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## Original Research Article

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3 Environmentally friendly agent against fall armyworm (*Spodoptera*  
4 *frugiperda*): antifeedant potency of *Mentha spicata* aqueous extracts

5

6 **Abstract:** The rising trends of insect resistance, coupled with escalating environmen-  
7 tal pollution from synthetic pesticides, heighten the need for a more effective and,  
8 non-hazardous agents to control insect/pests. Different aqueous extracts of *Mentha*  
9 *spicata* were screened for their phytochemical constituents and their antifeedant ac-  
10 tivities against *Spodoptera frugiperda*. Screening of the different aqueous extracts of  
11 *Mentha spicata* obtained by cold maceration revealed the presence of phenolics and  
12 tannins. The concentration of phenols and tannins in the water, glycerine, and glyce-  
13 rine plus water (glycerine-water) extracts were significantly different ( $p < 0.05$ ). *Men-*  
14 *tha spicata* water extract had a greater antifeedant activity against *Spodoptera frugiperda*  
15 as compared to glycerine and glycerine-water (60:40, v/v) extracts at a concentration  
16 of 5g/100 mL. The estimated % antifeedant activity recorded were 97 as against 8.21  
17 and 49.81, respectively. Aqueous neem seed water extracts gave an estimated % an-  
18 tifeedant activity of 93.07 and it served as a control. Saponins were absent in all ex-  
19 tracts and only water extracts had alkaloids present. The simple, non-hazardous, and  
20 cost-saving extraction method demonstrated could be applied in both commercial  
21 and subsistent farming to counteract the damnable effects of *Spodoptera frugiperda* in-  
22 festation.

23 **Keywords:** Phytochemicals, *Mentha spicata*, phenols, tannins, *Spodoptera frugiperda*,  
24 insect-pest control

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### 27 1. Introduction

28 The effects of insects and pests on agricultural production and crop yields have been  
29 substantial, although efforts to control its infestation have been enforced. Approx-  
30 imately 3000 known species of insect-pests are still prevalent worldwide, and re-  
31 sponsible for lowering field yield, decreasing crop quality and viability. *Spodoptera*  
32 *frugiperda*, commonly found in Sub-Saharan Africa, Ghana, is one of the worst cul-  
33 prits, attacking over 20,000 hectares of farmlands and costing the government \$64  
34 million to control [1].

35 *Spodoptera frugiperda* (Fall armyworm, FAW) is a polyphagous insect pest that attacks  
36 more than 80 plant species, including maize, sorghum, millet, sugarcane, and vege-  
37 table crops [2]. Although, it can reside in a repertoire of 80 crop/plant species, its

38 preferred host is maize, which is the staple food consumed by more than 300 million  
39 African smallholder farm families [3].

40 International development organisations have launched efforts against *Spodoptera*  
41 *frugiperda*, but due to its rapid evolution and adaptability to changing climatic condi-  
42 tions, these efforts have barely yielded any significant result [4]. Attempts to control  
43 this insect has seen the development of insecticides, herbicides, and fungicides but  
44 these have only resulted in a fractional decline in the population of *Spodoptera frugi-*  
45 *perda* [5]. Moreover, synthetic insecticides have been documented to affect reproduc-  
46 tion, suppress the immune, and contaminate the environment. Other consequences  
47 are phytotoxicity, destruction of beneficial organisms, disruption of agro-ecosystem,  
48 human health hazard and environmental persistence [6,7, 8, 9, 10]. Given these de-  
49 trimental effects, there is an urgent need to develop safer, more environmentally  
50 friendly, and efficient alternatives that have the potential to replace synthetic pesti-  
51 cides [8].

52 Recently, efforts have been revamped to use natural products in plants instead of the  
53 synthetic ones. This is because they are relatively safe, have less eco-toxicological  
54 properties and biodegradable [9, 11,12]. Plants play key roles in the ecological systems  
55 and constitute a rich source of bioactive compounds called phytochemicals— meta-  
56 bologically produced chemicals which are generally important for fighting against bac-  
57 teria, fungi, and plant virus infections. These phytochemicals which include flavo-  
58 noids, polyphenols, phenolic acids, carotenoids and polyphenols and stil-  
59 benes/lignans, are usually extracted and isolated from the original plant and their ef-  
60 ficacy against pathogens is tested on invivo and invitro experiments, as well as cell  
61 cultures. Thus far, there are more than 25000 phytochemicals from over 1500 species  
62 of plants. Emphatically, crude extracts and oil from leaf, stem, root, and seeds of  
63 various plant species possess unique properties which include antifeedant and insec-  
64 tical whereas others disrupt hormonal balance by inhibiting growth, metamorpho-  
65 sis, and reproduction [8, 13, 14, 15]. One of such powerful plant species that has been  
66 identified to possess potent phytochemicals is *Mentha Spicata*.

67 *Mentha spicata* is a creeping, rhizomatous and perennial herb with a rich supply of  
68 polyphenols and mostly cultivated in the tropical and temperate regions [16]. It is  
69 characterised by an aromatic smell and pungent giving sensation, hence in aroma-  
70 therapy, its potential as an insect repellent is promising. *Mentha spicata* has insecti-  
71 cidal value against ants, mosquitoes, wasps, hornets and cockroaches amongst others  
72 [9, 16].

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

## 77 **2. Materials and Methods**

### 78 **2.1 Solvents, Reagent and Materials**

79 Glycerine (VWR Chemicals BDH, USA), Ferric chloride (BDH Laboratory Supplies,  
80 England), HCl (AvonChem, U.K), Na<sub>2</sub>CO<sub>3</sub> (Wardle Chemicals, Calveley).

81

## 82 2.2 Sampling and Sample Preparation

83 The *Mentha spicata* leaves and the neem seeds were collected from the School of Bio-  
84 logical Sciences herbarium, University of Cape Coast, Ghana. The *Mentha spicata*  
85 leaves and the neem seeds were air dried for four weeks at laboratory conditions (26.4  
86 ± 2°C) to reduce the moisture content and was milled into fine powder to increase the  
87 surface area for extraction. The armyworms were collected from an unsprayed maize  
88 field at Kwaprow, a suburb in Cape Coast, Central Region, Ghana, and kept in a  
89 perforated container at laboratory conditions (26.4 ± 2°C). The army worms were fed  
90 with maize leaves collected for 12-30 days. About 50 4<sup>th</sup> instar larvae were collected  
91 and reared until a sufficient population of second-generation larvae was obtained for  
92 the study.

93

## 94 2.3 Preparation of reagents - Mayer's reagent

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

98

## 99 2.4 Extraction of Samples using Selective Solvents

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

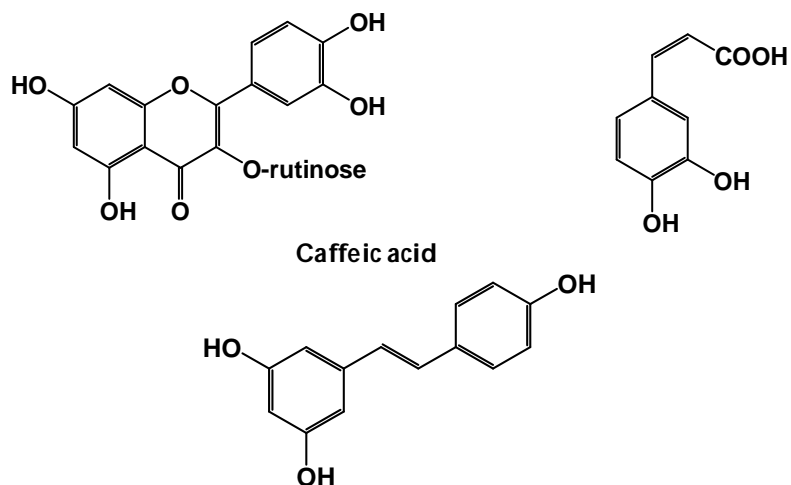
111

## 112 2.5 Phytochemical Screening of Extracts

### 113 2.5.1 Detection of phenolics

114 The detection of phenolics was done using a method described by Tiwari *et al.*, 2011.  
115 0.5 mL of the extract was measured into separate test tubes and 1 mL of 1% ferric  
116 chloride solution was added into each test tube. The formation of a bluish-black col-  
117 our indicated the presence of phenolic compounds. The chemical structures of com-  
118 mon plant derived phenolics, namely, Rutin, Caffeic acid and Resveratrol are shown  
119 in **Figure 1**.

**Comment [i1]:** if the method used neem seeds, please also explain in the introduction



120  
121 **Rutin**

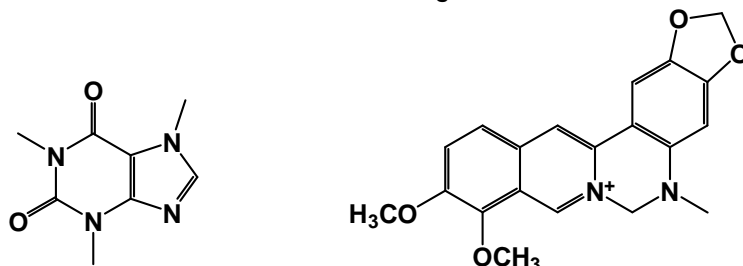
**Caffeic acid**

122  
123 **Resveratrol**

124 **Figure 1: Chemical structures of common plant derived phenolics (Rutin, Caffeic**  
125 **acid and Resveratrol)**

### 126 2.5.2 Detection of alkaloids

127 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**.



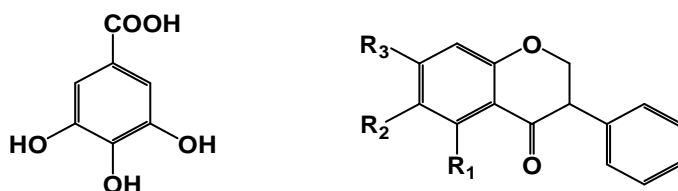
134  
135 **Caffeine**

**Berberine**

136 **Figure 2: Chemical structures of common plant derived alkaloids (Caffeine and**  
137 **Berberine)**

### 138 2.5.3 Detection of tannins

139 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 tannins, namely, Gallic acid, Daidzein, Genistein and Glycitein are shown in **Figure 3**.



147

148 Gallic acid

Daidzein:  $R_1=R_2=H$ ,  $R_3=OH$ 

149

Genistein:  $R_1=R_3=OH$ ,  $R_2=H$ 

150

Glycitein:  $R_1=H$ ,  $R_2=OCH_3$ ,  $R_3=OH$ 

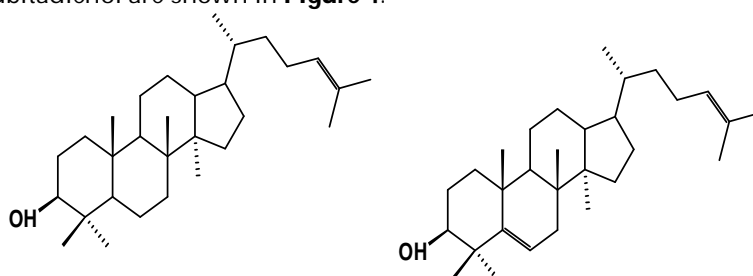
151

152 **Figure 3: Chemical structures of common plant derived tannis (Gallic acid, Daidzein, Genistein and Glycitein)**

153

#### 154 2.5.4 Detection of saponins

155 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. 156  
 157 The chemical structures of common plant derived saponins, namely, Dammarenediol and Cucubitiadienol are shown in **Figure 4**. 158  
 159



160

Dammarenediol

Cucubitiadienol

161

162 **Figure 4: Chemical structures of common plant derived saponins (Gallic acid, Daidzein, Genistein and Glycitein)**

163

#### 164 2.6 Quantitative Analysis of Extract

165 For each parameter, three repetitions were carried out to determine the concentration of the phyto constituents. 166  
 167

##### 168 2.6.1 Total Tannins Content (TTC)

169 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 170  
 171 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 172  
 173 set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100)  $\mu g/mL$  were prepared in the same manner as described earlier from a stock solution of 100  $\mu g/mL$  174  
 175 (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 spectropho- 176  
 177 tometer (UVD-3200, Labomed Inc., Los Angeles, California, USA). The tannin content was determined from the gallic acid standard curve. 178  
 179

### 180 2.6.2 Total phenolic content

181 The phenolic content detected using a method described by Singleton and Rossi [21].  
182 1.5 mL of the extracts was put into separate test tube and 0.2 mL of 0.1% Folin Cio-  
183 calteu reagent was added. The test tubes were incubated at room temperature for 4  
184 mins. 0.5 mL of 20% sodium carbonate was added and kept in the dark for 30 mins.  
185 The absorbance was read at a wavelength of 650 nm. Gallic acid of concentration: (20,  
186 40, 60, 80 and 100) ug / mL were used as standard for constructing calibration curve  
187 and water was used as the blank. The phenolic content was determined from the gal-  
188 lic acid standard curve.

### 189 2.6.3 Test for Antifeedancy

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

% Antifeedancy

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

\* 100

## 201 3. Results

### 202 3.1 Qualitative Phytochemical Analysis of Extracts

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

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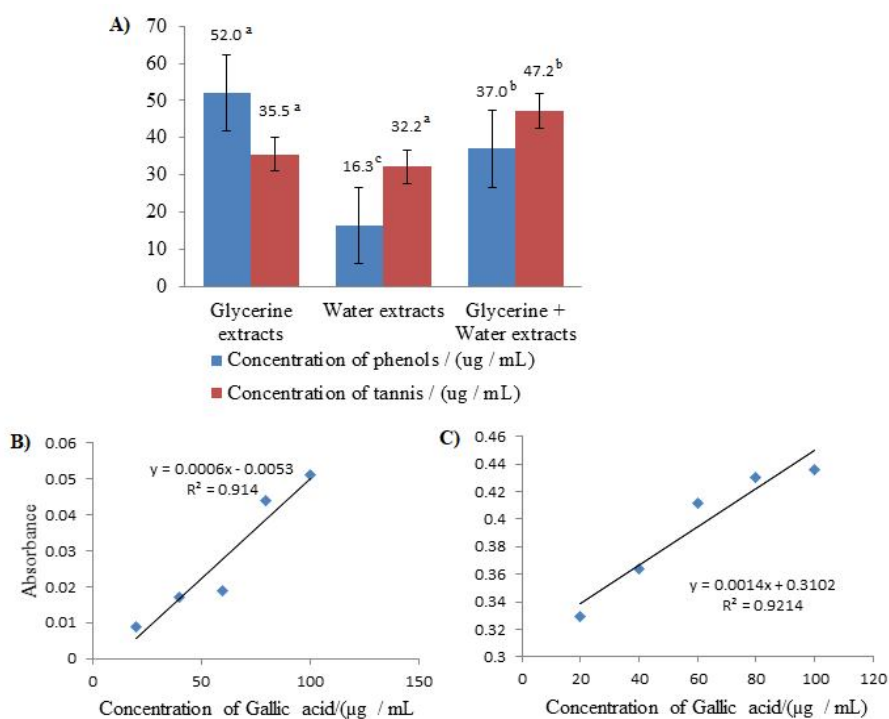
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213

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

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

215 The concentration of phenols and the concentration of tannins in the various aqueous  
 216 (water, glycerine, and glycerine plus water) extracts of *Mentha spicata* are shown in  
 217 **Figure 5A**. A one-way analysis of variance (ANOVA) confirmed significance differ-  
 218 ences in the concentration of phenols and tannins extracted by the various aqueous  
 219 extraction methods with empirical support ( $F(2,6) = 1319.906, p < 0.001$ ) and ( $F(2,6) =$   
 220  $64.495, p < 0.001$ ) respectively. With respect to phenols, the Bonferroni post hoc test  
 221 revealed that Glycerine extracts observed the highest concentration, followed by  
 222 Glycerine plus water extracts, with water extracts having the least concentration. With  
 223 regards to tannins, the post hoc test revealed no significant differences in the concen-  
 224 tration levels from Glycerine and water extracts. Glycerine plus water extracts ob-  
 225 served the highest concentration of tannins. The calibration curves for the extrapolation  
 226 of the concentration of total phenolics and tannins contents are shown in **Figure 5** (B,  
 227 C).

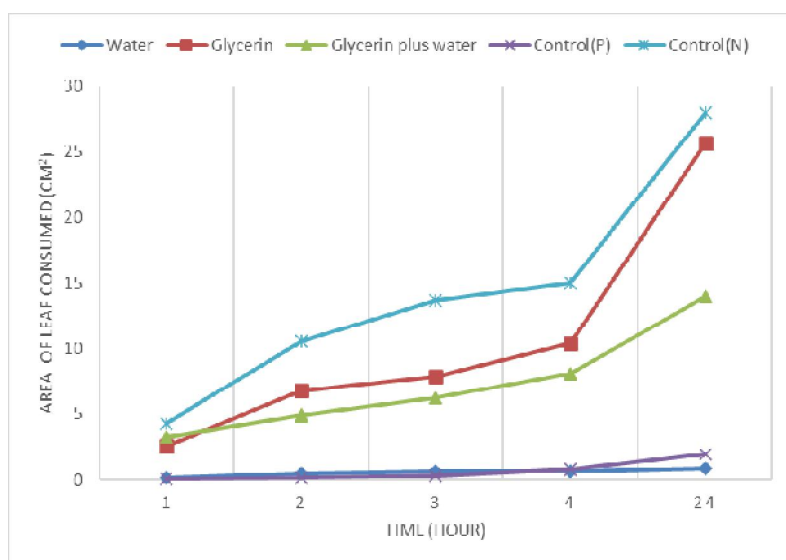


228

229 **Figure 5:** A) Concentration of phenols and tannins in different aqueous extracts of  
 230 *Mentha spicata*. B) Calibration curve of tannins C) Calibration curve for phenols

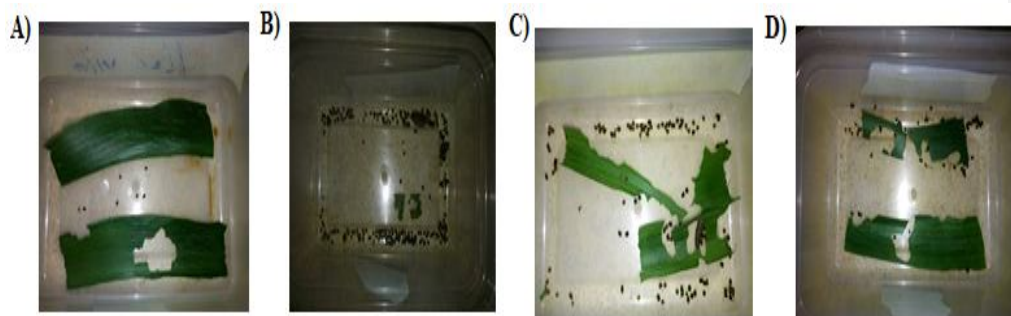
### 231 3.2 Efficacy of the different aqueous extracts of *Mentha spicata* as antifeedants

232 The rate of antifeedancy of the armyworms for 24 hours exposure to leaves with the  
 233 extracts applied is shown in **Figure 6**. A positive control and negative control were set  
 234 using neem seed water extracts and ordinary distilled water, respectively. Comparatively,  
 235 there was less feeding activity on leaves onto which the extract with water was  
 236 applied, demonstrating high efficacy of antifeedancy. Extracts from glycerine plus  
 237 water were more productive in antifeedancy compared to that of glycerine only. Out  
 238 of the total leaf area of 28 cm<sup>2</sup>, the army worm consumed 13.17 cm of the leaves in  
 239 glycerine plus water in 24 hours, whereas the total leaf in glycerine extract decreased  
 240 by 34.86 cm<sup>2</sup> following a 24-hr army worm exposure (see **Figure 6** and **7**).



241 **Figure 6:** Rate of leaf consumption by armyworms (cm<sup>2</sup>/hour)

243



244 **Figure 7:** Observed area of consumption of maize leaves by *Spodoptera frugiperda* A)  
 245 Water extracts B) Glycerine extracts C) Glycerine plus water extract D) Control (Neem  
 246 seed)  
 247

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

251 **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

#### 252 4. Discussion

253 The discovery of less-expensive and non-hazardous alternatives for the management  
 254 of insect-pests remains an urgent need to mitigate the rising trends of insect resistance  
 255 and the harmful environmental pollution by synthetic insecticides. In this study, dif-  
 256 ferent aqueous extracts of *Mentha spicata* were screened for their phytochemical  
 257 properties including phenols, saponins, tannins and alkaloids. The effects of the var-  
 258 ious extracts on *Spodoptera frugiperda* were also explored. The result of the present  
 259 study has indicated that water extract, glycerine and water/glycerine (60:40, v/v) ex-  
 260 tracts all contained phenols and tannins but not saponins. Alkaloids were present in  
 261 water extract but absent in glycerine, and water/glycerine (60:40, v/v) (**Table 1**). The  
 262 highest phenolic content of 52.0 µg/mL was recorded for glycerine, followed by 37.0  
 263 µg/mL from glycerin (60%) plus water (40%) extracts, with water extract having the  
 264 lowest phenolic content of 16.3 µg/mL (Figure 1). Glycerine plus water extracts had  
 265 the highest concentration of 47.2 µg / mL tannin content, followed by glycerine (35.5  
 266 µg/mL) and water (32.2 µg/mL) (Figure 5A).

267 The antifeedant activity was clearly influenced by the solvents used for extraction.  
 268 Antifeedant effects of different aqueous extracts of *Mentha spicata* were evaluated by  
 269 observing the leaf area consumed by *Spodoptera frugiperda* after the application of the  
 270 extracts. Among the extracts tested, the water extracts of *Mentha spicata* was found to  
 271 be the most effective against *Spodoptera frugiperda* with antifeedant activity of 97% as  
 272 against the *Azadirachta indica* (neem) seed water extracts (positive control) with anti-  
 273 feedant activity of 93.07% (**Table 2**). The potency *Mentha spicata* water extracts proved  
 274 to be a better antifeedant against fall armyworms (**Figure 7**) compared to *Azadirachta*  
 275 *indica* seed extracts which has been proven to have antifeedancy against other *Spod-*  
 276 *optera spp*[22]. Isman [23] reported that antifeedants can be found amongst all the  
 277 major classes of secondary metabolites (e.g.,alkaloids, phenolics and terpenoids)  
 278 which are the most likely toxic substances against insects. The water extract tested  
 279 positive for the presence of alkaloids (**Table 1**), hence the maximum antifeedant ac-  
 280 tivity observed for the water extract may be attributed to the presence of alkaloids.  
 281 Glycerine in water extract of *Mentha spicata* had an average antifeedant activity of  
 282 49.96% against *Spodoptera frugiperda*, corresponding with a significantly consumed  
 283 maize leaves (**Table 2**). The area of maize consumed was more pronounce upon ex-

**Comment [i2]:** the mention of the *Azadirachta indica* (neem) should be in the introduction, then referred to as neem only or *Azadirachta indica*

284 posure with glycerine extract of *Mentha spicata* and the recorded antifeedant activity  
285 was 8.21% the antifeedant activity of against armyworms (**Figure 7, Table 2**). The  
286 poor antifeedant activity of the glycerine extracts may be attributed to the extraction  
287 time as well as the concentration of the sample which may be insufficient to extract  
288 the biological components required for antifeedancy [24]. The results indicated that  
289 the active phytochemicals present in the water extract of *Mentha spicata* modulate  
290 feeding behaviour and make the food unpalatable resulting in feeding deterrence. The  
291 water extract presented the overall least content of phytochemicals, phenols, and  
292 tannins, except for alkaloids which were in abundance, it exhibited the highest (most  
293 active) antifeedant effect. In a similar study, water extracts of some *Mentha spp* namely  
294 *M. piperita*, *M. pulegium*, and *M. spicata* exhibited significant nematicidal activity  
295 against *Meloidogyne incognita*, with the *M. spicata* water extracts exhibiting an EC50  
296 value of 300 mg/L after 72 hours of exposure of *Meloidogyne incognita* to the extract  
297 [25].

298 This observation may be attributed to the possibility of the abundant phytochemicals  
299 i.e active components present in the water extract. Since polyphenols and tannins  
300 have many polar hydroxyl groups, the water extracts should have given the highest  
301 concentration. However, this was not the case in our study. According to Tiwari et al.,  
302 [24], the decrease in the phenolic and tannin content of the water extract may be at-  
303 tributed to the activity of the enzyme, polyphenol oxidase, which degrades poly-  
304 phenols in aqueous media.

305 The cost of synthetic insecticides and pesticides is high, even after Government sub-  
306 sidies. The extraction method proposed in the study could easily be implemented by  
307 farmers to combat the infestation of *Spodoptera frugiperda*. Thus, our research offers a  
308 unique alternative and a cheaper option for local farmers who struggle to purchase  
309 synthetic insecticides. In addition, insecticides derived from a synthesised natural  
310 product like the one highlighted in this research, are environmentally friendly and a  
311 safer for agricultural produce. Optimising the extraction method and synthesising  
312 these products on a large scale will alleviate the financial burden on local farmers,  
313 while maximising crop yield. As a limitation, the study only focused on specific ratio  
314 of the water-glycerine mixtures and would suggest that various ratio concentrations  
315 of the water-glycerine mixtures are compared to determine which concentrations will  
316 deliver the best antifeedant activity. Also, the search for promising antifeedants  
317 against fall armyworms should be expanded to other variety of plants. *Mentha spp*  
318 although not commercially cultivated in Ghana, they are readily accessible and has  
319 very promising prospects in the scientific and agricultural fields.

## 320 5. Conclusions

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

328 **Institutional Review Board Statement:** Not applicable.

329 **Informed Consent Statement:** Not applicable.

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

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Comment [i3]: if possible replaced  
with a newer reference