pests that would occur in the absence of pesticides, would spell economic and human disaster for many developing countries around the world (Saxena et al., 2014). However, global concern has been raised in recent times against the utility of synthetic insecticides in households and fields (Barua et al., 2016). Pesticides accumulate toxic residues on food grains used for human consumption and this may lead to health issues, in addition to the very high worldwide mortality rate due to

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pesticide poisoning (Singh & Kaur, 2018). Besides, pesticide residues may constitute a significant source of contamination of air, water and soil, and a large quantity of pesticides is released into the atmosphere during application, thereby inducing adverse climatic changes (Saxena et al., 2014). That's why for the future, it is necessary to develop a more environmentally friendly agriculture that will decrease inputs in chemicals and generate fewer harmful outputs such as pesticide residues (Wójcicka, 2010).

Consequently, identification and exploring for nature-originating pest control agents could become a possible substitute for the synthetic chemical pesticides (Kumar et al., 2022). Natural insecticides are chemical compounds or substances obtained from living organisms (Barua et al., 2016). Natural compounds known for their bioactivity against insects are considered safe, economical, biodegradable and easy to use (Singh and Kaur 2018). Among these, compost and compost teas have been shown, in previous studies, to possess control properties against a wide range of different plant pests and diseases (Bandara et al., 2010; Edwards et al., 2010; Alao et al., 2011; Shalaby et al., 2012; Pane et al., 2012; Martín, 2014; López-Martín et al., 2018; Morales-Corts et al., 2018; Suwandi et al., 2020; González-Hernández et al., 2021, 2022, among others). Concretely, compost teas are organic solutions obtained by the fermentation of compost in a liquid phase for a few days, with or without aeration (Al-Dahmani et al., 2003). The physical and biochemical quality of compost teas depends on the characteristics of the starting compost, as well as on other parameters that affect its production i.e., compost-to-water ratio, and aeration (Ingham, 1999; Scheuerell & Mahaffee, 2002; Martin et al., 2012).

On the other hand, the application of computational modeling guided the experimental procedure by elucidating the

mechanisms of ligand-enzyme binding and greatly reducing the research time and costs (Badawy et al., 2022). Molecular docking is an *in silico* method which predicts the placement of small molecules or ligands within the active site of their target protein (Surabhi & Singh, 2018). The nature of the interaction between ligand and receptor depends on a balance in the chemical/physical forces between them and the forces between each of these molecules and the solvent or environment (Krumrine et al., 2003).

Thus, the current study's objective is to evaluate *in vitro* the effectiveness of compost tea solutions against *Aphis craccivora*, as well as to screen the insecticidal activity of its components, using molecular docking approach.

Materials and Methods

Compost tea preparation

The compost tea (CT) derived from composting gardening wastes (mainly a mixture of green and pruning debris, i.e., mainly leaves and branches of cypress (*Cupressus* sp.), willow (*Salix* sp.) and poplar trees (*Populus alba* L.), reaching a C/N ratio of 30), which were obtained from public gardens of the Province of Salamanca (Spain). This process was performed in aerated piles of 15 × 2 × 2 m for 180 days in a garden center located in Salamanca (Spain) (40°57'23" N; 5°41'8" W, 775 m a.s.l.). Piles were turned twice per week for eight weeks and once a week during the rest of the biooxidative process. Moreover, the moisture of the piles was controlled once a week, and when it dropped below 55%, water irrigation was applied up to maintain the moisture average around 60% during the whole process. The mature compost was obtained under ambient conditions in March. The main characteristics of the garden waste compost were: pH 7.8, C/N 11.4, 41.4% total organic matter, 2.1% total N, 10.90% humic acids and 0.33 dS/m Electrical conductivity

(Registration No. F0004957/2031, Viveros El Arca enterprise, Salamanca, Spain).

Compost was mixed with tap water in a ratio of 1:5 (v/v) in polyethylene non-degradable 1000 L containers at room temperature (20 °C) for a brewing period of five days. Water had been previously aerated for 8 h to reduce chlorines concentration. The mixture was aerated for five hours every day by applying circular stirring and making fine bubbles of air using a pump (750 W-300 rpm). Then, it was filtered with a double-layered cheesecloth, and the aerated compost tea (CT) was stored in a dark container (50 L capacity at room temperature) until use.

Chemical and microbiological properties of the compost tea

The chemical properties of the CT were directly analyzed; pH, electrical conductivity (EC) and C:N ratio were determined as described by Morales-Corts et al. (2021). Furthermore, assimilable nutrient contents (NO_3^- , PO_4^{3-} , K_2O , SO_4^{2-} , Ca^{2+} and Mg^{2+}) were analyzed with the nutrient analysis photometer HANNA HI 83225. Finally, humic acids were determined following the alkali-acid method described by Pant et al. (2012). Salicylic acid and indoleacetic acid (IAA) were quantified by mass spectrometry (HPLC).

Microbial analysis of the CT was estimated using the serial dilution spread plate method. To determine the microbial population, different selective culture media and CT dilutions were used for microorganism isolation: nutrient agar and 10^{-3} dilution for total aerobic bacteria, Ashby medium, and 10^{-2} dilution for N-fixing bacteria, ISP² medium and 10^{-1} dilution for actinobacteria and modified potato dextrose agar medium but no dilution for total fungi and *Trichoderma* ssp. quantification (Wickerham, 1951; Sanchis Solera, 1996; Stella & Suhaimi, 2010; Vargas-Gil et al., 2006). Then, plates were inoculated by depositing on the agar surface

0.1 mL of the CT dilution, which was spread on the media surface with sterile glass beads. Moreover, non-inoculated plates were included as a negative control. Petri dishes were incubated in the dark at 28 °C for 3 to 15 days, depending on the medium. After this time, colony-forming units (CFU) were counted to estimate the cultivable microorganism's population. This experiment was conducted using five replications.

In vitro assays

Five CT concentrations (20, 40, 60, 80 and 100%) were tested, in addition to the negative control (distilled water) and a positive control represented by a chemical aphicide 'Lazer' (produced by Ortiva, represented in Algeria by Phytoplus and registered under the homologation number 07 45 036) containing two active molecules (Lambda-cyhalothrine and Pirimicarb, 5%+10%, which are sodium channel modulators and acetylcholinesterase inhibitor, respectively (IRAC, 2022)).

Faba bean leaves, bearing *Aphis craccivora* aphids (of the same colony), were collected from a commercial field, that had not undergone any chemical treatment. The leaves along with wingless insects were left for few hours in the plastic box. Aphids were then collected by means of a small brush and used for the bioassay.

For screening the insecticidal activity under laboratory conditions (T = 25 °C; Relative Humidity= 36%), cut pieces of leaves were dipped in each concentration of the compost for 2-5 seconds and then the solution was drained out and pieces were kept for drying. Three replications of each concentration along with positive and negative controls were taken for bio-assay studies. Treated leaves were kept in the Petri dish with 10 aphids in each replicate. Mortality was observed after 24 h of treatment. Percent mortality was corrected through Abbots formula (1925):

Corrected mortality rate = $[(T_{mp} - C_{mp}) / (100 - C_{mp})] \times 100$

where T_{mp} : mortality rate of the treatment and C_{mp} : mortality rate on the control.

Furthermore, ANOVA analysis followed by Student-Newman-Keuls test were performed by the means of SPSS software, to compare mean aphid mortalities.

Concerning the test of repellency *in vitro*, three replications for each concentration, in addition to the conventional pesticide, were considered. For each replicate, two leaf discs free of aphids were cut. After that, one leaf disc was dipped for 2-5 seconds in the corresponding solution. Each Petri dish was divided into two equal parts. Leaf discs dipped in treatment solution were placed in one side and untreated ones on the other side. 10 wingless aphids were placed at the center of the dish and left for 24 hours and then the number of aphids on each side was recorded. The repellency percentage was calculated according to the following equation:

$PR = [(C - T) / (C + T)] \times 100$ (Pavela et al., 2009)

where: C = the number of aphids on control disc, and T = the number of aphids on treated disc.

***In silico* study**

On the other hand, the evaluation of the insecticidal activity by molecular docking was carried out using the Molecular Operating Environment (MOE) software, version 2014.0901, designed by Chemical Computing Group Inc. (Canada). We studied *in silico* the ability of four ligands, including three compounds of the examined compost (salicylic acid, humic acid and indole-3-acetic acid), in addition to an active molecule of chemical pesticide (Pirimicarb), to inhibit a target enzyme of insects called acetylcholinesterase (AChE), having the PDB code 1QON.

Results

Compost tea properties

The studied characteristics (pH, EC, C-to-N ratio, assimilable macro and micronutrients, and humic acids) are shown in Table 1. Highlighted are the high NO_3^- and K_2O concentrations (2240.4 and 2851.2 ppm, respectively) and the low C-to-N ratio of the extract, as well as the relevant humic acids amount.

Concerning the microbiological analysis of the compost tea, its microbiological composition was: total aerobic bacteria 2.7×10^7 cfu mL⁻¹; N-fixing bacteria 2.7×10^7 cfu mL⁻¹; actinobacteria 7.4×10^4 cfu mL⁻¹; Trichoderma sp. and fungi were valued between 2.7 and 8.7×10^2 cfu mL⁻¹.

***In vitro* assays**

Results revealed that for the test of toxicity *in vitro*, there was a significant difference between the studied treatments. It seems that compost had very weak insecticidal effect against *A. craccivora* comparatively with the tested chemical pesticide. The corrected mortality did not exceed 24% for the different concentrations of compost tea (Table 2).

Results of the test of repellency *in vitro* showed that compost had some repellency effect against *A. craccivora* in comparison with the tested chemical pesticide which seems to have attractant effect (Table 3). All the tested concentrations of the compost were ranged in the class I of scale described by McDonald et al. (1970).

***In silico* study**

Concerning the results of the molecular docking, the best S score was recorded for Pirimicarb (active molecule of pesticides) followed by indole-3-acetic acid (Table 4). This result confirms that obtained for *in vitro* test, i.e. the tested pesticide was more toxic than compost solutions.

TABLE 1: Chemical characteristics of the studied compost tea. *Results are indicated as mean ± standard deviation*

pH	EC (dS/m)	C/ N	NO ₃ ⁻ (ppm)	P ₂ O ₅ (ppm)	K ₂ O (ppm)	SO ₄ ²⁻ (ppm)	Ca ²⁺ (ppm)	Mg ²⁺ (ppm)	Humic acids (mg/L)	Salicylic acid (mg/L)	IAA (ng/L)
7.16±0.1	1.2±0.0	7.1	2240.4±186.	149.7±18.	2851.2±234.	43±16	280±2	20±13	198±3	5.85±1.23	80±10
4	9		6	3	5		0		7		

TABLE 2: Corrected mortality percentages of aphids on different solutions

Treatments	Corrected mortality (M ± SE)
20 %	0.00 ± 0.00 a
40 %	0.00 ± 0.00 a
60 %	23.70 ± 7.73 a
80 %	4.63 ± 3.34 a
100 %	9.72 ± 9.72 a
Chemical pesticide	100.00 ± 0.00 b
Signification	0,000 *

* Signification at $P < 0.05$

TABLE 3: Percentages of repellency of aphids by different solutions

Treatments	Percentages of Repellency (M ± SE)
20 %	8.15 ± 39.34
40 %	17.04 ± 11.92
60 %	6.67 ± 48.07
80 %	18.52 ± 29.60
100 %	10.37 ± 5.78
Chemical pesticide	-14.07 ± 2.96

TABLE 4: S score of the studied ligands with the target protein 1QON

PubChem code	Ligands	Properties	S score (Kcal mol ⁻¹)
31645	Pirimicarb	Inhibitor of acetylcholinesterase	- 6.42
802	Indole-3-acetic acid	Compound of compost tea	- 5.23
90472028	Humic acid	Compound of compost tea	- 4.30
338	Salicylic acid	Compound of compost tea	- 4.64

Discussion

The extract's low C:N ratio, high NO_3^- and K_2O concentrations, and high humic acid content were important characteristics of the studied compost tea. There are numerous factors that affect the quality of vermicompost tea, which in-turn affect its ability for disease and pest suppressiveness, among them are included compost grade, compost maturity, aeration, temperature, microbial inoculants, and compost to water ratio (Yatoo et al., 2021). For instance, Nur et al. (2023) indicated differences in the C:N ratio and humic acid concentration between aerated and non-aerated compost tea made from wild lotus. Orosz et al. (2021) reviewed many mechanisms of action of different compost extract components allowing control of plant diseases. There is a lot of published literature concerning studies regarding the effects of humic acids and different biostimulants on the growth, productivity, and protection of vegetable species is available, whereas literature concerning the study of the effects of compost teas on vegetable crops is more limited (Pilla et al., 2023).

On the other hand, the present investigation revealed that the compost had weak insecticidal effect against *A. craccivora*, in comparison to the chemical pesticide containing pirimicarb, as well as limited repellency activity. Similarly, Abdu-Allah (2012) indicated that the toxicity index of pirimicarb shows higher aphicidal activity than three petroleum ether plant extracts. Additionally, Cantelo (1985) found that compost treated with experimental insecticides affecting acetylcholinesterase (such as diazinon and dimethoate) were effective against the dipteran pest *Megaselia halterata*.

In contrast, Bandara et al. (2010) found that brinjal aphids, mealy bugs and thrips were significantly reduced in treated plants

with different compost solutions, compared to the controls. Besides, other results have shown that organic compost liquid formulations acted as antifeedants for *Podagrica* sp., *Zonocerus variegatus* and *Bemisia tabaci*, while maize stover compost had a very good insect control (Alao et al., 2011). The compost extract containing amino acids showed a remarkable potential to develop into an effective biostimulant for protection from virus disease and its insect vector, *Aphis gossypii* (Suwandi et al., 2020).

Moreover, insect pests of the plant species *Telfairia occidentalis* that received foliar spray of compost extracts (from cassava peel and tithonia plant) were minimal compared with non-fertilized plants and those that received soil incorporated NPK fertilizer (Akanbi et al., 2007). Furthermore, Shalaby et al. (2012) mentioned that compost tea used as soil drench applied after sowing, followed by foliar application of compost tea proved to be the best treatment against insect infestations by *Pegomia mixta* Vill, *Scrobipalpa ocellatella* Boyd and *Cassida vittata* Vill. Moreover, Edwards et al. (2010) indicated that all examined vermicompost extracts suppressed pest establishment on the plants, and their rates of reproduction for all three species of pests, significantly. The same researchers concluded that the most possible cause for the unacceptability of the plants to pests was the uptake of soluble phenolic materials from the vermicompost extracts into the plant tissues and these compounds are known to make plants unattractive to pests.

Likewise, previous studies confirmed the influence of compost as soil fertilizer on aphid populations (Ponti et al., 2007; Stafford et al., 2012).

On the other hand, the results of the molecular docking were consistent with the in vitro toxicity assay. The pirimicarb molecule proved to be the more effective in

the inhibition of acetylcholinesterase, recording the best S score ($-6.42 \text{ kcal mol}^{-1}$), compared to the compost compounds. Other *in silico* studies showed not very different S score results. For instance, Lebbal et al. (2021) noticed that α -Thujene ligand has the best score with IQON target enzyme (-4.77), among seven compounds contained in *Thymus algeriensis* and *T. numidicus*, whereas, Badawy et al. (2022) indicated the docking energies in the AChE enzyme of -9.52 , -9.38 , and $-8.52 \text{ kcal mol}^{-1}$ for chlorpyrifos-methyl, diazinon, and malathion, respectively. Furthermore, Malak et al. (2022) found that among 38 studied bioactive phytochemicals, some compounds

recorded docking scores of 9.11 to 7.14 against cattle tick *Rhipicephalus microplus* acetylcholinesterase protein.

Conclusion

In summary, the present study confirmed through *in vitro* and *in silico* screenings that the compost had low aphicidal effect. In addition, it has better repellent activity compared with the tested chemical aphicide. Nevertheless, its use as fertilizer should be combined with other control methods to reduce efficiently the negative impact of aphid populations.

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Εντομοκτόνος δράση του εκχυλίσματος κομπόστ απορριμμάτων κήπου κατά των αφίδων *Aphis craccivora* (Koch): *In vitro* και *in silico* μελέτη

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ΠΕΡΙΛΗΨΗ

Οι αφίδες αποτελούν σημαντικούς εχθρούς πολλών καλλιεργειών. Η χρήση χημικών εντομοκτόνων έχει προκαλέσει σημαντικά περιβαλλοντικά προβλήματα και προβλήματα στην ανθρώπινη υγεία. Έτσι, η αποτίμηση των φυσικών προϊόντων αποτελεί μια ενδιαφέρουσα εναλλακτική. Τα εκχυλίσματα κομπόστ (τσάγια κομποστοποίησης) είναι φυσικά προϊόντα που θεωρούνται υποκατάστατα της χρήσης κοινών φυτοφαρμάκων. Η παρούσα μελέτη στοχεύει να εξετάσει την πιθανή εντομοκτόνο δράση του εκχυλίσματος κομπόστ απορριμμάτων κήπου, μέσω προσεγγίσεων *in vitro* και *in silico*. Πέντε συγκεντρώσεις του εκχυλίσματος κομπόστ (20, 40, 60, 80 και 100%) δοκιμάστηκαν έναντι των αφίδων *Aphis craccivora*, σε σχέση με αρνητικούς και θετικούς μάρτυρες. Οι δοκιμές απόθησης και τοξικότητας πραγματοποιήθηκαν σε εργαστηριακές συνθήκες. Επιπλέον, πραγματοποιήθηκε αξιολόγηση της ικανότητας αναστολής ορισμένων ενώσεων κομποστοποίησης έναντι της ακετυλοχολινεστεράσης με χρήση μοριακής σύνδεσης. Τα αποτελέσματα αποκάλυψαν ότι το κομπόστ είχε πολύ ασθενή εντομοκτόνο δράση του *A. craccivora* (όπου η διορθωμένη θνησιμότητα δεν ξεπέρασε το 24%) σε σύγκριση με το χημικό εντομοκτόνο μάρτυρα. Επιπλέον, η δοκιμή απόθησης έδειξε ότι το κομπόστ είχε κάποια αποθητική δράση σε σύγκριση με το χημικό εντομοκτόνο μάρτυρα που φαίνεται να έχει ελκυστική δράση. Όσον αφορά τα αποτελέσματα της μοριακής δέσμωσης, το pirimicarb (δραστική ουσία του χημικού εντομοκτόνου) κατέγραψε καλύτερη βαθμολογία S από τις τρεις ενώσεις κομποστοποίησης.