Folia Hort. 35(2) (2023): 287–296

DOI: 10.2478/fhort-2023-0021

Folia Horticulturae

Published by the Polish Society
for Horticultural Science since 1989

ORIGINAL ARTICLE | Open access | https://sciendo.com/journal/FHORT

The use of stinging nettle tea (*Urtica* sp.) to control *Aulacorthum solani* and *Macrosiphum euphorbiae* on *Ranunculus asiaticus*

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ABSTRACT

Natural and environmental-friendly approaches to control pests and diseases in horticultural production systems are showing an increasing trend. Biological alternatives, such as botanical extracts and basic substances, show promise in the reduction of the necessity for conventional plant protectants. In ornamentals, less is known about the usage, behaviour and potential of botanicals and basic substances for plant protection. In two trials, we investigated the effect of a weekly foliar spray of an aqueous extract of nettle (*Urtica* sp.; dried material 15 g · L^{-1}) on two aphids, *Macrosiphum euphorbiae* and *Aulacorthum solani*. Experiments were conducted in the greenhouse with *Ranunculus asiaticus* as hostplant. The *Urtica* tea and azadirachtin (NeemAzal-T/S) as a standard used in common horticultural production were compared with the water control. Both previously infested and noninfested plants were built up to examine the effect on aphid population growth and migration. As a result, we showed that both treatments, *Urtica* tea and azadirachtin, reduce the aphid density significantly in comparison with the water control, although the effect of the *Urtica* tea was not as substantial as that of azadirachtin. Contrarily, treatments could not prevent aphid migration on previously noninfested plants. The study demonstrates that the basic substance *Urtica* tea can be suitable for the reduction of aphid pests in ornamental plant production, and thus, reduce the amount of synthetic plant protectants in horticulture.

Keywords: aphids, basic substance, bioprotectant, insecticide, ornamentals, Persian buttercup, plant protection

INTRODUCTION

Since the demand for natural and environmentally friendly approaches to control pests and diseases in horticulture has increased, also biological and more sustainable alternatives such as botanical extract, the conservation of beneficial organism, crop diversification and basic substances have become increasingly interesting (Feldmann and Vogler, 2021). In terms of biocontrol, different strategies such as the use of a beneficial microorganism, for instance *Trichoderma* sp. or *Bacillus* sp., are available. These approaches can be based on direct effects such as biocontrol agents producing antimicrobial enzymes, and competition for place and resources, as well as more indirect effects such as priming and alternating plant phytohormones

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(Lahlali et al., 2022). Although the risks associated with some strategies in biocontrol are characterised by controversy (Deising et al., 2017), in general, botanicals are accepted as biodegradable, environmentally safe and less hazardous in comparison with chemical treatments (Sergeeva, 2016; Feldmann and Carstensen, 2018).

Plant-based insecticides obtain more attention in science and are already existing in the European market (Isman and Grieneisen, 2014). The activities of some insecticides derived from native plants of the Mediterranean region, such as *Artemisia absinthium* or *Rosmarinus officinalis*, have already been summarised (Karkanis and Athanassiou, 2021). One widely known example for bioinsecticides is extracts from the oil of seeds of the neem tree, *Azadirachta indica* (Brahmachari, 2004; Pavela, 2016). In the case of extracts from *A. indica*, many different active ingredients with very variable concentrations can be found, but only a few were promising for industrialisation (Isman, 2006). In the late 20th century, the first commercial products, Margosan-O, Azatin EC or Turplex, were introduced in various markets, and since then products with the active ingredient azadirachtin have been commercially successful (Ermel and Kleeberg, 1995). From this time onwards, it has been clear that azadirachtin as an active ingredient is useful for controlling a wide range of insect pests (Schmutterer and Singh, 1995). The artificial synthesis of azadirachtin has also widely become an object of interest, and after 22 years of work, the syntheses of the natural compound were completed in 2007 (Jauch, 2008). Azadirachtin is effectively systematically and widely distributed within the plant through the water transport in the xylem (McKenzie et al., 2010; Pavela et al., 2013). Two main effects are associated with the active ingredient. First, a disordering effect on an insect moulting hormone and subsequently an interrupted development of juveniles and sterility effect on adult individuals; and second, the ingredient works as a strong antifeedant (Isman, 2006; Pavela, 2016).

Similar to many botanicals, basic substances are considered and proven not to be harmful to human or animal health as well as to the environment (EU, 2009; Orconneau et al., 2022). These properties qualify them to enrich plant protection in industrial developed regions (Mamun and Ahmed, 2011; Sergeeva, 2016; Romanazzi et al., 2022). In particular, in organic and other sustainable horticulture cropping systems, these approaches bring about substantial improvements to the existing structures (Marchand, 2017; Đurić et al., 2019; Costantini and La Torre, 2022). However, the assumption underlying the enrichment of modern pest management systems through organic means is not always based on valid data for the effect on plant pathogens or pests. For the authorisation process, no data for efficacy are demanded (Feldmann and Carstensen, 2018). Different basic substances, for example *Equisetum arvense*, lecithine, hydrogen peroxide or chitosan, are already shown to be promising for implementation in plant protection strategies (Copes, 2009; El Hadrami et al., 2010; Jolly et al., 2018; Trebbi et al., 2021; Wulf et al., 2022, 2023). For other basic substances such as *Urtica* sp., only a meagre amount of data are available. *Urtica* sp. is authorised as a basic substance by the Commission Implementing Regulation (EU) 2021/1165 of 15 July 2021 in combination with the appropriate Review Report of January 2017 (EU, 2017, 2021). Plants of the genera *Urtica* are perennial and flowering herbs, occurring almost all over the world, such as Europe, North America, North Africa and parts of Asia (Bhusal et al., 2022). These plants are widely used for human and animal food and feed as well as for traditional medicine and modern pharmaceutical aspects (Kregiel et al., 2018; Goswami et al., 2022). Additionally, the fibres from *Urtica dioica* are promising concerning the use as raw material in textile production and as a substitute for artificial fibres (Viotti et al., 2022).

Many botanical extracts are loaded with plenty of secondary metabolites such as lectins, flavonoids or phenolic compounds. Aqueous, distilled or fermented extract of *Urtica* sp. is known to be carrying high amounts of secondary metabolites, for example flavonoids, other phenolic compounds or lectins (Joshi et al., 2014; Sehari et al., 2020; Langa-Lomba et al., 2021; Majedi et al., 2021; Devkota et al., 2022; Shreejana et al., 2022). Some of the secondary metabolites found in nettle extracts are known to have insecticidal effects on aphids, for example the flavonoid quercetin or the group of lectins (Vandenborre et al., 2011; Goławska et al., 2014; Hikal et al., 2017). These metabolites can also be used as a tool in plant protection against fungal pathogens (Jamiołkowska et al., 2023). Several studies concerning the antifungal effect of an *Urtica* spp. extract are summarised by Costantini and La Torre (2022).

Assuming insecticidal or repellent effects of an extract of *Urtica* sp., in our study we evaluate weekly foliar treatments against two aphid species, *Macrosiphum euphorbiae* and *Aulacorthum solani*. For this purpose, we set up infested and noninfested plants in two experimental trials and compare the population density and the settlement of aphids after an *Urtica* treatment with a water control and the use of a commercially available insecticide containing azadirachtin. For use in the experimentation, NeemAzal-T/S, a naturally derived azadirachtin that is formulated as an emulsion concentrate, was chosen as the practical standard.

MATERIAL AND METHODS

Plant material and aphid infestation

For the study, two greenhouse experiments were conducted. Seedlings of *Ranunculus asiaticus* 'Magic' F$_1$ were received from a commercial breeding company (Volmary GmbH, Münster, Germany). Plants for the first experiment were potted in early October 2021 and for the second in early November 2021. A peat substrate with wood fibre and compost (gramoProfi Basic, Gramoflor

GmbH & Co. KG, Vechta, Germany) was used. The first experiment was conducted from November to December 2021 and the second from January to February 2022. Plants were cultivated at 12°C and irrigated using a retention irrigation system. The experimental site was located at longitude 10°05′00.2′′ E, latitude 53°30′33.5′′ N. The aphid infestation on the plants occurs naturally by chance. A mixed plant sample was used to determine the aphid species at the beginning and the end of both experiments on randomly selected plants via light microscopy. Thus, in the first experiment the natural infestation occurred by the foxglove aphid (*A. solani*) and in the second experiment by the potato aphid (*M. euphorbiae*).

Greenhouse set-up and data collection

The experiments were conducted in the greenhouse and four repetitions were arranged in a randomised block design. Each repetition consisted of 20 plants. Within a plot, 10 plants were previously infested with aphids whereas the other 10 plants were not. To achieve the possibility of colonisation of the noninfested plants, the pots were placed systematically alternated within the repetition without any distance between the plants (Figure 1). Plots were sized of 0.7 m × 0.8 m and plants with aphids were noted before the commencement of the experiment. Data collection was always performed on the same five initially colonised or noncolonized plants. Thereby, the number of aphids was counted weekly.

Treatments

In both trials, three treatments were established: (i) water control, (ii) practical standard and (iii) *Urtica* tea applying the same amount of water with 100 mL · m^{-2}. As practical standard, a formulated insecticide containing azadirachtin (NeemAzal-T/S, Trifolio-M GmbH, Lahnau, Germany; Reg. No. 024436-00) was used. The product NeemAzal-T/S is also authorised for ecological plant production in the EU and was applied with 0.3 mL · m^{-2}. *Urtica* tea was sprayed with an amount of 100 mL · m^{-2}. For the preparation, 15 g dried plant material of *Urtica* spp. (Krauterie GmbH, Dänischenhagen, Germany) was poured with 1 L boiling water and cooled down before use. A total of four applications each were performed at an interval of 7 days. For the foliar treatment, a backpack sprayer (Profi Star 3, Birchmeier Sprühtechnik AG, Stetten, Switzerland) was used.

Statistical analysis

To determine significant differences, the program R (version 4.2.1, R Core Team 2022, Vienna, Austria) in combination with RStudio (version 1.3.1093, RStudio Team 2022, Boston, US-MA) was used. Based on the Q–Q-plot, a normal distribution was assumed. The practical standard and the treatment with *Urtica* tea were compared with the water control. For the comparisons, the R package emmeans (version 1.7.0) was used. The effect of the randomised block design was included in the statistical model.

RESULTS

Infestation and population growth on water treated plants

During the trial period, the plants undergo natural infestation with *A. solani* Kaltenbach in the first period and with *M. euphorbiae* Thomas in the second (Figure 2). The infestation took place naturally and by chance. During both trails only anholocyclic forms of the aphids occurred and were studied. In addition, a small amount of *Myzus ascalonicus* Doncaster was found during the

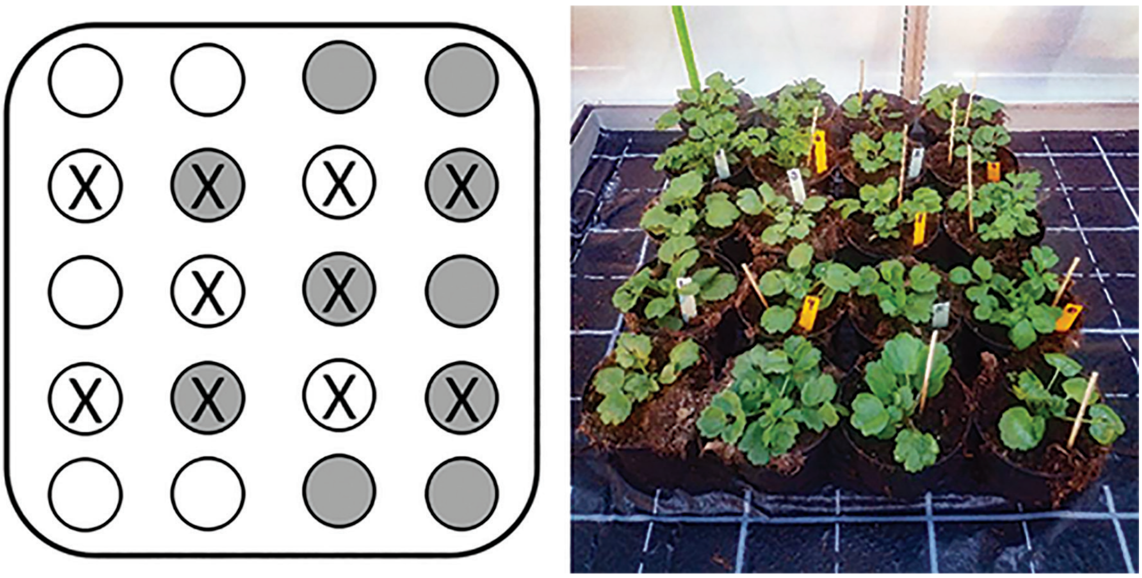

Figure 1. Plant were placed within a plot according to the scheme (left) in the greenhouse (right); shaded circles show previously infested plants and marked (×) circles show plants set for data assessment.

first trial. The percentage distribution of aphid species on the plants was not determined.

A high infestation with aphids occurred within the plants treated solely with water. The infested plants in the control treatment directly before the first application started with 54.5 ± 11.95 aphids per plant in the first experiment and with 55.85 ± 8.22 aphids per plant in the second trial (Figure 3 and Figure 4). In the further course of cultivation, the number of aphids increased (first trial, day 14: 106.95 ± 10.17; second trial, day 15: 78.2 ± 8.25) and ended up with 250.89 ± 22.78 aphids per plant in the first trial (day 35) and 179.13 ± 15.25 aphids per plant in the second trial (day 29).

In contrast, the noninfested plants started without an infestation with aphids in both trials. However, throughout the experiment, natural dispersal and migration of aphids led to a colonisation and infestation of those previously noninfested plants (first trial, day 14: 4.25 ± 2.02; second trial, day 15: 74.65 ± 14.45). At the last acquisition date, in both periods, a comparatively high infestation was found (first trial, day 35: 103.2 ± 16.5; second trial, day 29: 302.05 ± 26.08).

Effect of azadirachtin on aphid populations

The use of azadirachtin resulted in a significant reduction of aphids on the plants. On the previously infested plants in the first experiment, a significant reduction was found from day 14 (49.55 ± 10.67, $p < 0.01$; Figure 3) onwards to the end of the acquisition time (day 35: 12.95 ± 3.26, $p < 0.01$). During the second experiment, a significantly

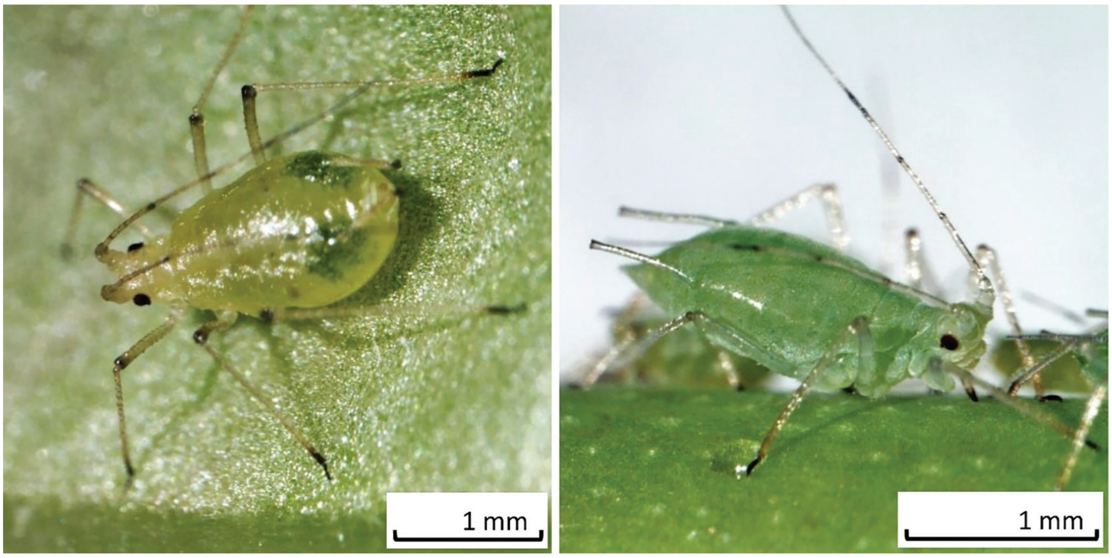

Figure 2. Within the experiments, plants of *R. asiaticus* undergo natural infestation with *A. solani* (left) and *M. euphorbiae* (right).

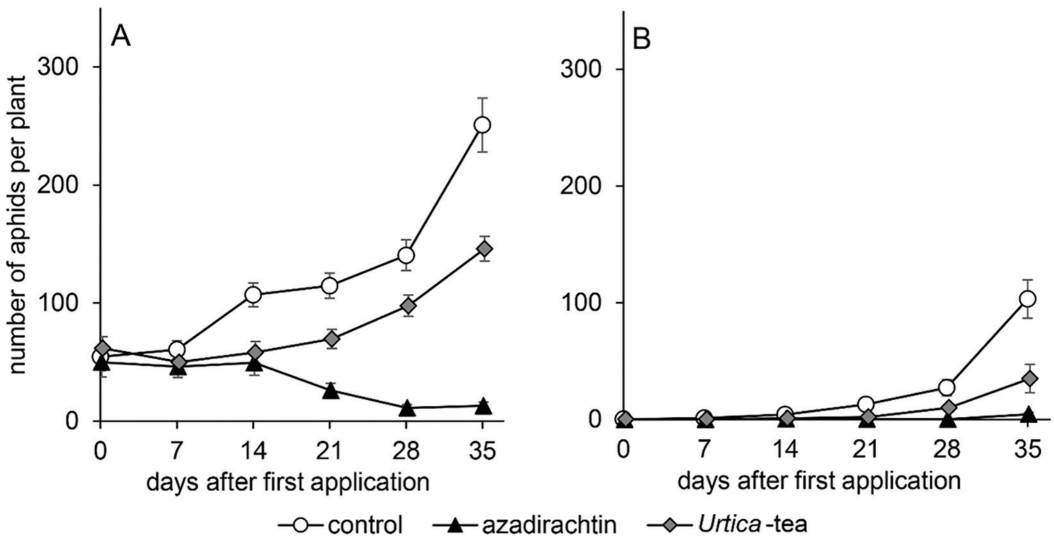

Figure 3. Number of *A. solani* (means ± SE, $n = 20$) during the first trial on the previously infested (A) and the noninfested (B) plants of *R. asiaticus* after four foliar treatments with water (control), azadirachtin (NeemAzal-T/S) and *Urtica* tea, each in an interval of 7 days.

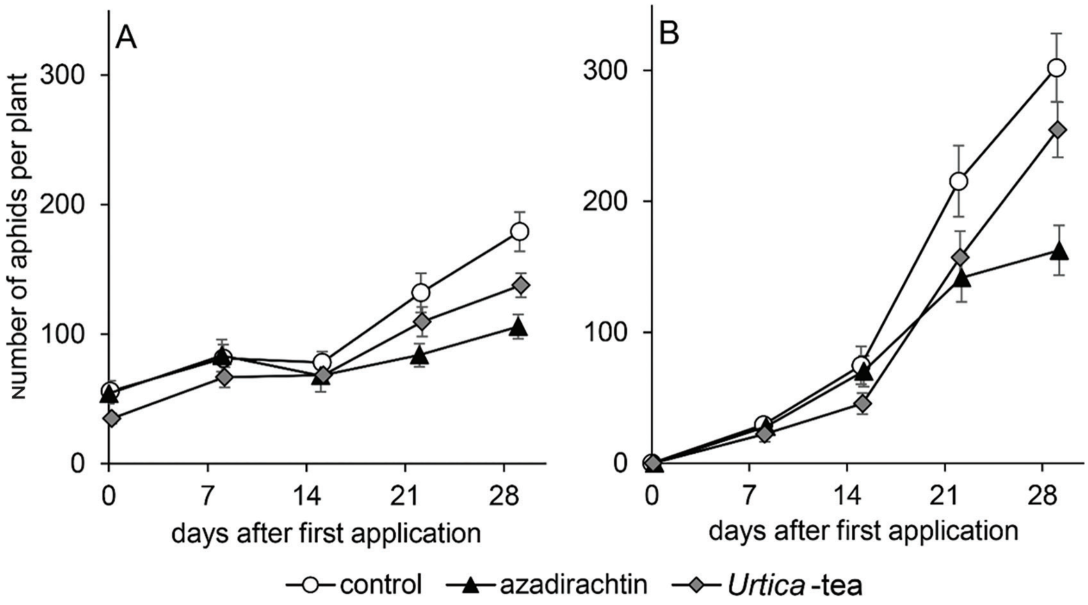

Figure 4. Number of *M. euphorbiae* (means ± SE, $n = 20$) during the second trial on the previously infested (A) and the noninfested (B) plants of *R. asiaticus* after four foliar treatments with water (control), azadirachtin (NeemAzal-T/S) and *Urtica* tea, each in an interval of 7 days.

reduced number of aphids was found on the infested plants at the last two dates, day 22 (83.83 ± 18.57, $p < 0.01$) and day 29 (105.82 ± 9.43, $p < 0.01$).

The previously noninfested plants in the first experiment stayed at a very low level of infestation and showed a significantly smaller number of aphids at the last two acquisition dates (day 28: 0.25 ± 0.18, $p < 0.01$; day 35: 4.4 ± 1.74, $p < 0.01$). Although the infestation in the second trial increases more drastically, the treatments with the insecticide led to a significant reduction of aphids at the last two dates of acquisition (day 22: 141.65 ± 18.57, $p < 0.01$; day 29: 162.55 ± 19.04, $p < 0.01$).

Effect of Urtica tea on aphid populations

The weekly treatment with *Urtica* tea resulted in a slight reduction of aphids. A significantly decreased aphid density in the previously infested plants was found in both trials. In the first, from day 14 onwards to the end of the experiment, the significant reduction could be confirmed (day 14: 58.37 ± 9.12, $p < 0.01$; day 35: 146.06 ± 10.53, $p < 0.01$; Figure 3). In the second, only once was the reduction significant (day 29: 137.76 ± 9.3, $p < 0.05$).

The previously noninfested plants showed also a significantly reduced aphid density at some dates. In the first days of the first experiment, the population growth was nearly the same compared with the treatment with azadirachtin. From day 28 onwards, a significant reduction was confirmed (10.05 ± 3.04, $p < 0.05$) and the lower population stayed significant until the end of the experiment (day 35: 35.0 ± 12.05, $p < 0.01$). In the noninfested plants of the second experiment, only once could a significantly reduced number of aphids be found. At day 22, aphid density was significantly lower after the *Urtica* tea treatment compared with the water control (157.26 ± 19.92, $p < 0.05$).

DISCUSSION

In the future, new environmental-friendly strategies are needed to control insect pests. This will be the case for not only ecological but also integrated plant protection (Brzozowski and Mazourek, 2018). Up until the present, less has been known about alternative systems such as basic substances in most horticultural production systems. Currently, in common practice of ornamental plant protection, basic substances do not play a role. Nevertheless, they may have the potential to fill the gap caused by upcoming deficits of chemical plant protectants (Richter et al., 2021).

In our study, we could find a significantly reduced population growth of *A. solani* and *M. euphorbiae* due to the weekly sprayed *Urtica* tea. This was the case for both the previously infested and, at the later experimental time, the noninfested plants. The effect of the *Urtica* treatment on the population was higher for *A. solani* in the first experiment than for *M. euphorbiae* in the second experiment. The population growth of *M. euphorbiae* on the noninfested plants of the second trial was much higher than the increase of individuals of *A. solani* on the noninfested plants in the first trial. De Conti et al. (2011) had already reported similar developmental parameters for *A. solani* and *M. euphorbiae* on *Lactuca sativa* at 16°C. Differences between the aphid populations might be explained by the aphid species, the plant species or the interaction between them. Our study could not show a reduced settlement of both aphids on *R. asiaticus*. The weekly spraying of *Urtica* tea directly on the insects may result in upcoming effects that could not be attributed to a reduced aphid migration.

As predicted, the use of azadirachtin resulted in a reliable reduction of the aphid density in both trials. This was the case on the previously infested and as well as on the noninfested plants. This is in line with previous studies, already demonstrating the effect of azadirachtin on the aphids *Macrosiphoniella sanborni*, *Macrosiphum rosae* and *Brevicoryne brassicae* (Koul, 1999; Pavela et al., 2004). The experiments underline the efficacy of the plant protection product, in particular for the direct insecticidal effect. In the second period, the efficacy of the plant protectant was not as strong as that in the first experiment. In both previously infested and noninfested plants, a high number of aphids was able to develop. Due to the high complexity of the natural compound, the risk of an insect resistance to azadirachtin seems to be small (Kilani-Morakchi et al., 2021). Thus, the smaller success in controlling *M. euphorbiae* might be based on the aphid species, the host plant or their interactions.

Plants of the genus *Urtica* sp. are loaded with many different secondary plant metabolites such as lectins, phenols, flavonoids or alkaloids (Pinelli et al., 2008; Otles and Yalcin, 2012; Orčić et al., 2014; Kregiel et al., 2018). Regarding plant metabolites, the group of lectins shows promise as agents for plant protection. Vandenborre et al. (2011) discussed different modes of action of plant lectins, such as binding on glycoproteins, which results in negative effects in the insect midgut. Lectins are shown to disrupt larval development by exercising adverse effects on their survival, weight, feeding ability and pupation (Vandenborre et al., 2011; War et al., 2012). Additionally, adult stages of *Callosobruchus maculatus* were affected with a reduced oviposition rate (Sadeghi et al., 2006). In the case of a leaf lectin of *Allium sativum*, the *A. sativum* leaf agglutinin (ASAL), studies with transgenic plants have already shown an insecticidal effect on a broad spectrum of insects, such as *Aphis craccivora*, *Myzus persicae*, *Nilaparvata lugens* and *Nephotettix virescens* (Dutta et al., 2005; Saha et al., 2006; Chakraborti et al., 2009). Furthermore, an antifungal effect of aqueous and alcoholic nettle extract has been demonstrated in several studies (Hadizadeh et al., 2009; Nabrdalik and Grata, 2015).

For achieving effectiveness, in terms of insecticidal effects, of the secondary metabolites applied for biological control of plant pests, it is necessary to ensure their appropriate content and composition as well as fibre content and other characteristics, and also essential is the proper preparation of the extracts. However, these characteristics vary depending on the biotic and abiotic environmental influences, in particular habitat and season (Koczka et al., 2015; Kőszegi et al., 2020; Repajić et al., 2021, Yigit et al., 2021). Furthermore, Koczkodaj et al. (2023) reported a high variability of secondary metabolite content within and between different populations in Poland without a strict correlation with the geographical location. Moreover, the content of different plant metabolites, for example phenols or flavonoids, differs within the aerial parts of *Urtica* sp. (Pinelli et al., 2008; Otles and Yalcin, 2012). Thus, this knowledge can be used when preparing the extract of *Urtica* sp.

Although *Urtica* sp. has been used as plant protectant and strengthener for a long time, mostly in organic farming and private gardens (Mamun and Ahmed, 2011; Garmendia et al., 2018), less is known about the effect and the efficacy of the treatment regarding insects and in particular aphids. Bozsik (1996) showed small and insignificant effects of an aqueous extract and a fermented product of *U. dioica* on the aphids *Hyalopterus pruni*, *Cryptomyzus ribis* and *Aphis spiraephaga* on *Prunus domestica*, *Ribes rubrum* and *Spiraea vanhuottei*. Gaspari et al. (2007) reported a reduced fecundity without a substantial lowering of the population increase of the aphid *M. persicae*. Additionally, their results suggest no direct adverse effect on the aphid (Gaspari et al., 2007). Furthermore, a small but significant reduction of the coleopteran pests of the genera *Algarobius* and *Amblycerus* on *Prosopis laevigata* and *Acanthoscelides obtectus* has been reported (Jovanović et al., 2007; González-Macedo et al., 2021). Concerning acari, the experiments comprised in the study of Dąbrowski and Seredyńska (2007), dealing with *Tetranychus urticae*, demonstrated a higher intensity of probing behaviour, as well as a significantly higher mortality, subsequent to the mites feeding on treated leaf discs of common bean. On the other hand, different studies reported no significant effect regarding plant protection. Thus, the treatment with an *Urtica* extract or fermentation was not successful for the insect pests *Plutella xylostella* and *Frankliniella occidentalis* and had also no negative effect on the beneficial *Orius laevigatus* (Attia et al., 2012; Bonsignore and Vacante, 2012; Cerda et al., 2017). Additionally, controversial results are reported concerning plant strengthening effects on *Brassica oleracea, Solanum tuberosum, Delosperma cooperi* and *Sedum rubrotinctum* (Garmendia et al., 2018; Prisa, 2019; Godlewska et al., 2020).

CONCLUSIONS

All in all, the knowledge on the efficacy of the basic substance *Urtica* sp. as an aqueous extract or a fermented product is insufficient. Often, ornamental pests have to be controlled completely, and owing to the fact of zero tolerance by the consumer, no threshold can be applied (Daughtrey and Benson, 2005). Additionally, aphids are vectors for different viruses in ornamentals and in particular in *R. asiaticus* (Turina et al., 2006; Hayahi et al., 2018).

Our study shows the potential of the basic substance to reduce aphids under practical conditions. However, the data also show differences in effectivity of the stinging nettle tea regarding the examined aphid species.

Thus, the effect of the basic substance has to be tested on other herbivory insects as well. Nevertheless, the use of *Urtica* sp. as well as other basic substances seems not to be able to replace conventional plant protectants in their entirety. Further investigations on the kind of usage and especially on the characteristics of the used raw materials need to be carried out. Finally, basic substances, and in particular extract of *Urtica* spp., may become part of a modern plant protection strategy (Romanazzi et al., 2022). This strategy must include the application of alternative products, climate control or sanitation, although further research would be required to derive a reliable approach.

FUNDING

This research was founded by the Ministry of Economy and Innovation, Free and Hanseatic City of Hamburg, Germany (project AZ 734.650.004/014B).

AUTHOR CONTRIBUTIONS

F.W., M.B. and M.R. contributed to conceptualisation. F.W. and J.P. contributed to methodology and investigation. F.W. performed formal data analysis, data curation and visualisation and wrote the first draft. F.W. and M.B. contributed to validation. F.W., J.P., M.B., C.B. and M.R. reviewed the final draft. C.B. contributed to funding acquisition and supervision. C.B. and M.R. contributed to project administration and resources.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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Received: December 21, 2022; accepted: July 14, 2023