

Aphicidal and antifeedant activity of *Synandropadix vermitoxicus* extracts against *Myzus persicae* on potato plants

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Research Article

Keywords: Aphididae, Araceae, botanical insecticides, electrical penetration graph, natural products, *Solanum tuberosum*

Posted Date: March 5th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-3997619/v1>

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Journal of Pest Science on February 20th, 2025. See the published version at <https://doi.org/10.1007/s10340-024-01849-z>.

Abstract

Potato crops are usually kept virus-free through intensive aphid vector control schemes that require multiple treatments with insecticides. The generalist aphid *Myzus persicae* developed different mechanisms of resistance to insecticides, and there is a need for sources of novel insecticides. *Synandropadix vermitoxicus* (Griseb.) Engl., an Araceae family native to the northwestern region of Argentina, Paraguay, and Bolivia has been locally described as having insecticidal properties against insect larvae and could be a potential source of new natural insecticides against aphids. We tested the antifeedant and aphicidal effects of two extracts from the tubers of *S. vermitoxicus*, ethanolic and hexanic, on *M. persicae*. First, we treated potato leaves with the extracts to assess their antifeedant effect by measuring host preference changes in *M. persicae*. Then, we evaluated its aphicidal effect by offering the extracts to aphids through artificial diets and the aphid probing behaviour by electrical penetration graph. We also analysed the extracts for the main classes of secondary metabolites. We found that both extracts have antifeedant effects, with the hexanic being the strongest and accordingly, aphid probing behaviour was affected on leaves treated with hexanic extract. While the ethanolic extract affected the survival of aphids fed on artificial diets, the hexanic extract did not. The analysis of *S. vermitoxicus* extracts shows an array of flavonoids and triterpenoids compounds. Therefore, our results show that the tubers of this plant could be a source for a novel product with potential use on the control of *M. persicae* on potato crops.

Key Message

- Extracts obtained from tubers of a native South American plant, *Synandropadix vermitoxicus*, have negative effects on aphids.
- While the ethanolic plant extract has insecticidal properties, the hexanic plant extract has a potent antifeedant effect.
- This plant could be a source of new natural insecticides with potential use to control aphids such as *Myzus persicae*, an important vector in potato crops.

INTRODUCTION

On potato crops, aphids are the most efficient vectors of viruses (Castle and Berger 1993; Salazar 1996) and therefore, they are one of the major target insects of insecticides treatments. The green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae) is a generalist cosmopolitan insect recognized as the mayor pest infesting potato crops (Kuroli and Lantos 2006; Hill 2008). *Myzus persicae* is a piercing–sucking insect that ingests plant phloem fluid through modified mouthparts, the stylets. To select the host plant, *M. persicae* follows a sequence of behavioural steps including from alighting to penetration of stylets in the plant tissue. This process of inserting their stylets between the cell walls of the epidermis and the mesophyll, and piercing parenchyma cells salivating and ingesting small amounts of saliva/cytoplasm mixture in the way to the phloem to test the cells content and presumably for cytoplasmic feeding (Tjallingii and Hogen Esch 1993; Martin et al. 1997; Alvarez and Griffing 2023), made aphids the most efficient vectors of plant viruses (Robert and Bourdi, 2001; Radcliffe and Ragsdale 2002). In the long process of first interaction, the assessment of physical and/or chemical cues can result in the rejection or acceptance of the host plant, and therefore, phytochemical information is particularly important in determining aphid behaviour at many stages of the host selection process (reviewed by Powell et al. 2006).

Potato crops are usually kept virus-free by means of intensive aphid control schemes that require multiple insecticides applications, but *M. persicae* developed different mechanisms of resistance to insecticides (Devonshire and Field 1991; Moores et al. 1994; Devonshire et al. 1998; Martinez-Torres et al. 1999; Foster et al. 2000; van Toor et al. 2008; Silva et al. 2012; Shi et al. 2023). For instance, on a sample of 72 lineages of *M. persicae* collected from potato fields in 31 sites in New Zealand, 60% contained mechanisms of resistance to one or more of the four classes of insecticides tested, i.e., organophosphates, carbamates, pyrethroids and neonicotinoids (van Toor, et al. 2008). Although *M. persicae* was occasionally registered as resistant to neonicotinoids, potato crops and especially seed crops, are commonly treated with the neonicotinoid imidacloprid (van Toor et al. 2008). Moreover, the exposure to sublethal concentrations of imidacloprid, sulfoxaflor and azadirachtin was found to stimulate reproduction and fecundity in *M. persicae* in a phenomenon called hormesis (Cutler et al. 2009; Ayyanath et al. 2013; Ullah et al. 2019; Wang et al. 2023).

The increased use of synthetic insecticides produces environmental impacts that are unforeseen at the time of their introduction, including poisoning of humans; destruction of wildlife; disruption of natural biological control and pollination; soil and groundwater contamination, and the evolution of resistance to pesticides in pest populations (Isman 2006; Ekstr and Ekbohm 2011). As an

alternative strategy to control aphids with less negative impact to environment and health, research has focused on searching for substitutes for hazardous insecticides among natural products such as botanical secondary metabolites (Isman 2000; Carlini and Grossi-de-Sá 2002; Liu et al. 2014; Martin et al. 2024) to develop botanical insecticides as biopesticides. A low proportion of the biopesticides with insecticide action available in US (11 out of 85, 13%) and in Europe (3 out of 20, 15%) came from plant origin (Bailey et al. 2010). Isman (2015) stated that even though plants produce a wide variety of metabolites to deter herbivores, countries like Brazil, India, or China, have an enormously diverse and unexplored flora to discover and develop new botanical insecticides. Also, in Argentina there are vast ecoregions holding new plant sources that have been reported as potential bactericidal, fungi-static and insecticide agents (Delbón et al. 2007; Palacios et al. 2007; Sosa and Tonn 2008; Silva et al. 2012; Del Corral et al. 2014; Díaz Napal et al. 2016; Sosa et al. 2019).

Synandropadix vermitoxicus (Griseb.) Engl. is a plant of the Araceae family, tribe Spathicarpae, genera *Synandropadix*, autochthonous from the northwestern region of Argentina, Paraguay, and Bolivia. There are records describing that a solution prepared from the tubers of *S. vermitoxicus* is applied topically on animals' injuries to protect them against larval insect pests (Bogner and Nicolson 1988; Novara 1993; Gonçalves et al. 2007; Arenas 2016), however this plant potential as source of new insecticides has not been study. Here, we tested the effects that two *S. vermitoxicus* extracts, ethanolic and hexanic, have on the host preference and survival of aphids. Plant extracts were obtained from successive steps of extraction of natural compounds with different organic solvents. We treated potato leaves with the extracts to evaluate its antifeedant effect by measuring the changes on *M. persicae* host preference. Then we evaluated the extracts aphicidal activity by offering the extracts to aphids through artificial diets and studied the probing behaviour by electrical penetration graph (EPG). Finally, we analysed the *S. vermitoxicus* extracts composition looking for the principal classes of secondary metabolites with biological activity i.e alkaloids, terpenoids, and phenolics.

MATERIALS AND METHODS

Botanical extracts

Plants of *Synandropadix vermitoxicus* (Griseb.) Engl were collected in December 2011, at Coronel Moldes, Salta, Argentina (25°14'21.1'' S, 65°25'41.7'' W). A voucher sample was deposited at the Museum of Natural Science of Salta, Argentina (number 11904-MCNS) (Fig. 1).

Fresh tubers were cut into 2–3 cm cubes (total weight 6 kg) and were macerated with ethanol at room temperature for 24 h, then the solvent was evaporated at reduced pressure to yield 50 g of ethanolic extract (Sv-E). A 20 g fraction of Sv-E was suspended in ethanol-water (3:1) solution and extracted successively with hexane (3 x 300 ml) to yield 1.092 g of hexanic extract (Sv-H) (Fig. 2). Both extracts were kept at 4°C until use in biological assays and to test for presence of flavonoids, alkaloids, sugars. The Sv-E extract (200 mg) was suspended in 96% ethanol to assess the preliminary phytochemical analysis for flavonoids and alkaloids.

The presence of flavonoids was assessed by the Shinoda's test. Metallic magnesium (0.1 g) was added to 5 mL of the Sv-E extract followed by a few drops of concentrated hydrochloric acid; a reddish, violet, or orange colour indicates the presence of flavonoid (Lock de Ugaz 1994; Carvajal et al. 2008).

The presence of alkaloids was evaluated by three tests: (1) Dragendorff's reagent (Iodine in potassium bismuth iodide), (2) Meyer's reagent (potassium mercuric iodide solution), and (3) Warner's reagent (Iodine in potassium iodide). Briefly, a solution of 5% HCl is added to 5 mL of Sv-E extract. Then, four drops of each reagent are added to 1 mL of this solution. For the Dragendorff's reaction a yellow precipitate indicates the presence of alkaloids, while for the two other reagents a white precipitate indicates its presence (Lock de Ugaz 1994; Wagner and Blandt 1996).

Purification of Sv-H extract

The hexanic extract (1.092 g) was fractionated by silica gel flash chromatography and eluted with a gradient of increasing polarity of CH₂Cl₂ and mixtures of CH₂Cl₂:CH₃OH to reach 95 fractions which were combined according to its similar profiles in thin layer chromatography analyses (TLC), visualized with UV-light, vanillin solution and sulfuric acid:ethanol mixture (1:4). Fractions eluted with CH₂Cl₂:CH₃OH (3:0.5) were purified on TLC with CH₂Cl₂:CH₃OH mixtures of different polarities to yield compounds number 7 and a mixture of 8 and 9, selected for analysis by 1H NMR.

Analysis of plant extracts by spectrometry (NMR) and chromatography (HPLC)

NMR spectra were recorded on a Bruker AVANCE II AV-400 operating at 400.13 MHz for ¹H. Chemical shifts are given in ppm (δ) downfield from the TMS internal standard. Qualitative and quantitative analyses of sugars were carried out on an Agilent 1100 System, with an Agilent Hi-Plex Ca (Dúo) column, 300 x 6.5 mm (PL1F70-6850) 80°C. The injection volume for Sv-E extract (2 mg in 200 μ L Milli-Q water) and the standard was 10 μ L, and the separation was carried out at a flow rate of 0.4 mL min⁻¹, and eluent of 100% Milli-Q water. Sugars were detected by refractive index at 30°C (Ball and Lloyd, 2011) using standards of sucrose, glucose, and fructose (Sigma®).

Qualitative and quantitative analyses of flavonoids were carried out on an Agilent 1100 System N° G1365, with an Eclipse XDB-C18 column. The sample was prepared as follows: 2 mg of dry Sv-E was dissolved on 200 μ L of methanol/hexane, 1:0.5 mL, v/v, then, it was centrifuged 10 min at 13.000 rpm. The hexane layer was discarded, and the methanol fraction was dried completely by rotavap. Sample was then dissolved for analysis in 200 μ L methanol (modified after Torres et al. 2005). The extract and standard injection volume were 5 μ L, and separation was carried out at a flow rate of 1 mL min⁻¹, using a mobile phase of water/acetic acid of 99.9:0.1, v/v; and a linear gradient of 15%-36.5%, 30 min of acetonitrile/acetic acid (99.9:0.1, v/v). Flavonoids were detected at 270 nm using standards of galic acid, chlorogenic acid, catechin, rutin, ferulic acid, quercetin, naringenin and kaempferol (Sigma®). All eluents used were of HPLC grade (Sintorgan). Sugar and flavonoid contents were quantified on the basis of standard curves constructed using area ratio of respective standard. Least-squares linear regression was used for this purpose.

Insects and plants

Myzus persicae Sulzer colonies were reared on radish (*Raphanus sativus* L). Aphids used in the experiments came from a colony maintained at the Faculty of Natural Science, National University of Salta, Argentina. This colony was initiated from a single virginoparous apterous individual collected in field in 2009. The colony was reared in a climate chamber at 22 $\pm$ 2°C, 30%-40% RH, and 16L:8D h photoperiod to induce parthenogenesis. A new colony was started every week, and newly moulted apterae adult aphids were used for the experiments.

Potato plants of *Solanum tuberosum* (L) cultivar PO 97.11.9 were provided by the germplasm bank of INTA-EEA Balcarce, (Balcarce, Buenos Aires, Argentina). Plants were propagated *in vitro* on Murashige and Skoog with vitamins, 3% sucrose w/v, pH 5.8. After two weeks on agar, the plantlets with developed roots were transferred to soil in 500 g plastic pots and maintained in a chamber at 22 $\pm$ 2°C and 16L:8D h photoperiod.

Choice test to evaluate antifeedant activity

Antifeedant activity of the extracts was evaluated by studying the aphid's preference over potato leaves treated with Sv-E or, Sv-H at different concentrations vs. control leaves, with a choice test. Choice tests were carried out in plastic cages, 100 mm diameter and 50 mm high, with four 15 mm diameter lateral ventilation holes sealed with non-woven mesh. The cage was closed at the top and bottom by a removable polystyrene Petri dish. The top Petri dish had a layer base of water-agar 1.5% (to prevent the leaves from dehydration) over which four leaves previously treated on the abaxial side, were attached in cross by its adaxial side to the agar. Then, to support the leaves, the dish containing the leaf was covered with a plastic lid with four 15 mm diameter holes to expose the leaves and finally placed on top of the cage (Lucatti, 2014) (Fig. 3). The treatments were: (A) Treated potato leaves sprayed with Sv-E extract at concentrations of 2.5, 5 and 10 mg/mL in a solution of Tween-20 at 0.05% v/v in water (emulsifier solvent); (B) Treated potato leaves sprayed with Sv-H extract previously diluted with acetone 5% at concentrations of 2.5, 5 and 10 mg/mL in a solution of Tween-20 at 0.05% v/v in water. For treatments (A) and (B) the control were leaves treated with each of the solvents respectively; and (C) Blank leaves, which consisted of natural untreated leaves. Blank treatment was included to test the effect that the Tween-20, 0.05% solution used as emulsifier has on aphids. Treated leaves were sprayed with 100 μ L of the corresponding solution and air dried for 60 minutes to allow the evaporation of the solvent. Then, on each cage, two equally treated leaves were placed opposite and on cross side to two control leaves (Fig. 3a and 3b). Forty recently molted apterae adults of *M. persicae* were gently placed with a paintbrush at the centre of a polystyrene lid (arena) that was immediately used to close the plastic cage from the bottom. The number of aphids settled on each treated leaf was recorded at 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 180 and 240 minutes after the start of the experiment. Each treatment was repeated (biological replicates) 12–14 times for Sv-E, and 8 times for Sv-H. The experiment was performed in room under controlled conditions at 22 $\pm$ 2°C, 30%-40% RH, and normal light.

A settling inhibition index (%SI) was calculated for all the treatments at each time scored as $\%SI = [1 - (\%T/\%C)] \times 100$, where %T is the percentage of aphids on the treated surface, and %C is the percentage of aphids on the control surface (Gutiérrez et al. 1997). For those cases where the control had lower values than the extracts treatments (then the negative values represent attraction for the extracts treatments, and the index can range from -1 to -100), the index was modified as $\%SI = \{[1 - (\%C/\%T)] \times 100\} \times -1$.

Toxicity trial to evaluate aphicidal activity

The aphicidal activity of Sv-E, Sv-H on *M. persicae* was assessed by measuring the toxic effect over adult aphids and their nymphs maintained on artificial diet containing the Sv-E or Sv-H extracts. Three concentrations of each extract, 50, 100 and 1000 µg/mL, were administrated through aphid's artificial diets (150 mM amino acids, 500 mM sucrose, vitamins, and minerals (diet composition modified by Douglas after Prosser and Douglas 1992). The control treatment consisted of aphids fed on artificial diet without the extracts. Five recently moulted apterae adult aphids were confined on plastic cages (3 cm diameter and 2 cm high) sealed on top with a diet sachet containing 100 µL of diet solution between two layers of Parafilm, and sealed at the bottom with a mesh (Fig. 3c and 3d) (Prosser and Douglas 1991; Koga et al. 2007; Machado-Assef et al. 2015). Aphids were placed on the diet cages (14 and 15 replicates per control and treatments respectively) and maintained during the experiment in a climate chamber at $22 \pm 2^\circ\text{C}$, 30%-40% RH, in darkness. After 96 h of treatment on artificial diets, the adult survival and the total number of offspring produced were counted per cage, then the mean number of nymphs per adult were calculated.

EPG to evaluate the probing behaviour

The EPG technique (McLean and Kinsey 1964; Tjallingii, 1978, 1985a,b, 1987) was used to monitor the probing behaviour of adult *M. persicae* aphids on potato leaves treated with Sv-E or Sv-H extracts. Two leaves per plant was sprayed with 1 mL each of Sv-E or Sv-H extract, previously diluted with acetone 5%, at a concentration of 10 mg/mL in a solution of Tween-20 at 0.05% v/v in water (emulsifier solvent). The control were leaves treated with the solvents and the blank leaves consisted of natural untreated leaves. Blank treatment was included to test the effect that the emulsifier solvent has on aphids. After sprayed on both sides, treated leaves were air dried for 60 minutes to allow the evaporation of the solvent. A Giga-8 EPG-DC amplifier (EPG Systems, Wageningen, The Netherlands) was used to monitor eight wingless wired aphids placed on the stem of the plant, one per plant was recorded simultaneously for 4 h. The number of replicates obtained was 10. Signals were acquired and analysed using Stylet + software (EPG Systems).

During probing (periods where the insect is with the stylets inside the plant tissue, calculated without distinction of activities), six activities (EPG waveforms) were distinguished, and related to: (1) stylet pathway (C), reflecting the first electrical stylet contact with the epidermis, intercellular sheath salivation in epidermis and mesophyll, and extracellular stylet penetration movements in all plant tissues; (2) potential drop (pd), reflecting brief intracellular stylet punctures during C; (3) derailed stylet mechanics (F), reflecting stylet penetration difficulties; and (4) xylem ingestion (G), reflecting active sap ingestion from xylem elements. The phloem phase appears after an abrupt voltage drop caused by the intracellular penetration of sieve elements (SE) by the stylet tips and includes (5) SE salivation (E1), and (6) phloem sap ingestion (E2) with concurrent salivation (Tjallingii 1978b). An event was considered as an uninterrupted period of a waveform. A number of 127 standardized EPG variables were calculated automatically using an EXCEL Data Workbook CSIC-UAL elaborated by Garzo E. (Institute of Agricultural Sciences, CSIC, Spain) and Alvarez A.J. (University of Almería, Spain). The variables related to non-probing, probing, pathway, and phloem fall into five categories: (1) 'number of', mean number of times that an activity occurred per insect, (2) 'mean duration of', mean duration of an activity per insect per event, (3) 'total time on', total time on an activity per insect, (4) 'time to', mean time to the first occurrence per insect of an activity from the start of the experiment, and (5) 'proportion of', number or percentage of aphids that showed a particular activity.

Statistical analysis

In the choice test results, the differences between extracts treatments and control, and control vs. blank were analysed by the Binomial test, assuming the null hypothesis of no preference for the treatments; the analyses were performed using SISA online statistical analysis (<https://www.quantitativeskills.com/sisa/distributions/binomial.htm>) (Uitenbroek 1997).

The %SI were analysed by Kruskal–Wallis nonparametric analysis of variance (ANOVA), $P \leq 0.05$, at one way of classification, followed by multiple comparisons with Conover test between means of treatments with Bonferroni's correction ($P \leq 0.008$.) The toxicity of the extract was analysed by means of adults' survival and their nymphs' survival, using the Kruskal–Wallis ANOVA, $P \leq 0.05$ followed by multiple comparisons with Conover test with Bonferroni's correction ($P \leq 0.002$) (Weisstein 1999).

The dose that kills 50% of the aphids in the population (LD50) of each extract was obtained using GraphPad Prism 6.01 (Swift 1997). To do so, an inhibition graph was made indicating the percentage mortality of *M. persicae* adults as a function of dose concentration.

The EPG variables were analysed with a Mann-Whitney U test, with $P < 0.05$. The Fisher's exact test was used to evaluate the difference in proportions of individuals performing each type of activity. The statistical analyses were performed using Infostat Profesional v2015 (<http://www.infostat.com.ar>) (Di Rienzo et al. 2015).

RESULTS

Analysis of *S. vermitoxicus* botanical extract

The qualitative analysis of the *Sv-E* extract using the Shinoda test revealed the presence of flavonoids that were subsequently determined and quantified by HPLC. The compounds found were, naringenin (0.01 mg/g) (1), catechin (0.274 mg/g) (2), quercetin (0.006 mg/g) (3), rutin (0.068 mg/g) (4), gallic acid (0.001 mg/g) (5); chlorogenic acid (0.048 mg/g) (6) (Fig. 4a). Also, a HPLC of *Sv-E* was performed to quantify the sugars. The sugars found on mg. g⁻¹ were, sucrose 5.419; glucose 8.268; fructose 6.954. The *Sv-E* extract did not show presence of alkaloid with any of the three qualitative methods, i.e., Dragendorff's, Meyer's, and Warner's reagents.

The *Sv-H* extract of *S. vermitoxicus* was fractionated with a combination of chromatographic techniques, showing the known triterpene alcohols compounds β -sitosterol (7), and a mixture of 24-methylenecycloartanol (8), and cycloartanol (9) (Fig. 4b). The ¹H-NMR spectrum of β -sitosterol (Fig. 5a) showed a doublet signal for one olefinic methine proton at δ 5.35 ppm ($J = 4.4$ Hz), and a multiplet signal at δ 3.53 corresponding to H-6 and H-3 respectively. Also, the spectrum showed six methyl signals at δ 1.00 (s, H-19), 0.93 (d, $J = 6.7$ Hz, H-21), 0.85 (t, $J = 7.0$ Hz, H-29), 0.84, 0.82 (two doublets, $J = 7.0$ Hz, H-26, H-27) and 0.69 (s, H-18).

In the ¹H-NMR spectrum shown in Fig. 5b, the compounds 8 and 9 were identified in a 1:1 mixture. The compound 24-methylenecycloartanol (8), was characterized by two singlet signals at δ 4.68 and 4.73 corresponding to the olefinic methylene protons (H-28), the multiplet signal at δ 3.30 (H-3), the methyl signals at δ 1.04, 1.02 (d, $J = 2.2$ Hz, H-26, H-27), 0.98 (s, H-30, H-31), 0.91 (s, H-29), 0.90 (d, $J = 6.6$ Hz, H-21), 0.82 (s, H-18) and the methylene protons of cyclopropane at δ 0.33 and 0.55 ppm (H-19). Some signals in the spectrum were superimposed to the cycloartanol (9), i.e., H-19, H-3, C-4-methyl groups and H-18, the others methyl signals corresponding to cycloartanol were observed between δ 0.86 and 0.89. All the compounds were characterized by comparing their ¹H NMR spectral data with literature (Suttiarporn et al. 2015).

Choice test to evaluate antifeedant activity

The preferences of aphids for *Sv-E* and *Sv-H* treated leaves vs. control leaves, or control leaves vs. blank untreated leaves performed with a choice essay are shown in Fig. 6. In the choice test between control leaves vs. *Sv-E* and *Sv-H* treated leaves, aphids preferred control leaves over *Sv-E* and *Sv-H* treated leaves at all concentrations throughout the experiment (Fig. 6). Also, aphids preferred blank leaves over control leaves throughout the experiment, except after 240 min of experiment started for control of *Sv-E* and at 5 min of control of *Sv-H* respectively (Fig. 6, control vs. blank).

Tables 1 and 2 shows the %SI index calculated for *Sv-E* and *Sv-H* treated leaves at different concentrations. Each scored time was contrasted with the %SI for control vs. blank, and the effect was significant at about 15 min of starting the experiment. Figure 7 depicts the results on box plots, the leaves treated with *Sv-E* at a concentration of 10 mg/mL, and with *Sv-H* at 5 and 10 mg/mL had the strongest effect over aphids; in these treatments the %SI medians are above the 75% threshold value defined by Gutiérrez et al., (1997).

Table 1 *Myzus persicae* settling behavior inhibition (%SI). %SI are on control leaves vs blank untreated leaves at each time, and %SI on leaves treated with *Synandrosadix vermitoxicus* ethanolic extract (Sv-E) at different concentrations vs control at each time during the preference test.

Time	Control vs Blank		Sv-E (mg/mL) vs Control ^a						H ^b	P ^c
min	n	n	Sv-E 2.5	n	Sv-E 5	n	Sv-E 10			
5	14	32 ± 13	14	58 ± 11	13	40 ± 12	14	67 ± 11	7.55	0.056
10	14	31 ± 12	14	62 ± 7	13	52 ± 13	14	78 ± 6	10.16	0.017
15	14	40 ± 9 a	14	63 ± 6 a	13	48 ± 12 a	14	84 ± 5 b	14.72	0.002 *
20	14	46 ± 7	14	61 ± 7	13	54 ± 13	14	81 ± 5	11.35	0.010
25	14	43 ± 8 a	14	69 ± 8 ac	13	54 ± 11 ab	14	83 ± 4 c	15.44	0.002 *
30	14	34 ± 10 a	14	67 ± 9 bc	13	46 ± 13 ab	14	81 ± 6 c	14.79	0.002 *
45	14	39 ± 9 a	14	68 ± 7 bc	13	55 ± 11 ab	14	81 ± 8 c	14.6	0.002 *
60	14	39 ± 10 a	14	67 ± 6 ab	13	56 ± 12 a	14	78 ± 8 b	13.13	0.004 *
90	14	28 ± 11 a	14	69 ± 5 b	13	54 ± 11 ab	14	86 ± 4 c	22.2	<0.001 *
120	14	27 ± 12 a	14	65 ± 7 b	13	61 ± 10 b	14	79 ± 6 b	14.47	0.002 *
180	14	30 ± 10	14	64 ± 5	12	64 ± 6	14	68 ± 8	10.53	0.015
240	14	9 ± 13 a	14	52 ± 6 b	12	42 ± 10 ab	14	74 ± 6 c	17.3	0.001 *

^aValues are means ± SEM. ^bH, Kruskal-Wallis non parametric ANOVA, P≤0.05, followed by multiple comparisons between means of treatments with Bonferroni correction; values in a row with different letters indicate significant differences at P≤0.008

Table 2 *Myzus persicae* settling behavior inhibition (%SI). %SI are on control leaves vs untreated leaves at each time, and %SI on leaves treated with *Synandrosadix vermitoxicus* hexanic extract (Sv-H) at different concentrations vs control at each time during the preference test.

Time	Control vs Blank		Sv-H (mg/mL) vs Control ^a						H ^b	P ^c
min	n	n	Sv-H 2.5	n	Sv-H 5	n	Sv-H 10			
5	12	22 ± 21	14	43 ± 20	8	60 ± 25	13	60 ± 21	3.89	0.232
10	14	34 ± 15 a	15	37 ± 14 a	15	70 ± 10 b	15	78 ± 9 b	11.82	0.007 *
15	14	38 ± 14 a	15	48 ± 11 a	15	69 ± 13 b	15	89 ± 4 b	18.12	0.000 *
20	14	43 ± 13 a	15	50 ± 9 a	15	86 ± 7 b	15	84 ± 5 b	21.09	<0.001 *
25	14	38 ± 13 a	15	55 ± 10 a	15	84 ± 6 b	15	86 ± 3 b	20.15	<0.001 *
30	14	34 ± 14 a	15	58 ± 8 a	15	90 ± 2 b	15	84 ± 5 b	22.04	<0.001 *
45	14	39 ± 12 a	15	60 ± 7 a	15	84 ± 4 b	15	86 ± 4 b	21.53	<0.001 *
60	14	40 ± 10 a	15	58 ± 8 a	15	84 ± 4 b	15	85 ± 3 b	21.64	<0.001 *
90	14	33 ± 12 a	15	64 ± 5 a	15	83 ± 4 b	15	87 ± 4 b	25.24	<0.001 *
120	14	38 ± 11 a	15	65 ± 5 a	14	82 ± 6 b	15	88 ± 3 b	26.22	<0.001 *
180	14	32 ± 11 a	15	62 ± 6 a	14	86 ± 3 b	15	88 ± 3 b	32.09	<0.001 *
240	14	26 ± 11 a	15	61 ± 7 a	14	86 ± 4 b	15	90 ± 3 b	34.87	<0.001 *

^aValues are means ± SEM. ^bH, Kruskal-Wallis non parametric ANOVA, P≤0.05, followed by multiple comparisons between means of treatments with Bonferroni correction; values in a row with different letters indicate significant differences at P≤0.008

Table 3 Aphicidal effect in *M.persicae* of *Synandropsadix vermitoxicus* ethanolic (Sv-E) and hexanic (Sv-H) extracts, incubated 96 h on artificial diets.

Treatment	n	Adults alive	Nymphs alive
Control	14	4.71 ± 0.16 b	51.43 ± 4.84 c
Sv-E 50 µg/mL	15	4.47 ± 0.32 b	42.73 ± 6.71 bc
100 µg/mL	15	4.00 ± 0.50 b	34.87 ± 5.64 b
1000 µg/mL	15	2.60 ± 0.41 a	12.00 ± 3.72 a
Sv-H 50 µg/mL	15	4.67 ± 0.19 b	49.00 ± 5.11 bc
100 µg/mL	15	4.53 ± 0.19 b	43.27 ± 5.01 bc
1000 µg/mL	15	4.40 ± 0.24 b	41.00 ± 5.28 bc

Numbers are means (± SEM) of aphids, adults and nymphs. Statistics, Kruskal-Wallis non parametric ANOVA at $P \leq 0.05$, followed by multiple comparisons between means of treatments with Bonferroni correction at $P \leq 0.002$; values in a column with different letters indicate significant

Toxicity trial to evaluate aphicidal activity

The toxicity of Sv-E and Sv-H was evaluated used artificial diets containing three different concentrations of the extracts, i.e. 50, 100 and 1000 µg/mL (Table 3). Aphid adult survival after four days' treatment was significantly affected on diets containing Sv-E at 1000 µg/mL compared to control diet (mean number of adults were 2.6 and 4.7 respectively, $H = 16.09$ $P = 0.001$). The number of nymphs significantly decreased on diets containing Sv-E at 100 and 1000 µg/mL of compared to control diet (mean number of nymphs were 34.9, 12.0 and 51.4 respectively, $H = 27.42$, $P = 0.0001$). The aphid survival, adults and nymphs on Sv-H extract diets did not differ from control at any concentration (Table 3). adults; nymphs,

The LD50 of the Sv-E extract was 1,300 ug/ml with a 95% confidence limit of 657.8-2,571 ug/ml (Fig. 1S); for the Sv-H extract it was not possible to calculate the LD50 value since there was no toxic effect in the evaluated doses (Table 3).

EPG to evaluate the probing behaviour

The analysis of the feeding behaviour of *M. persicae* on plants during 4 h of EPG recording showed that aphids on control plants differed from aphids on plants treated with Sv-H extract in the number of probes, mean duration of probing, number of short probes, mean duration of derailed stylet mechanics, time to first probe, total duration of no probing before first E, number of probes and brief probes after first E, and percentage of probes with at least one pd (%_Pr_pd, 61.56 ± 2.8 and 74.63 ± 5.4 , control mean and hexenic mean respectively, $W=74.5$, $P=0.02$) (Table 1S, Table 2S supplementary information and Fig. 8). With respect to the effect of the solvents of the extracts, the feeding behaviour of aphids on control plants differed from aphids on blank plants only in the duration of the first probe; and from aphids on plants treated with Sv-E extract differed only in the number of pd in the first probe (Table 1S).

Figure 8 Probing behaviour of *M. persicae* aphids on potato plants during 4 h electrical penetration graph (EPG) monitoring. Sv-H were leaves treated with *S. vermitoxicus* hexanic extract at a dose of 10 mg/mL in an emulsifier solvent of Tween-20 at 0.05% v/v in water. Control were leaves treated with the solvents. Error bars represent the standard error of the mean, $n = 10$. (a) Number of probes, n_Pr; number of short probes, n_bPr; number of probes after first E, n_Pr.after 1E; number of brief probes after first E, n_bPr.after 1E (b) mean duration of probing, a_Pr; mean duration of derailed stylet mechanics, a_F; time to first probe t > Pr; total duration of no probing before first E, s_np.1E. Asterisk are based on Mann-Whitney U test for paired samples, $P \leq 0.05$.

DISCUSSION

Our results revealed three facts regarding the botanical extract of *S. vermitoxicus* over *M. persicae* aphids, (1) the aphids challenged with *S. tuberosum* leaves treated with Sv-E or Sv-H preferred the control leaves, although when challenged with control leaves vs. blank untreated leaves they preferred the blank; (2) aphids feeding on artificial diets supplemented with Sv-E had reduced survival compared with the control, while aphids feeding on the diet supplemented with Sv-H did not and; (3) aphid probing behavior was affected on Sv-H-treated leaves.

In the choice-test, we found that both extracts had antifeedant effect on *M. persicae*, and this effect increases with concentration, the leaves treated with Sv-E or Sv-H at 5 and 10 mg/mL had the strongest effects on the settling of aphids, it started after 5 min and throughout the 240 min of the experiment (Fig. 6). Overall, the antifeedant effect of Sv-H is stronger than that of Sv-E (Fig. 7), therefore the antifeedant effect increased with the extraction process (Tables 1 and 2). The settling inhibition index (%SI) of *M. persicae* in potato leaves treated either with Sv-E or Sv-H, shows that both extracts affected the settling behaviour compared to the control. Aphids were able to perceive the compounds and reacted sensitively to some –or a blend of – compounds, present in these extracts. On leaves treated with Sv-H at 10 mg/mL throughout the experiment after 10 min even the lowest %SI value obtained was above the %SI threshold of 75%, which is considered as an effective settling inhibition (Gutiérrez et al. 1997).

The reduced survival of adults and nymphs of aphids feeding on artificial diets containing the Sv-E suggests that there is a detrimental effect at post ingestion level on aphids, referred to as long-term effects involving various aspects of digestion and absorption of food. Our results show that Sv-E include an array of flavonoids, the presence of terpenoids and sugars; also show that these crude blends are bioactive against *M. persicae*. Further activity-guided fractionation studies of each compound are needed to identify the active compounds and to study the synergy of the additive effect of each component of the blend.

In addition, our results show that the Sv-E has a negative antifeeding effect on aphids, interestingly this extract has a blend of naringenin (1) (flavanone) and quercetin (3) (flavonol) (Fig. 4a), Golawska et al. (2014) found that both flavonoids increase the pre-reproductive period, decreased fecundity, increased mortality, and impeded the normal feeding behaviour of *Acyrtosiphon pisum*. Flavonoids are secondary metabolites of mayor importance in the interaction of plants with other organisms; particularly insects can discriminate between the different flavonoids' profiles (Simmonds 2001). Also, studies on the feeding behaviour of aphids on artificial diets (with electrical penetration graph monitoring, EPG) found that flavonoids luteolin and genistein have antifeedant effect on the pea aphid *Acyrtosiphon pisum* (Golawska and Lukasik 2012).

The probing behaviour of *M. persicae* on Sv-H treated plants was affected, the time to first probe for aphids on leaves treated with Sv-H was increased to approximately 26 min, which is six-fold greater than in control aphids. Also, the number of probes was reduced (Fig. 8). This behaviour reflects the effects of deterrent stimuli at surface level that may help to prevent the transmission of non-persistent virus (Ragsdale et al. 2001). Interestingly, aphids on Sv-H-treated leaves showed a lower number of probes but of longer duration. Probing activities include all activities when the styles are inside the plant tissues, even mechanical stylet derailments. Thus, the longer duration of mechanical problems presumably increased the duration of the probing time, but with a negative impact on overall normal probing behaviour.

The analysis of Sv-E by spectroscopy shows the presence of triterpenoids (Fig. 4b), β -sitosterol (7) is among the most abundant phytosterols found in plants (Piironen et al. 2000), and 24-methylenecycloartanol (8) and cycloartenol (9) are precursors for plants synthesizing and accumulating significant amounts of steroids (Piironen et al. 2000; Szakiel et al. 2017). The 24-methylenecycloartanol (8) and cycloartenol (9) were identified in several plant genera including *Cheilocostus* (Kumar et al. 2018), *Dioscorea* (Szakiel et al. 2017), *Euphorbia* (Giner et al. 2000; Haba et al. 2007), *Epidendrum* (Rosa et al. 2007), *Ficus* (Nair et al. 2020), *Oryza* (Ohta and Shimizu 1958; Narumi and Takatsuto 2000) and several genera of the Solanaceae family (Itho et al. 1977); although the biological activities described for these triterpenoids are related to effects in human health (Suttiarporn et al. 2015; Jolfaie et al. 2016), phytosterols act as substrates for a wide variety of secondary metabolites such as, glycoalkaloids, cardenolides and saponins (Piironen et al. 2000), e.g. spinasterol and 22, 23-dihydrospinasterol from plant resources, have effective aphicidal activity against the aphids *Brevicoryne brassicae* L. (Ahmed et al. 2020). Overall, the effect of triterpenoids on aphids requires further analysis.

Considering that the major hazards that *M. persicae* aphids can do to potato plants are related to its ability to transmit viruses, then a product with antixenotic (non-preference) and repellence type of action will be desirable to prevent virus transmission. Once the aphid lands on a plant, to avoid virus transmission, a product that modifies the behaviour in the sense avoiding the probing of plant tissue by 'repellence and deterrents' (antifeedants) will be desirable for aphid management in potato crops. Taking all together, we found that both extracts, Sv-E and Sv-H, have a potent antifeedant effect against *M. persicae*, and that the Sv-E extract, but not Sv-H, has a strong aphicidal effect that reduces aphid survival. Our results show that the toxic effect found in the first extraction, i.e., Sv-E, was not present after fractionation with hexane, viz., in the Sv-H extract, and therefore, next studies should be focused on the aqueous extract. Finally, we conclude that *S. vermitoxicus* extracts have strong bioactive components against aphids that merit further analysis.

Declarations

AUTHOR CONTRIBUTION

GLI, MLU and AEA conceived research. GLI conducted experiments. CMA, MGR and MLU contributed material and analysed data. GLI and CMA analysed data and conducted statistical analyses. GLI and AEA wrote the manuscript. AEA and MLU secured funding. All authors read and approved the manuscript.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any conflict of interest.

Data Availability Statement

The data that support the findings of this study are openly available in [Zenodo], Version 1. <http://doi.org/10.5281/zenodo.4448037>

ACKNOWLEDGEMENTS

This research was financially supported by Consejo de Investigación de la Universidad Nacional de Salta (project 1967) and CONICET (doctoral fellowship program). We thank the valuable support of Dr. Marcelo Huarte and Dra. Olga Martinez. We thank Dr. Pablo Ortega Baes for his suggestions and ideas. We are grateful to Arq. Lilian Maroto and Arq. Luis Novoa for their valuable contribution to the illustrations.

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Figures

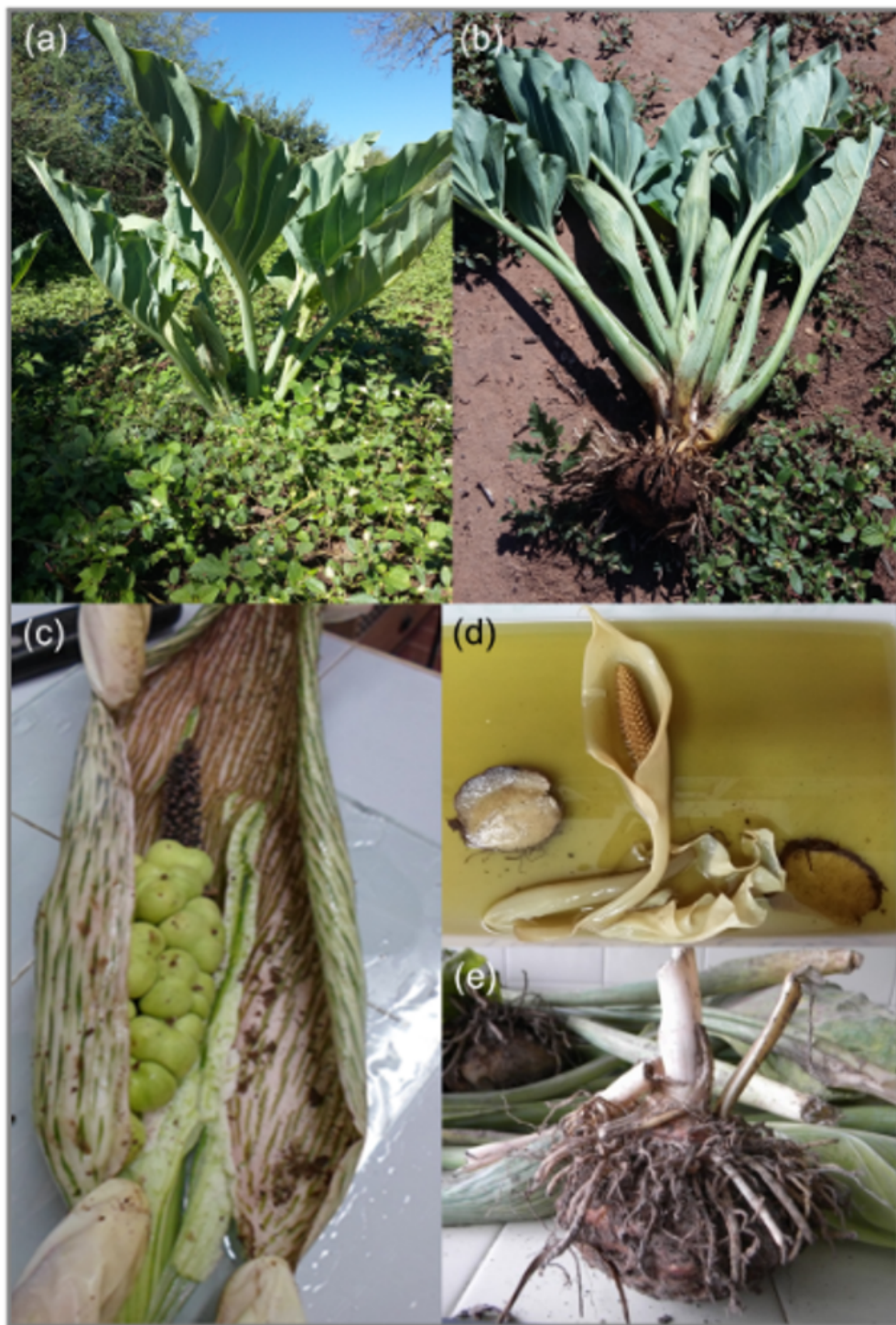


Figure 1

Synandropadix vermitoxicus (Griseb.) Engl. Family Araceae, tribe Spathicarpae, (a) Plant in situ. (b) Removed from soil, see stem tubers. (c) Inflorescence *spadix* 'surrounded by a single bract spathe'. (d) Inflorescence and stem tubers in ethanol 70% from the voucher sample at the Museum of Natural Science of Salta, Argentina (number 11904-MCNS). (e) Stem tuber used on the extraction process.

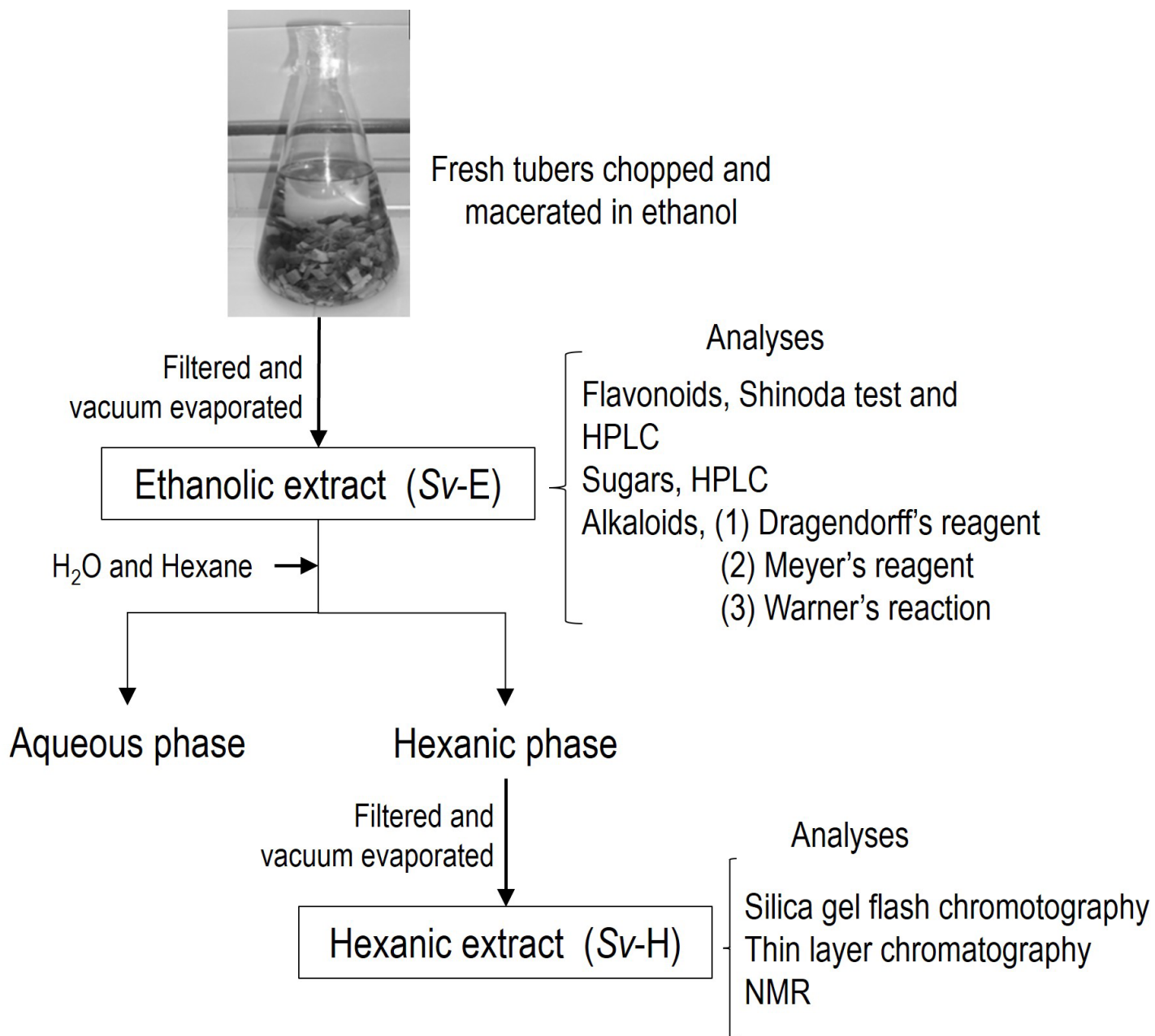


Figure 2

Extraction process from tubers of *Synandropadix vermitoxicus*. Ethanolic (Sv-E) and hexanic (Sv-H) extracts were used for biological essays. The chemical analysis performed to each extract are listed to the right (for details see Materials and Methods).

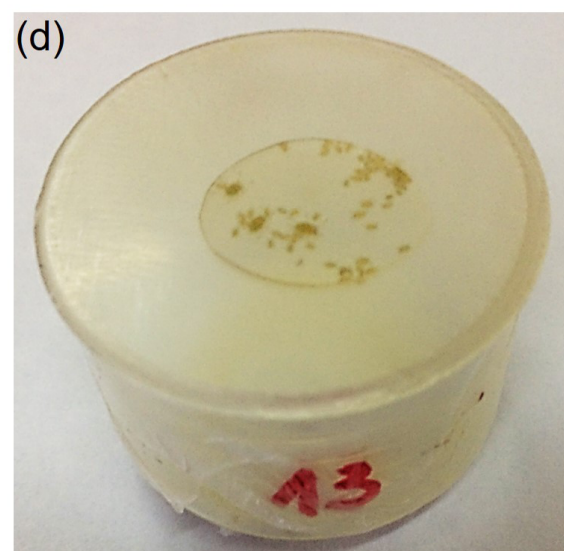
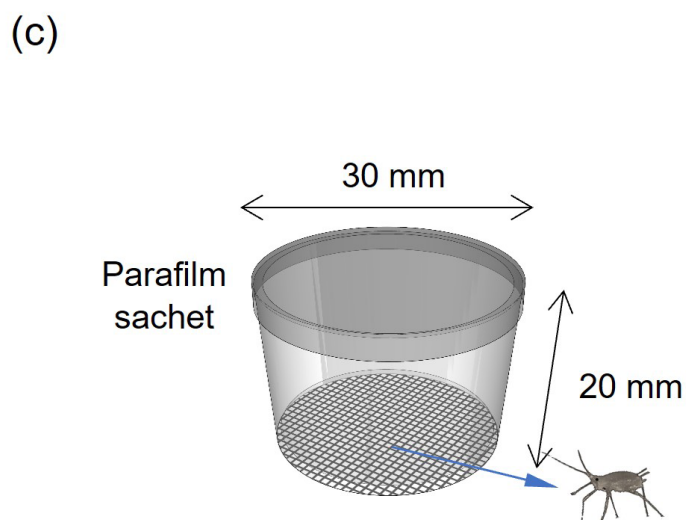
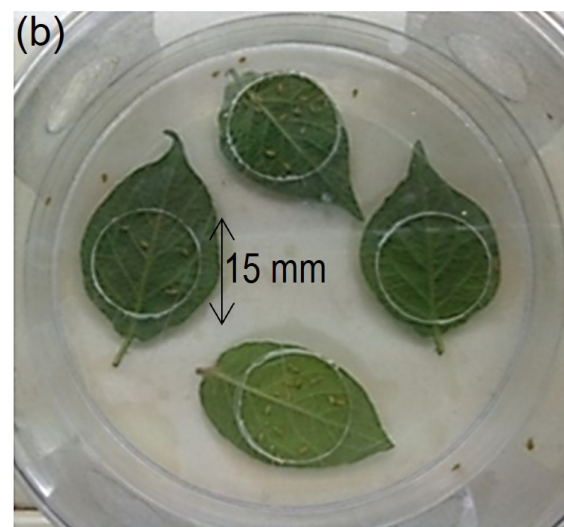
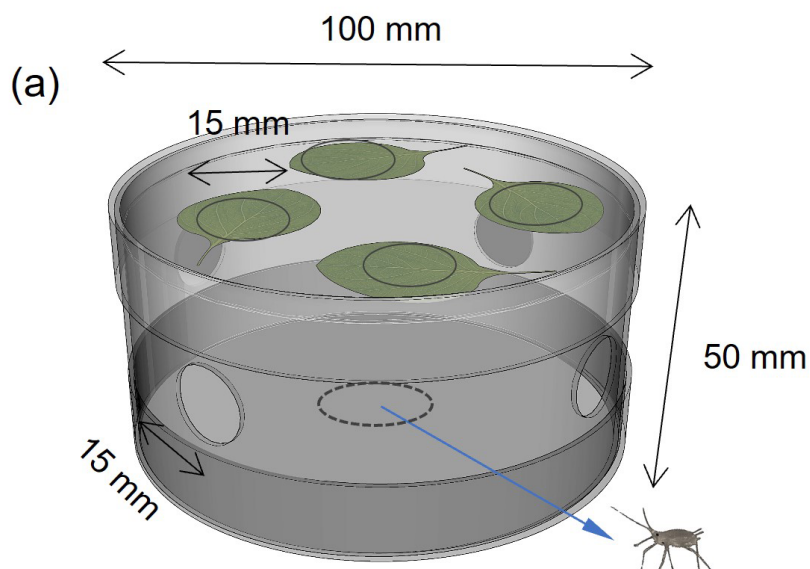


Figure 3

Experimental setup. (a) Antifeedant trial, illustration of choice-cage with four lateral ventilation holes sealed with mesh; the cage was closed at the top and bottom by a removable Petri dish; (b) inside view of Petri dish with two leaves treated on the abaxial side with the *Synandropadix vermitoxicusextracts* and placed in cross with two control leaves by its adaxial side to a layer of agar; to support the leaves the dish was covered with a plastic lid with four holes to expose the leaves and used to seal the cage from top; 40 aphids were placed on the centre 'arena' of the bottom lid and the number of aphids settled on each treated leaf was recorded during time. (c) Aphicidal trial, illustration of diet-cage; (d) cage view from top, five adults aphids were confined inside the cage and sealed on top with a sachet of Parafilm containing 100 μ L of diet solution, after 94 h the number of adults and nymphs were recorded (see Materials and Methods for details).

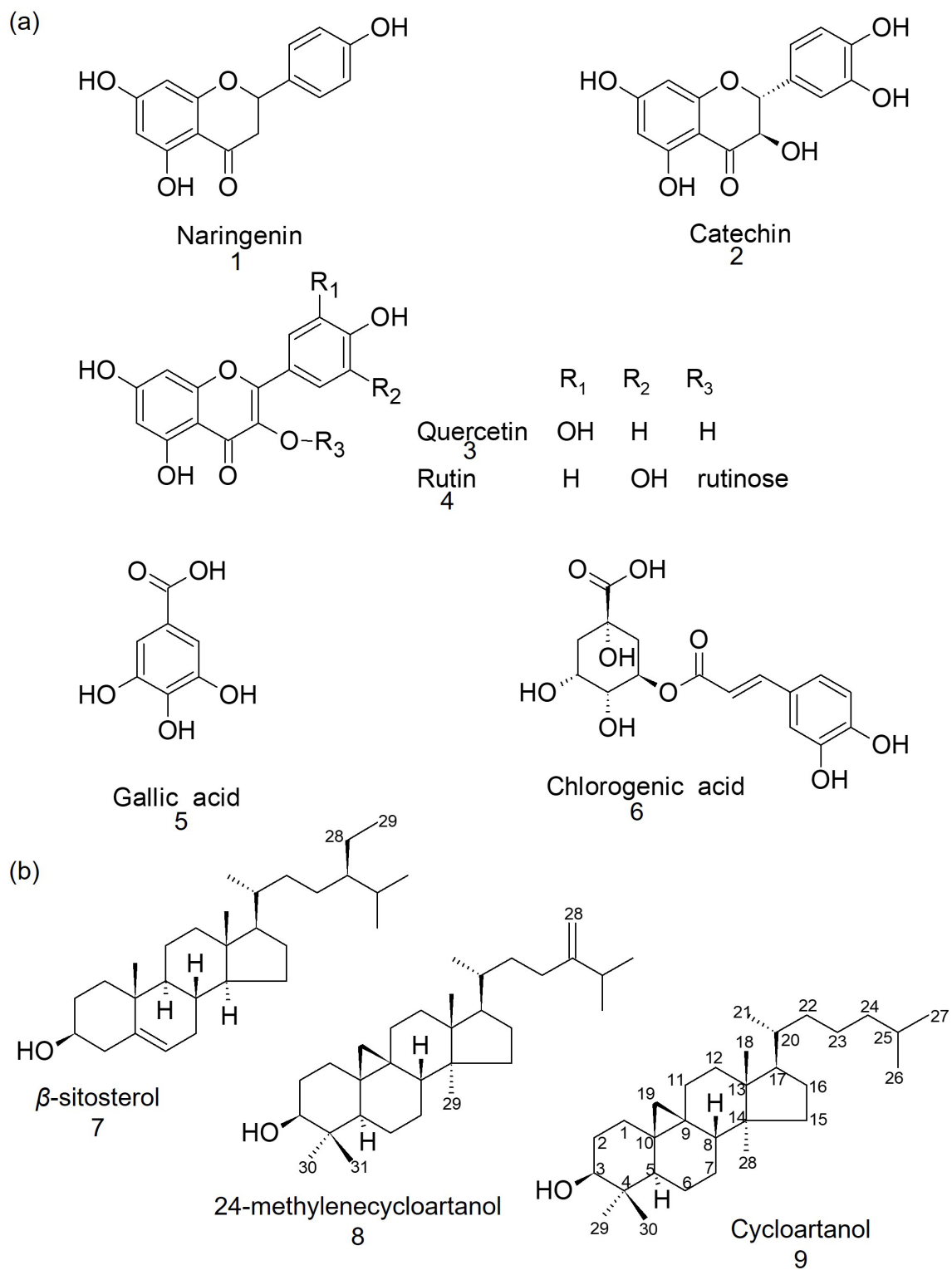


Figure 4

Identified compounds from *Synandropadix vermitoxicus*. (a) flavonoids, naringenin, 1; catechin, 2; quercetin, 3; rutin, 4; gallic acid, 5; chlorogenic acid, 6. (b) triterpenoids, β -sitosterol, 7; 24-methylenecycloartanol, 8; Cycloartanol, 9.

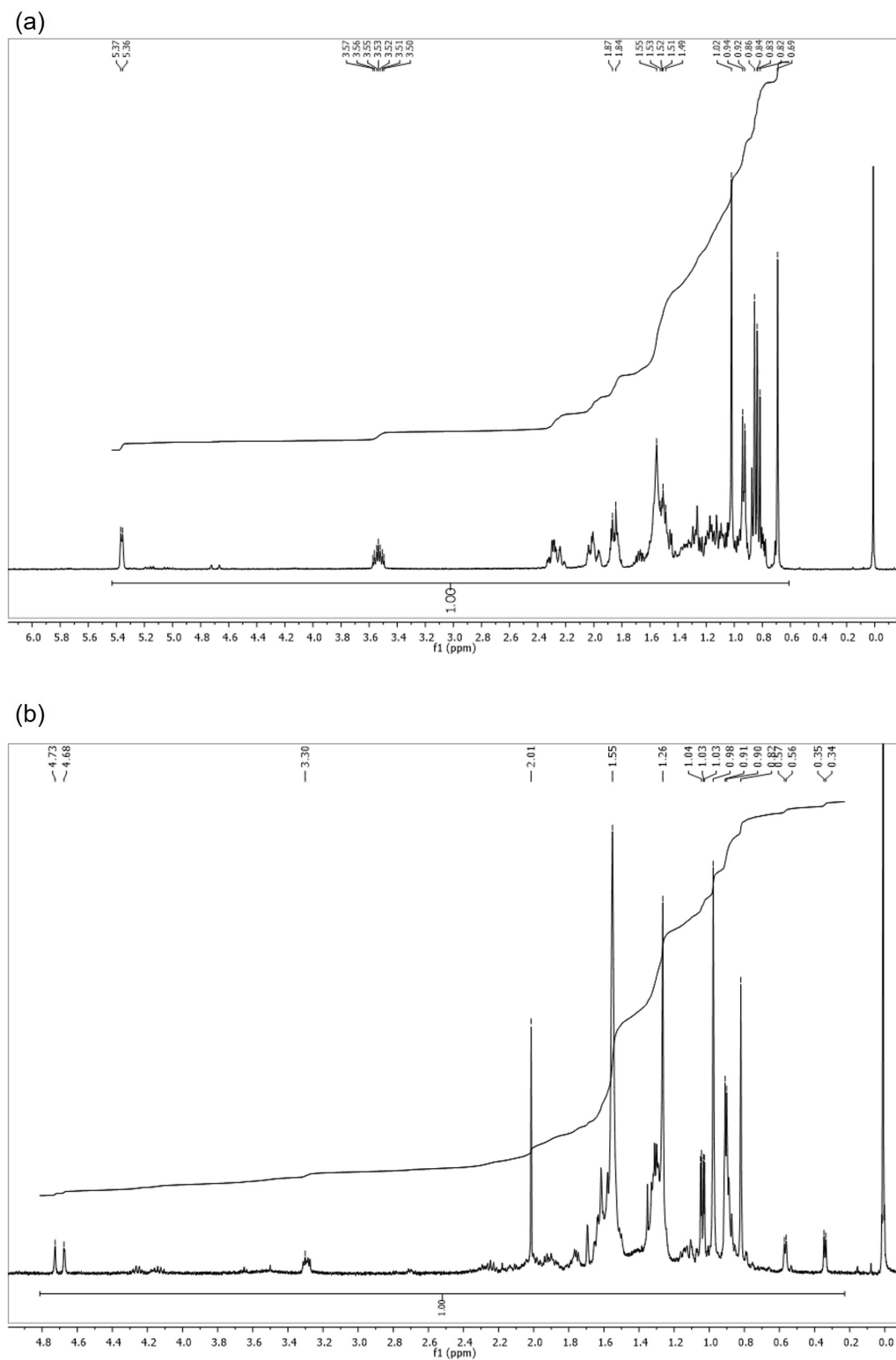


Figure 5

^1H NMR spectra performed on 400 MHz equipment in CDCl_3 . (a) β -sitosterol. (b) cycloartanol and 24-methylenecycloartanol in mixture.

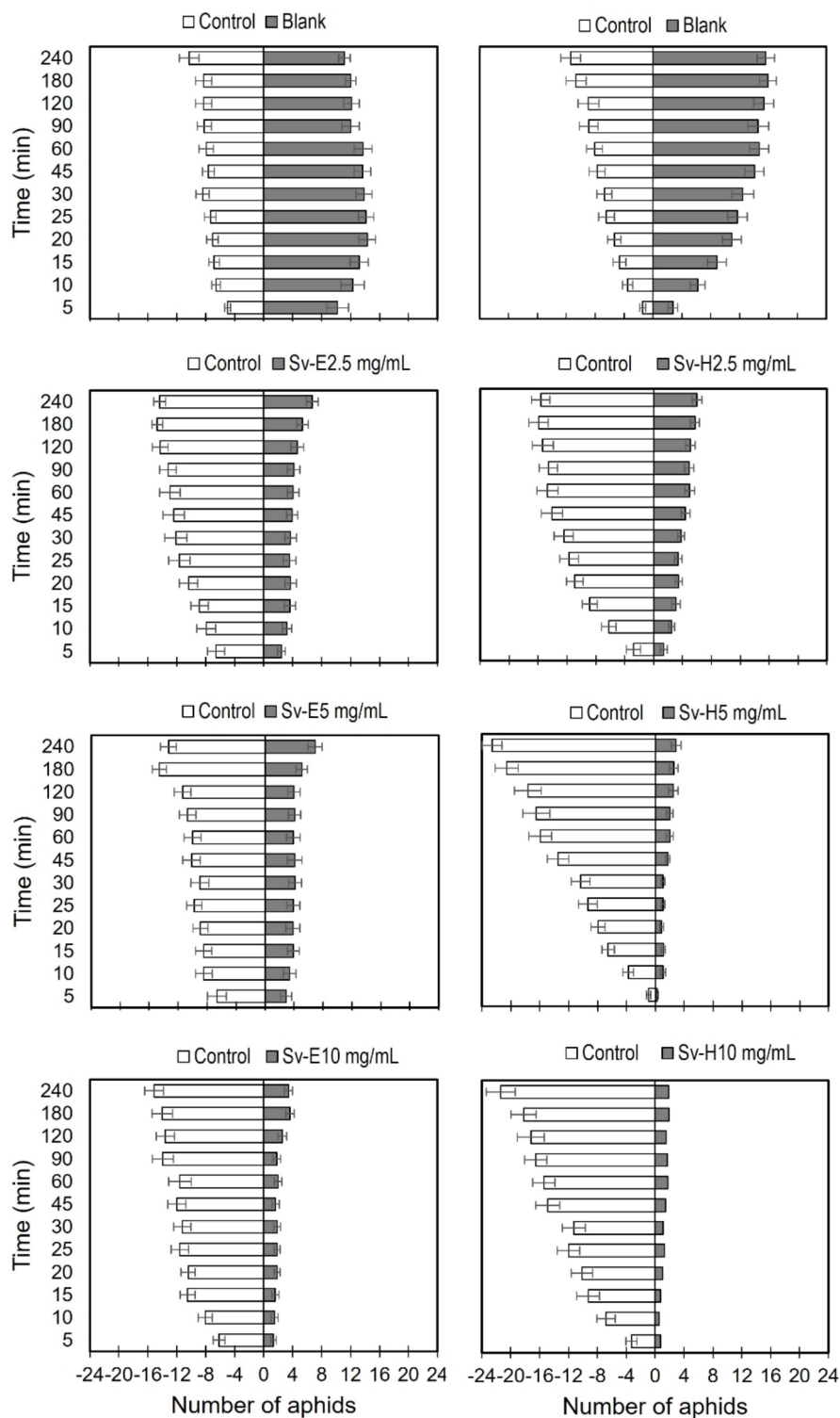


Figure 6

Antifeedant effect of *Synandropadix vermitoxicus* ethanolic (Sv-E) and hexanic (Sv-H) extracts to *Myzus persicae* on potato leaves. The aphid preference over potato leaves treated with Sv-E or, Sv-H at different concentrations vs. control leaves was assessed by a choice test. (a) Leaves treated with ethanolic extract at 2.5, 5 and 10 mg/mL vs. control leaves, and untreated (blank) vs. control. (b) Leaves treated with hexanic extract at 2.5, 5 and 10 mg/mL vs. control leaves, and untreated (blank) vs. control. Bars are means of number of aphids responding at different times, SEM are indicated next to each bar. All contrast, except those marked ns (not significant), where significant at $P < 0.05$ with binomial test.

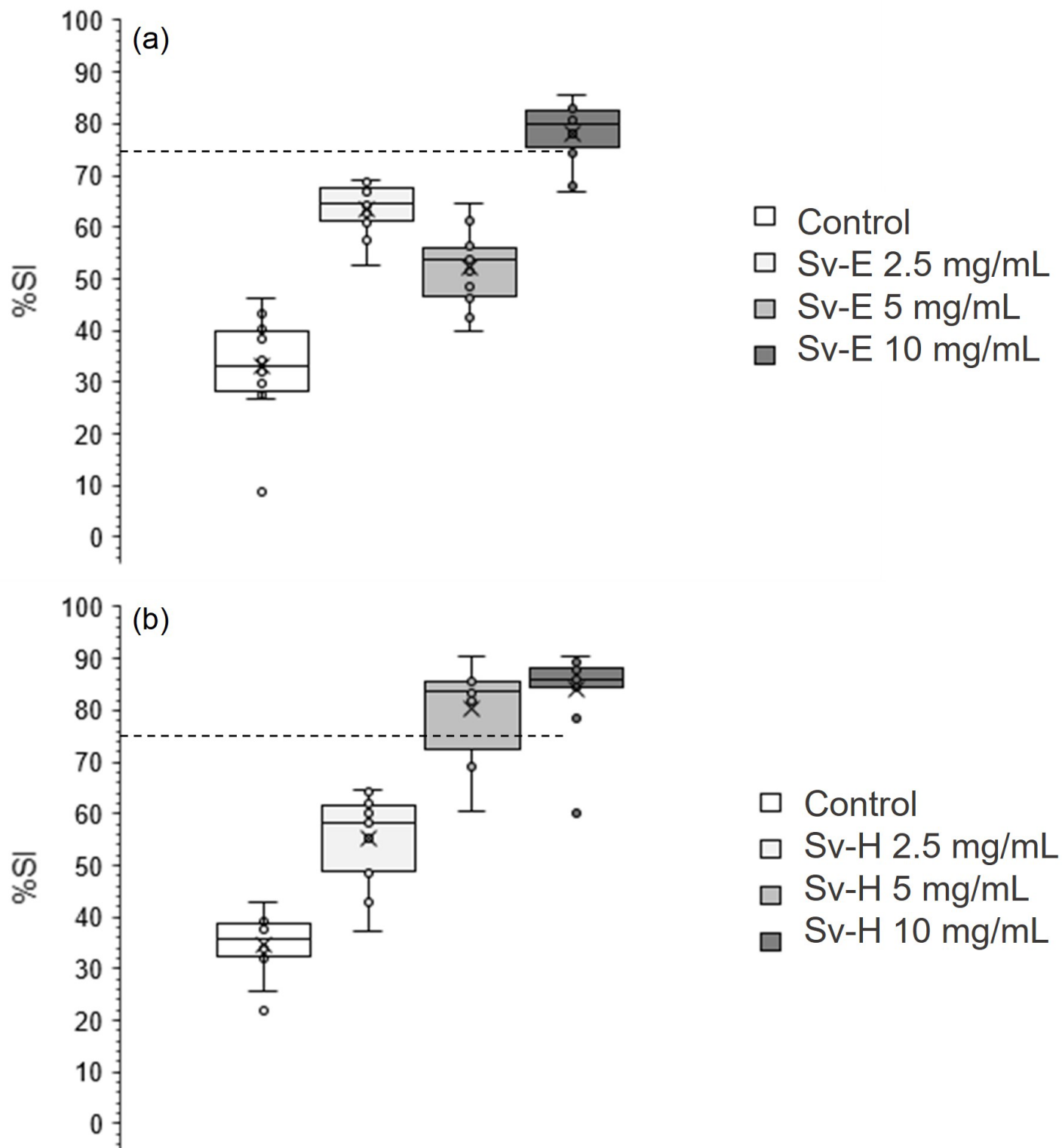


Figure 7

Settling behaviour inhibition (%SI) of *Myzus persicae* in preference test of potato leaves treated with *Synandropadix vermitoxicus* extracts at 2.5, 5 and 10 mg/mL vs. control leaves calculated at different times. (a) Ethanollic extract (Sv-E). (b) Hexanic extract (Sv-H). Boxes represents the lower and upper interquartile range and the central lines the medians; crosses, average; dots, means; whiskers, the 5% and 95% percentiles of $n = 8$ to 14 replicates per treatment. Dotted line is the minimum threshold for effective settling inhibition (Gutierrez et al. 1997).

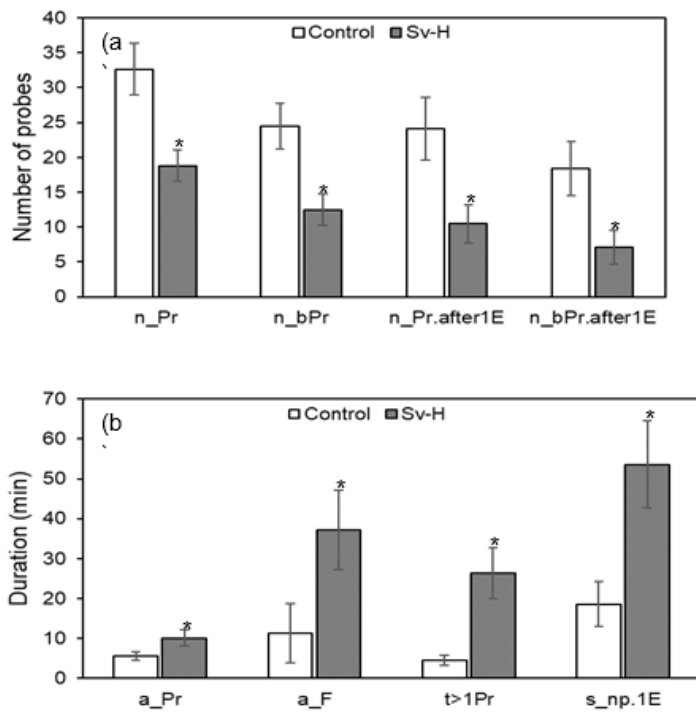


Figure 8

Probing behaviour of *M. persicae* aphids on potato plants during 4 h electrical penetration graph (EPG) monitoring. Sv-H were leaves treated with *S. vermitoxicus* hexanic extract at a dose of 10 mg/mL in an emulsifier solvent of Tween-20 at 0.05% v/v in water. Control were leaves treated with the solvents.

Supplementary Files

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- [SupplementaryFig1TablesS1andS2EPGvariables.docx](#)