Antifeedant potency of Mentha spicata aqueous extracts against fall armyworm (*Spodoptera frugiperda*)

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Research Article

Keywords: Phytochemicals, Mentha spicata, phenols, tannins, Spodoptera frugiperda, insect-pest con-trol

Posted Date: February 11th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1314042/v1

Abstract

The rising trends of insect resistance, coupled with escalating environmental pollution from synthetic pesticides, heighten the need for a more effective and, non-hazardous agents to control insect/pests. Different aqueous extracts of Mentha spicata were screened for their phytochemical constituents and their antifeedant activities against Spodoptera frugiperda . Screening of the different aqueous extracts of Mentha spicata obtained by cold maceration revealed the presence of phenolics and tannins. The concentration of phenols and tannins in the water, glycerine, and glycerine plus water (glycerine-water) extracts were significantly different ($p < 0.05$). Mentha spicata water extract had a greater antifeedant activity against Spodoptera frugiperda as compared to glycerine and glycerine-water (60:40, v/v) extracts at a concentration of 5g/100 mL. The estimated % antifeedant activity recorded were 97 as against 8.21 and 49.81, respectively. Aqueous neem seed water extracts gave an estimated % antifeedant activity of 93.07 and it served as a control. Saponins were absent in all extracts and only water extracts had alkaloids present. The simple, non-hazardous, and cost-saving extraction method demonstrated could be applied in both commercial and subsistent farming to counteract the damnable effects of Spodoptera frugiperda infestation.

Introduction

The effects of insects and pests on agricultural production and crop yields have been substantial, although efforts to control its infestation have been enforced. Approximately 3000 known species of insect-pests are still prevalent worldwide, and responsible for lowering field yield, decreasing crop quality and viability. *Spodoptera frugiperda*, commonly found in Sub-Saharan Africa, Ghana, is one of the worst culprits, attacking over 20,000 hectares of farmlands and costing the government $64 million to control [1].

Spodoptera frugiperda (Fall armyworm, FAW) is a polyphagous insect pest that attacks more than 80 plant species, including maize, sorghum, millet, sugarcane, and vegetable crops [2]. Although, it can reside in a repertoire of 80 crop/plant species, its preferred host is maize, which is the staple food consumed by more than 300 million African smallholder farm families [3].

International development organisations have launched efforts against *Spodoptera frugiperda*, but due to its rapid evolution and adaptability to changing climatic conditions, these efforts have barely yielded any significant result [4]. Attempts to control this insect has seen the development of insecticides, herbicides, and fungicides but these have only resulted in a fractional decline in the population of *Spodoptera frugiperda* [5]. Moreover, synthetic insecticides have been documented to affect reproduction, suppress the immune, and contaminate the environment. Other consequences are phytotoxicity, destruction of beneficial organisms, disruption of agro-ecosystem, human health hazard and environmental persistence [6, 7, 8, 9, 10]. Given these detrimental effects, there is an urgent need to develop safer, more environmentally friendly, and efficient alternatives that have the potential to replace synthetic pesticides [8].

Recently, efforts have been revamped to use natural products in plants instead of the synthetic ones. This is because they are relatively safe, have less eco-toxicological properties and biodegradable [9, 11, 12]. Plants play key roles in the ecological systems and constitute a rich source of bioactive compounds called phytochemicals– metabolically produced chemicals which are generally important for fighting against bacteria, fungi, and plant virus infections. These phytochemicals which include flavonoids, polyphenols, phenolic acids, carotenoids and polyphenols and stilbenes/lignans, are usually extracted and isolated from the original plant and their efficacy against pathogens is tested on invivo and invitro experiments, as well as cell cultures. Thus far, there are more than 25000 phytochemicals from over 1500 species of plants. Emphatically, crude extracts and oil from leaf, stem, root, and seeds of various plant species possess unique properties which include antifeedant and insecticidal whereas others disrupt hormonal balance by inhibiting growth, metamorphosis, and reproduction [8, 13, 14, 15]. One of such powerful plant species that has been identified to possess potent phytochemicals is *Mentha Spicata*.

Mentha spicata is a creeping, rhizomatous and perennial herb with a rich supply of polyphenols and mostly cultivated in the tropical and temperate regions [16]. It is characterised by an aromatic smell and pungent giving sensation, hence in aromatherapy, its potential as an insect repellent is promising. Mentha spicata has insecticidal value against ants, mosquitoes, wasps, hornets and cockroaches amongst others [9, 16].

Although the literature has highlighted some unique properties of *Mentha spicata*, its insecticidal properties have not been fully investigated. Hence, the present study sought to profile a panel phytochemical constituent or extracts of *Mentha spicata* and explore its potential of as antifeedants on the insect-pest, *Spodoptera frugiperda*.

Materials And Methods

2.1 Solvents, Reagent and Materials

Glycerine (VWR Chemicals BDH, USA), Ferric chloride (BDH Laboratory Supplies, England), HCl (AvonChem, U.K), Na_2CO_3 (Wardle Chemicals, Calveley).

2.2 Sampling and Sample Preparation

The *Mentha spicata* leaves and the neem seeds were collected from the School of Biological Sciences herbarium, University of Cape Coast, Ghana. The *Mentha spicata* leaves and the neem seeds were air dried for four weeks at laboratory conditions (26.4 ± 2°C) to reduce the moisture content and was milled into fine powder to increase the surface area for extraction. The armyworms were collected from an unsprayed maize field at Kwaprow, a suburb in Cape Coast, Central Region, Ghana, and kept in a perforated container at laboratory conditions (26.4 ± 2°C). The army worms were fed with maize leaves

collected for 12-30 days. About 50 4th instar larvae were collected and reared until a sufficient population of second-generation larvae was obtained for the study.

2.3 Preparation of reagents - Mayer's reagent

The Mayer's reagent was prepared by dissolving 1.36 g of mercuric chloride and 5 g of potassium iodide in 100 mL of distilled water. This reagent would be used in the detection of alkaloids.

2.4 Extraction of Samples using Selective Solvents

A glass syrup bottle was loaded with 5 g of the blended *Mentha spicata* leaves powder and 100 mL of the aqueous solvent was poured over it to cover the samples. This was put under cold maceration for three days with intermittent agitations to ensure thorough diffusion of solvent into the plant tissues and to enhance solubilization of extracts. The residue was separated from the filtrate by suction filtration and the filtrate was stored in glass syrup bottle at laboratory conditions (26.4 ± 2°C) and kept away from sunlight until usage. This was done for all three selected aqueous solvents namely, distilled water, glycerine and glycerine plus water (in the ratio of 6:4). These solvents were chosen because their low cost, easy accessibility and their safety to the environment. The neem seeds powder was also taken through the same procedure using water as solvent.

2.5 Phytochemical Screening of Extracts

2.5.1 Detection of phenolics

The detection of phenolics was done using a method described by Tiwari *et al*., 2011. 0.5 mL of the extract was measured into separate test tubes and 1 mL of 1% ferric chloride solution was added into each test tube. The formation of a bluish-black colour indicated the presence of phenolic compounds. The chemical structures of common plant derived phenolics, namely, Rutin, Caffeic acid and Resveratrol are shown in **Figure 1**.

2.5.2 Detection of alkaloids

The detection of alkaloids was done using the Mayer's test (Potassium Mercuric Iodine solution) as described by Ramamurthy and Sathiyadevi [17]. Extracts were dissolved individually in 5 mL dilute HCl and filtered. To 1 mL of the filtrate, 0.5 mL of Mayer's reagent was added. The formation of a yellow cream precipitate indicated the presence of alkaloids. The chemical structures of common plant derived alkaloids, namely, Caffeine and Berberine are shown in **Figure 2**.

2.5.3 Detection of tannins

The detection of tannins was done using the method described by Kumar *et al.,* [18] with slight modifications. 0.5 mL of the extract was measured into separate test tubes and mixed with 2.5 mL of water. The mixture was heated for 10 minutes and filtered. 1 mL of 1% ferric chloride solution was then added into the filtrate in each test tube. The formation of a green colour indicated the presence of tannins. The chemical structures of common plant derived tannis, namely, Gallic acid, Daidzein, Genistein and Glycitein are shown in **Figure 3**.

2.5.4 Detection of saponins

The detection of saponins was done using the foam test as described by Ekwueme *et al*., [19]. 5 mL of the extracts was added to 5 mL of distilled water and shaken thoroughly. The persistence of foam for ten minutes indicated the presence of saponins. The chemical structures of common plant derived saponins, namely, Dammarenediol and Cucubitadienol are shown in **Figure 4**.

2.6 Quantitative Analysis of Extract

For each parameter, three repetitions were carried out to determine the concentration of the phyto constituents.

2.6.1 Total Tannins Content (TTC)

The quantitative tannin content in the extracts was estimated by Folin-phenol method a described by Tamilselvi *et al*, [20]. 0.1 mL of the sample/extract was added to 7.5 mL of distilled water and 0.5 mL of Folin-phenol reagent was added. After that 1.0 mL of 35 % Na_2CO_3 solution was added, and the mixture was diluted to 10mL with distilled water. The mixture was shaken well and kept at room temperature for 30 minutes. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100) µg/mL were prepared in the same manner as described earlier from a stock solution of 100 µg/mL (i.e. 2 mg/20 mL) and water was used as the blank. Absorbance for test and standard solutions were measured at a wavelength of 725 nm with an UV/VIS spectrophotometer (UVD-3200, Labomed Inc., Los Angeles, California, USA). The tannin content was determined from the gallic acid standard curve.

2.6.2 Total phenolic content

The phenolic content detected using a method described by Singleton and Rossi [21]. 1.5 mL of the extracts was put into separate test tube and 0.2 mL of 0.1% Folin Ciocalteu reagent was added. The test tubes were incubated at room temperature for 4 mins. 0.5 mL of 20% sodium carbonate was added and kept in the dark for 30 mins. The absorbance was read at a wavelength of 650 nm. Gallic acid of concentration: (20, 40, 60, 80 and 100) ug / mL were used as standard for constructing calibration curve and water was used as the blank. The phenolic content was determined from the gallic acid standard curve.

2.6.3 Test for Antifeedancy

Maize leaves of approximately 14 cm x 2 cm were cut out. The leaves were dipped into the various extracts. This was done for glycerine, water, glycerine (60%) plus water (40%) extracts and for *Azadirachta indica* (neem seed) extracts which served as a positive control. A negative control was also set using ordinary distilled water. The armyworms were exposed to the leaves in plastic containers with perforated lids. Per each treatment, one armyworm pre-starved for 2 hours was used to prevent cannibalism. The antifeedant activity was monitored for a period of 24 hours of exposure, i.e., first 4 hours continuum and left overnight until after 24 hours. After 24 hours, the antifeedant activity of the extracts was accessed based on the rate of feeding by the armyworms. Percentage (%) antifeedancy was observed and estimated using the formula:

$$\%\ \text{Antifeedancy} = \frac{\text{Total Area of Leaves} - \text{Total Area of Leaves Consumed after 24 hours}}{\text{Total Area of Leaves}} * 100$$

Results

3.1 Qualitative Phytochemical Analysis of Extracts

The various phytochemicals that were screened for in the different extracts of *Mentha spicata* are shown in Table 1. The presence of the phytochemicals in the extract was indicated by a plus sign (+) and the absence of the phytochemical in the extract was indicated by a minus sign (-). Screening of the different aqueous extracts of *Mentha spicata* revealed the presence of phenols and tannins in the water, glycerine only and glycerine and water extracts. Saponins were absent in all the different extracts and only water extracts had alkaloids present.

Table 1: Phytochemical screening of the different aqueous extracts of *Mentha spicata*

Extract	Phenol	Saponin	Tannin	Alkaloid
Glycerine	+	-	+	-
Water	+	-	+	+
Glycerine plus Water	+	-	+	-

The concentration of phenols and the concentration of tannins in the various aqueous (water, glycerine, and glycerine plus water) extracts of *Mentha spicata* are shown in Figure 5A. A one-way analysis of variance (ANOVA) confirmed significance differences in the concentration of phenols and tannins extracted by the various aqueous extraction methods with empirical support ($F(2, 6) = 131.906$, $p < 0.001$)

and ($F(2, 6) = 64.495$, $p < 0.001$) respectively. With respect to phenols, the Bonferroni post hoc test revealed that Glycerine extracts observed the highest concentration, followed by Glycerine plus water extracts, with water extracts having the least concentration. With regards to tannis, the post hoc test revealed no significant differences in the concentration levels from Glycerine and water extracts. Glycerine plus water extracts observed the highest concentration of tannis. The calibration curves for the extrapolation of the concentration of total phenolics and tannins contents are shown in Figure 5 (B, C).

3.2 Efficacy of the different aqueous extracts of *Mentha spicata* as antifeedants

The rate of antifeedancy of the armyworms for 24 hours exposure to leaves with the extracts applied is shown in Figure 6. A positive control and negative control were set using neem seed water extracts and ordinary distilled water, respectively. Comparatively, there was less feeding activity on leaves onto which the extract with water was applied, demonstrating high efficacy of antifeedancy. Extracts from glycerine plus water were more productive in antifeedancy compared to that of glycerine only. Out of the total leaf area of 28 cm2, the army worm consumed 13.17 cm of the leaves in glycerine plus water in 24 hours, whereas the total leaf in glycerine extract decreased by 34.86 cm2 following a 24-hr army worm exposure (see Figure 6 and 7).

The percentage antifeedant activity of the various extracts against armyworms were observed and estimated (Table 2). Extracts from water had the highest percentage antifeedant activity on armyworms, and was more effective in repelling army worms

Table 2. Percent Antifeedant activity of the different aqueous extracts on armyworms

Extract	% Antifeedant activity
Glycerine	8.21
Water	97
Glycerine + Water	49.96
Positive Control (Neem seed)	93.07
Negative Control	0

Discussion

The discovery of less-expensive and non-hazardous alternatives for the management of insect-pests remains an urgent need to mitigate the rising trends of insect resistance and the harmful environmental pollution by synthetic insecticides. In this study, different aqueous extracts of *Mentha spicata* were screened for their phytochemical properties including phenols, saponins, tannins and alkaloids. The effects of the various extracts on *Spodoptera frugiperda* were also explored. The result of the present study has indicated that water extract, glycerine and water/glycerine (60;40, v/v) extracts all contained phenols and tannins but not saponins. Alkaloids were present in water extract but absent in glycerine, and

water/glycerine (60;40, v/v) (Table 1). The highest phenolic content of 52.0 µg/mL was recorded for glycerine, followed by 37.0 µg/mL from glycerin (60%) plus water (40%) extracts, with water extract having the lowest phenolic content of 16.3 µg/mL (Figure 1). Glycerine plus water extracts had the highest concentration of 47.2 µg / mL tannin content, followed by glycerine (35.5 µg/mL) and water (32.2 µg/mL) (Figure 5A).

The antifeedant activity was clearly influenced by the solvents used for extraction. Antifeedant effects of different aqueous extracts of *Mentha spicata* were evaluated by observing the leaf area consumed by *Spodoptera frugiperda* after the application of the extracts. Among the extracts tested, the water extracts of *Mentha spicata* was found to be the most effective against *Spodoptera frugiperda* with antifeedant activity of 97% as against the *Azadirachta indica* (neem) seed water extracts (positive control) with antifeedant activity of 93.07% (Table 2). The potency *Mentha spicata* water extracts proved to be a better antifeedant against fall armyworms (Figure 7) compared to *Azadirachta indica* seed extracts which has been proven to have antifeedancy against other *Spodoptera spp* [22]. Isman [23] reported that antifeedants can be found amongst all the major classes of secondary metabolites (e.g., alkaloids, phenolics and terpenoids) which are the most likely toxic substances against insects. The water extract tested positive for the presence of alkaloids (Table 1), hence the maximum antifeedant activity observed for the water extract may be attributed to the presence of alkaloids. Glycerine in water extract of *Mentha spicata* had an average antifeedant activity of 49.96% against *Spodoptera frugiperda*, corresponding with a significantly consumed maize leaves (Table 2). The area of maize consumed was more pronounce upon exposure with glycerine extract of Mentha spicata and the recorded antifeedant activity was 8.21% the antifeedant activity of against armyworms (Figure 7, Table 2). The poor antifeedant activity of the glycerine extracts may be attributed to the extraction time as well as the concentration of the sample which may be insufficient to extract the biological components required for antifeedancy [24]. The results indicated that the active phytochemicals present in the water extract of *Mentha spicata* modulate feeding behaviour and make the food unpalatable resulting in feeding deterrence. The water extract presented the overall least content of phytochemicals, phenols, and tannins, except for alkaloids which were in abundance, it exhibited the highest (most active) antifeedant effect. In a similar study, water extracts of some *Mentha spp* namely *M. piperita, M. pulegium*, and *M. spicata* exhibited significant nematicidal activity against *Meloidogyne incognita*, with the *M. spicata* water extracts exhibiting an EC50 value of 300 mg/L after 72 hours of exposure of *Meloidogyne incognita* to the extract [25].

This observation may be attributed to the possibility of the abundant phytochemicals i.e active components present in the water extract. Since polyphenols and tannins have many polar hydroxyl groups, the water extracts should have given the highest concentration. However, this was not the case in our study. According to Tiwari et al., [24], the decrease in the phenolic and tannin content of the water extract may be attributed to the activity of the enzyme, polyphenol oxidase, which degrades polyphenols in aqueous media.

The cost of synthetic insecticides and pesticides is high, even after Government subsidies. The extraction method proposed in the study could easily be implemented by farmers to combat the infestation of

Spodoptera frugiperda. Thus, our research offers a unique alternative and a cheaper option for local farmers who struggle to purchase synthetic insecticides. In addition, insecticides derived from a synthesised natural product like the one highlighted in this research, are environmentally friendly and a safer for agricultural produce. Optimising the extraction method and synthesising these products on a large scale will alleviate the financial burden on local farmers, while maximising crop yield. As a limitation, the study only focused on specific ratio of the water-glycerine mixtures and would suggest that various ratio concentrations of the water-glycerine mixtures are compared to determine which concentrations will deliver the best antifeedant activity. Also, the search for promising antifeedants against fall armyworms should be expanded to other variety of plants. *Mentha spp* although not commercially cultivated in Ghana, they are readily accessible and has very promising prospects in the scientific and agricultural fields.

Conclusions

Glycerine, water, and water/glycerine (60:40, v/v) extracts of *Mentha spicata* showed the presence of phenols and tannins and the absence of saponins. Alkaloids were present in water extracts but absent in glycerine and combination of glycerine and water extracts. *Mentha spicata* water extract exhibited the highest potential as an antifeeding agent against Spodoptera frugiperda compared to the glycerine and combination glycerine and water extracts. Thus, *Mentha spicata* water extracts are potent antifeedants and may be used in the biocontrol of *Spodoptera spp*.

Declarations

Funding: This research received no funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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Figures

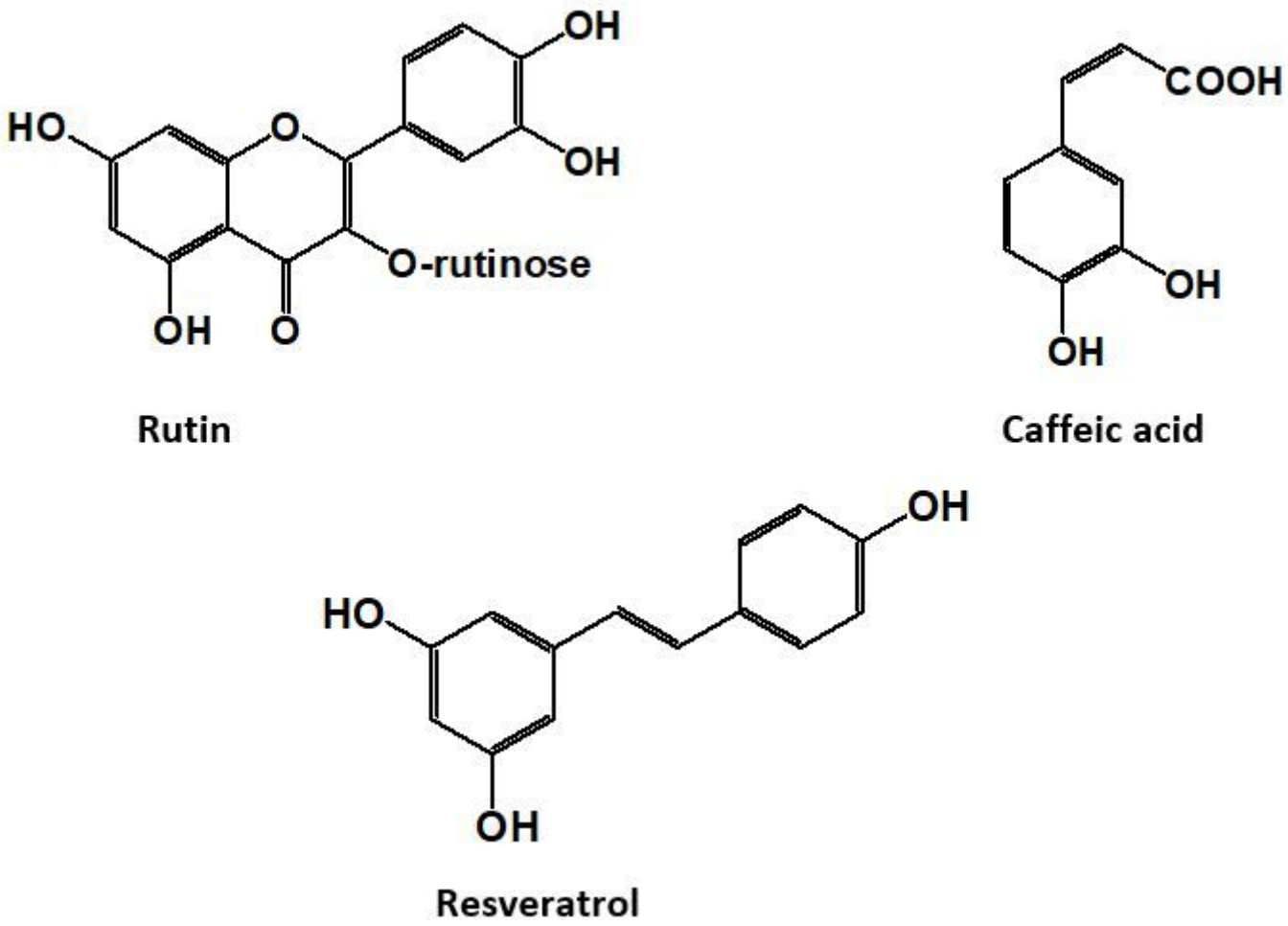

Figure 1

Chemical structures of common plant derived phenolics (Rutin, Caffeic acid and Resveratrol)

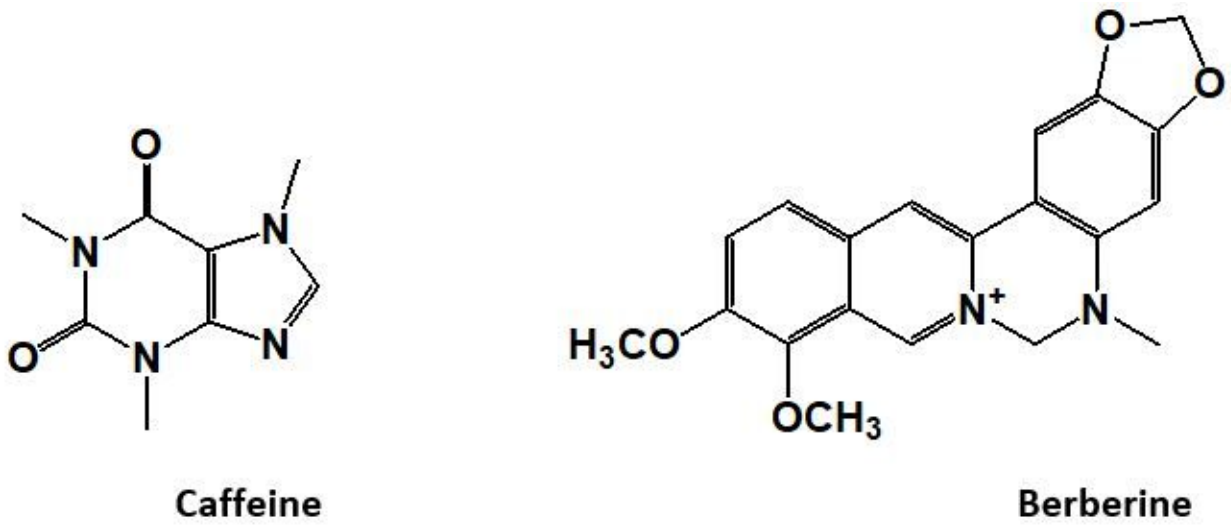

Figure 2

Chemical structures of common plant derived alkaloids (Caffeine and Berberine)

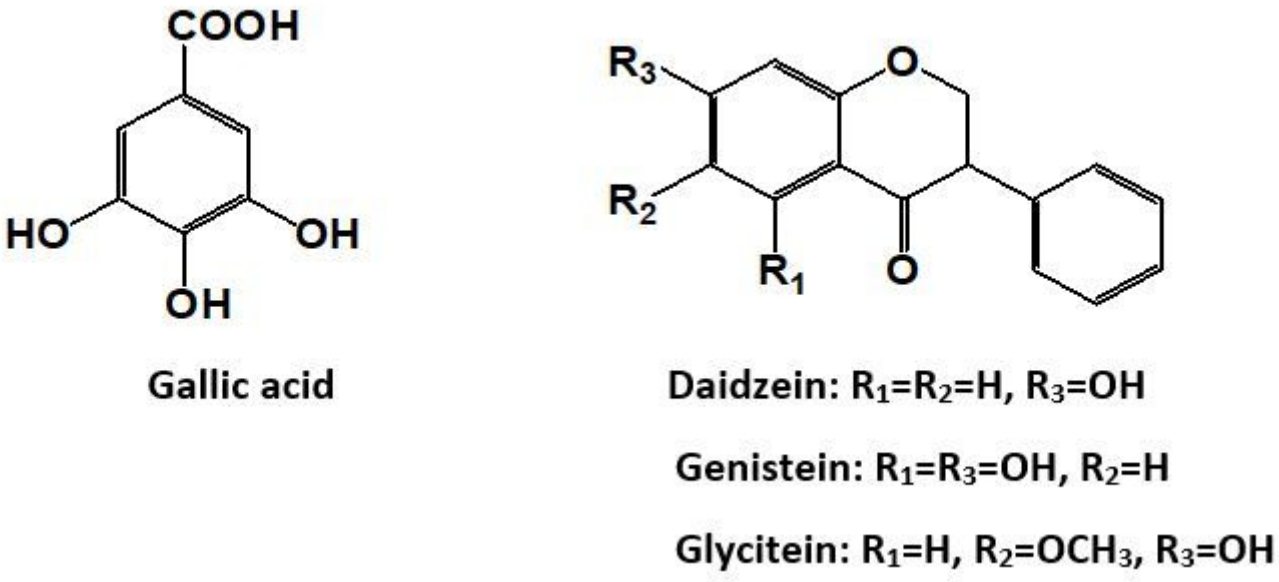

Figure 3

Chemical structures of common plant derived tannis (Gallic acid, Daidzein, Genistein and Glycitein)

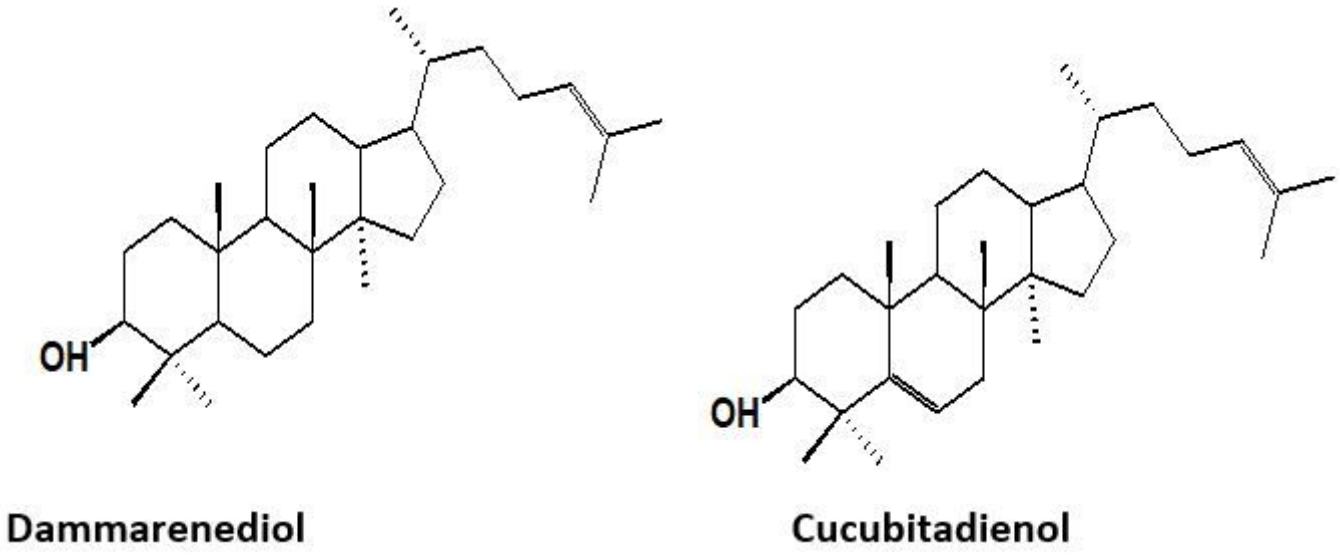

Figure 4

Chemical structures of common plant derived saponins (Gallic acid, Daidzein, Genistein and Glycitein)

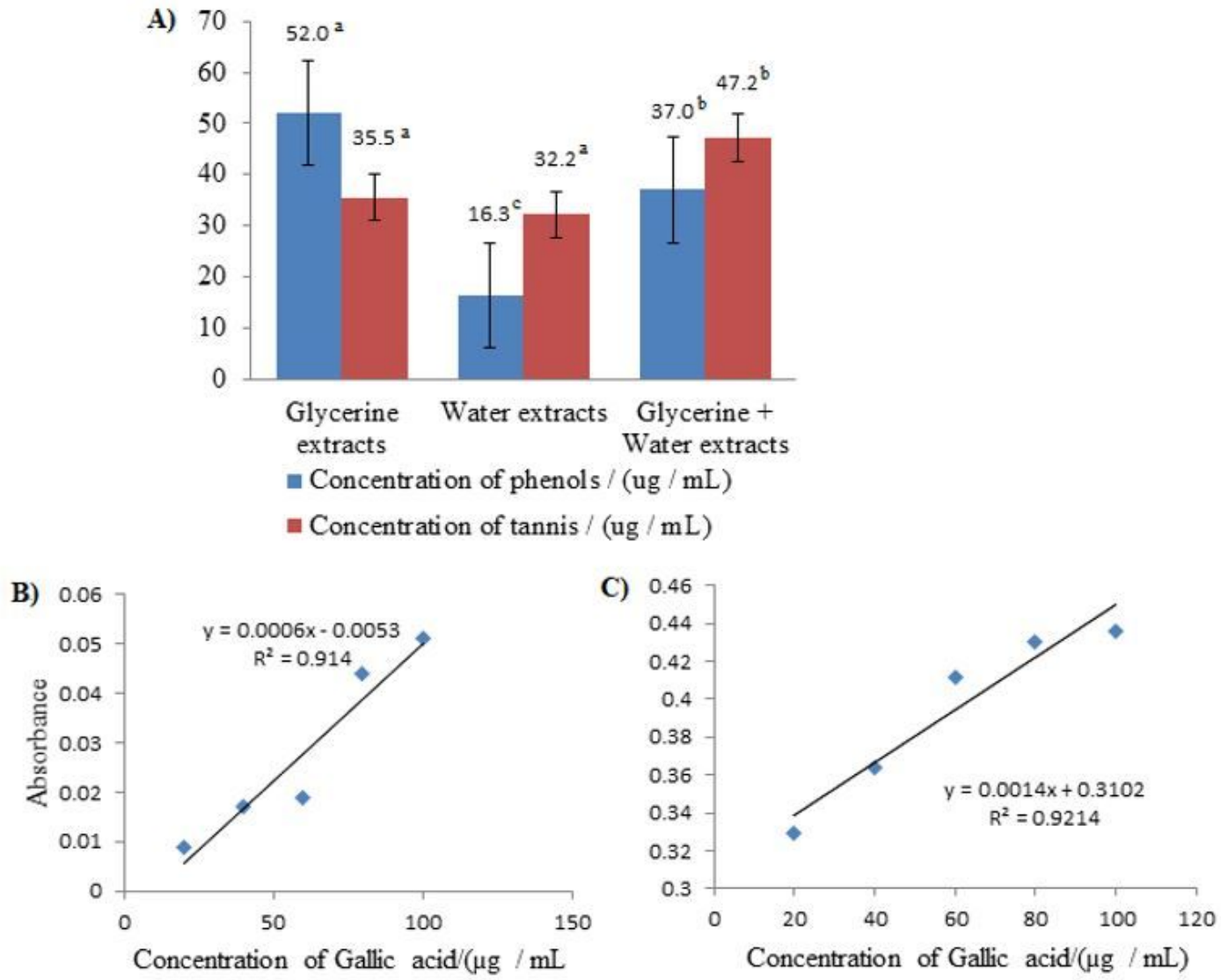

Figure 5

A) Concentration of phenols and tannins in different aqueous extracts of *Mentha spicata*. B) Calibration curve of tannins C) Calibration curve for phenols

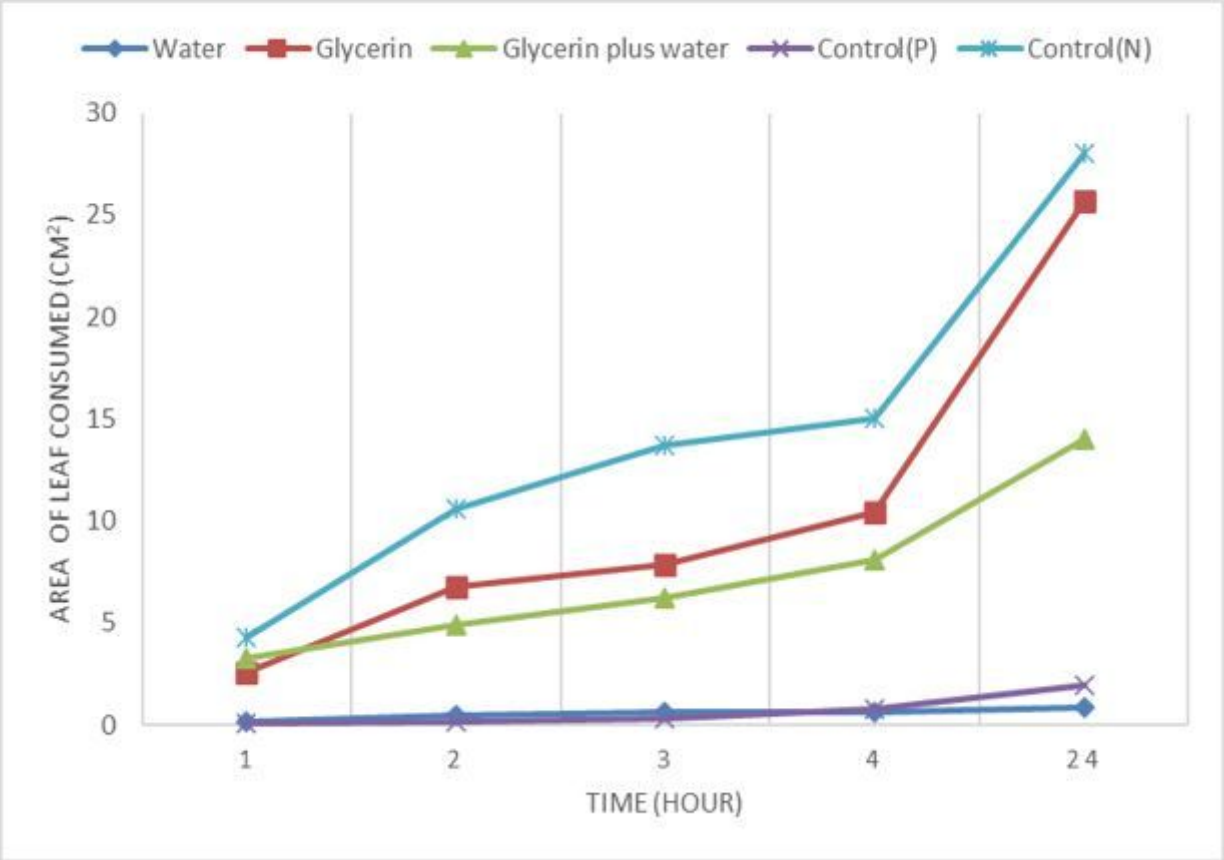

Figure 6

Rate of leaf consumption by armyworms (cm2/hour)

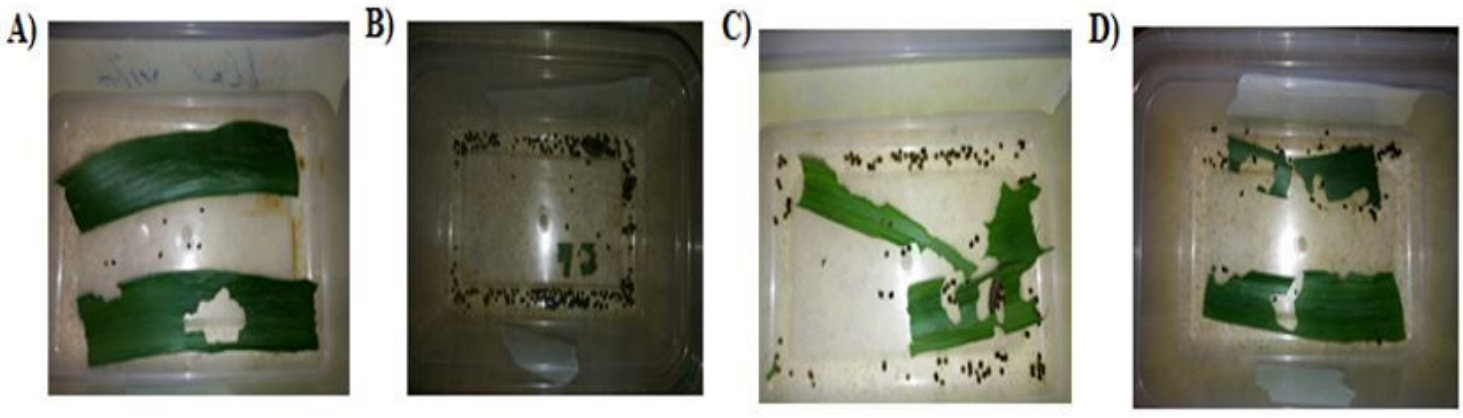

Figure 7

Observed area of consumption of maize leaves by *Spodoptera frugiperda* A) Water extracts B) Glycerine extracts C) Glycerine plus water extract D) Control (Neem seed)