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2
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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26
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 sys-
55 tems and constitute a rich source of bioactive compounds called phytochemicals—
56 metabolically produced chemicals which are generally important for fighting against
57 bacteria, fungi, and plant virus infections. These phytochemicals which include fla-
58 vonoids, 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 in vivo 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 ticial 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 [15]. 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, 15].

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), Na₂CO₃ (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 4th 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 [16]. 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**.

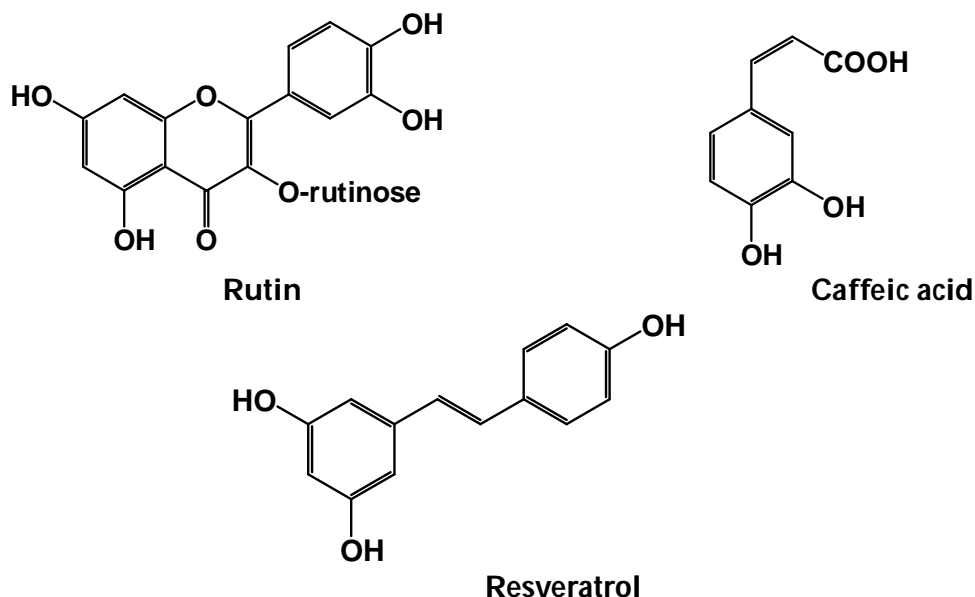


Figure 1: Chemical structures of common plant derived phenolics (Rutin, Caffeic acid and Resveratrol)

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**.

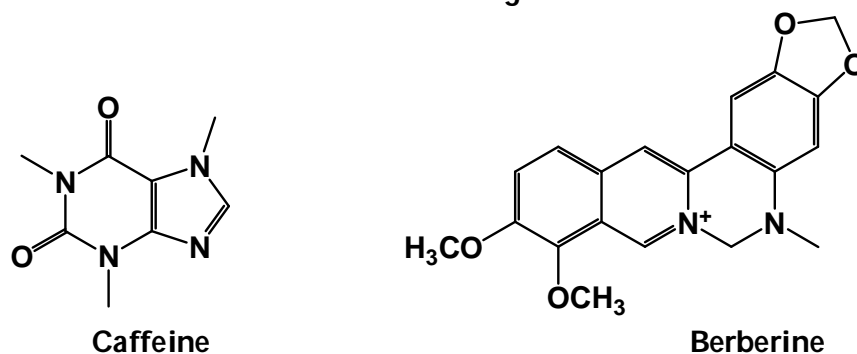
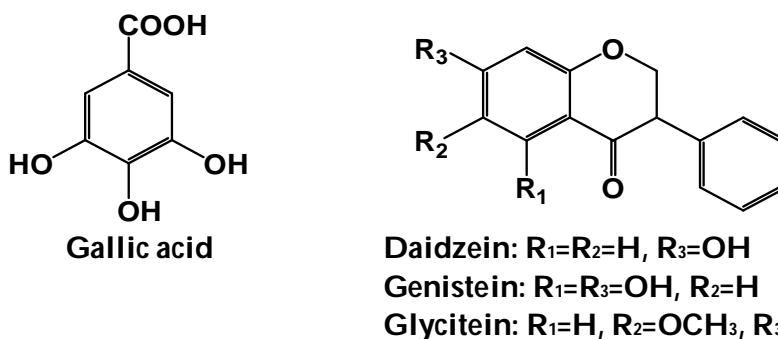


Figure 2: Chemical structures of common plant derived alkaloids (Caffeine and Berberine)

2.5.3 Detection of tannins

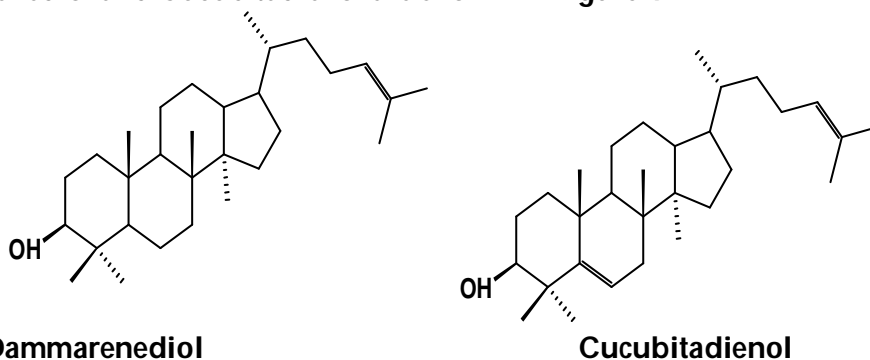
The detection of tannins was done using the method described by Kumar *et al.*, [18] with slight modifications. 0.5 mL of the extract was measured into separate test tubes and mixed with 2.5 mL of water. The mixture was heated for 10 minutes and filtered. 1 mL of 1% ferric chloride solution was then 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 tannins, namely, Gallic acid, Daidzein, Genistein and Glycitein are shown in **Figure 3**.



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

153 2.5.4 Detection of saponins

154
155 The detection of saponins was done using the foam test as described by Ekwueme *et*
156 *al.*, 2015 [19]. 5 mL of the extracts was added to 5 mL of distilled water and shaken
157 thoroughly. The persistence of foam for ten minutes indicated the presence of
158 saponins. The chemical structures of common plant derived saponins, namely,
159 Dammarenediol and Cucubitadienol are shown in **Figure 4**.



160
161
162 **Figure 4: Chemical structures of common plant derived saponins (Gallic acid,**
163 **Daidzein, Genistein and Glycitein)**

164 2.6 Quantitative Analysis of Extract

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

168 2.6.1 Total Tannins Content (TTC)

169 The quantitative tannin content in the extracts was estimated by Folin-phenol method
170 a described by Tamilselvi *et al.*, [20]. 0.1 mL of the sample/extract was added to 7.5 mL
171 of distilled water and 0.5 mL of Folin-phenol reagent was added. After that 1.0 mL of
172 35 % Na₂CO₃ solution was added, and the mixture was diluted to 10mL with distilled
173 water. The mixture was shaken well and kept at room temperature for 30 minutes. A
174 set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100) µg/mL were
175 prepared in the same manner as described earlier from a stock solution of 100 µg/mL
176 (i.e. 2 mg/20 mL) and water was used as the blank. Absorbance for test and standard
177 solutions were measured at a wavelength of 725 nm with an UV/VIS spectropho-
178 tometer (UVD-3200, Labomed Inc., Los Angeles, California, USA). The tannin content
179 was determined from the gallic acid standard curve.

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 cannibal-
 196 ism. 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:

$$201 \text{ \% Antifeedancy} = \frac{\text{Total Area of Leaves} - \text{Total Area of Leaves Consumed after 24 hours}}{\text{Total Area of Leaves}} * 100$$

202 3. Results

203 3.1 Qualitative Phytochemical Analysis of Extracts

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

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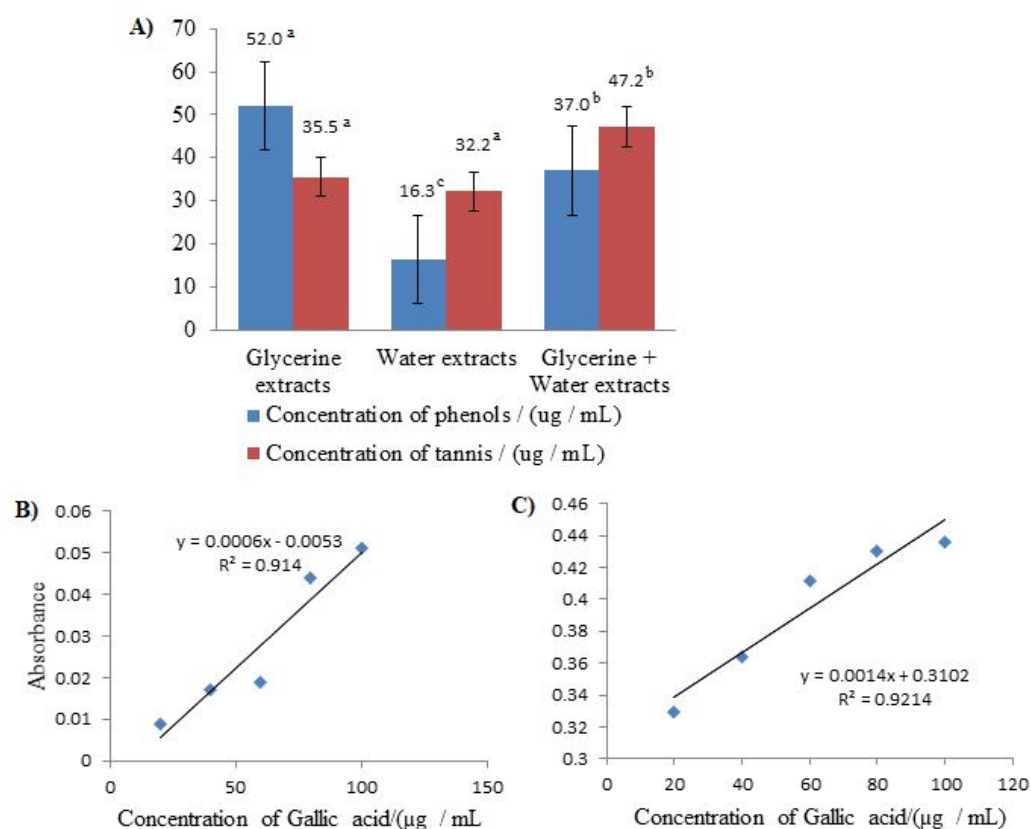
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215 **Table 1:** Phytochemical screening of the different aqueous extracts of *Mentha spicata*

Extract	Phenol	Saponin	Tannin	Alkaloid
---------	--------	---------	--------	----------

Glycerine	+	-	+	-
Water	+	-	+	+
Glycerine plus Water	+	-	+	-

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

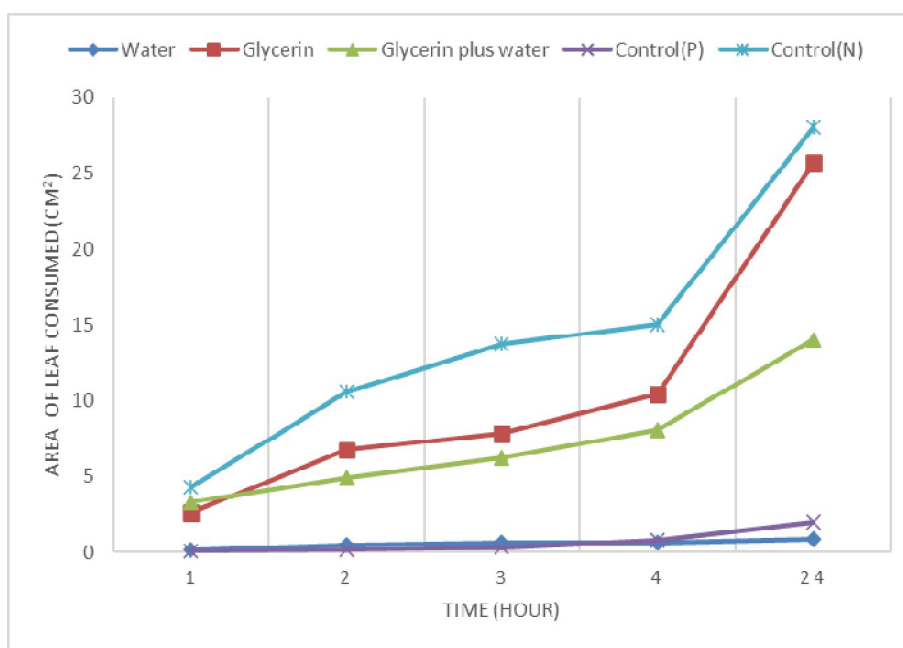


229

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

232 3.2 Efficacy of the different aqueous extracts of *Mentha spicata* as antifeedants

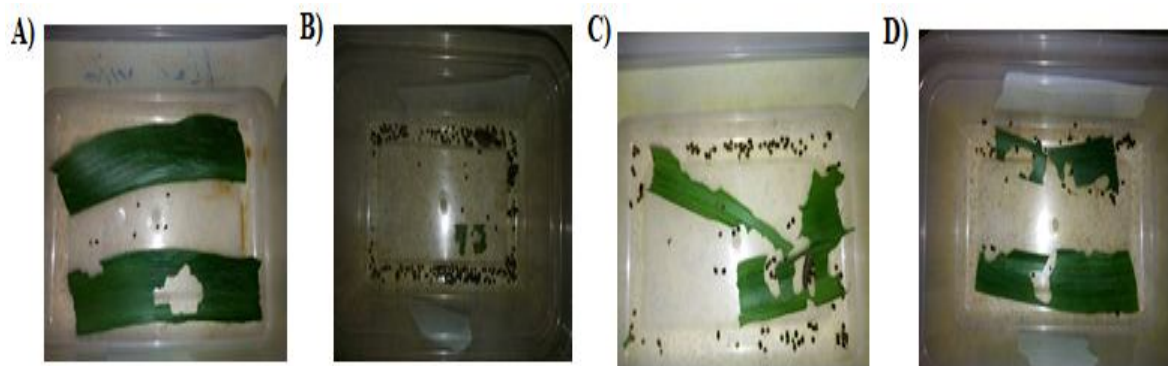
233 The rate of antifeedancy of the armyworms for 24 hours exposure to leaves with the
 234 extracts applied is shown in **Figure 6**. A positive control and negative control were set
 235 using neem seed water extracts and ordinary distilled water, respectively. Compara-
 236 tively, there was less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



242

243 **Figure 6:** Rate of leaf consumption by armyworms (cm²/hour)

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245

246

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

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

253 **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

254 4. Discussion

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

269 The antifeedant activity was clearly influenced by the solvents used for extraction.
 270 Antifeedant effects of different aqueous extracts of *Mentha spicata* were evaluated by
 271 observing the leaf area consumed by *Spodoptera frugiperda* after the application of the
 272 extracts. Among the extracts tested, the water extracts of *Mentha spicata* was found to
 273 be the most effective against *Spodoptera frugiperda* with antifeedant activity of 97% as
 274 against the *Azadirachta indica* (neem) seed water extracts (positive control) with anti-
 275 feedant activity of 93.07% (**Table 2**). The potency *Mentha spicata* water extracts proved
 276 to be a better antifeedant against fall armyworms (**Figure 7**) compared to *Azadirachta*
 277 *indica* seed extracts which has been proven to have antifeedancy against other *Spo-*
 278 *doptera spp* [22]. Isman [23] reported that antifeedants can be found amongst all the
 279 major classes of secondary metabolites (e.g., alkaloids, phenolics and terpenoids)
 280 which are the most likely toxic substances against insects. The water extract tested
 281 positive for the presence of alkaloids (**Table 1**), hence the maximum antifeedant ac-
 282 tivity observed for the water extract may be attributed to the presence of alkaloids.
 283 Glycerine in water extract of *Mentha spicata* had an average antifeedant activity of
 284 49.96% against *Spodoptera frugiperda*, corresponding with a significantly consumed
 285 maize leaves (**Table 2**). The area of maize consumed was more pronounce upon ex-

286 posure with glycerine extract of *Mentha spicata* and the recorded antifeedant activity
287 was 8.21% the antifeedant activity of against armyworms (**Figure 7, Table 2**). The
288 poor antifeedant activity of the glycerine extracts may be attributed to the extraction
289 time as well as the concentration of the sample which may be insufficient to extract
290 the biological components required for antifeedancy [16]. The results indicated that
291 the active phytochemicals present in the water extract of *Mentha spicata* modulate
292 feeding behaviour and make the food unpalatable resulting in feeding deterrence. The
293 water extract presented the overall least content of phytochemicals, phenols, and
294 tannins, except for alkaloids which were in abundance, it exhibited the highest (most
295 active) antifeedant effect. In a similar study, water extracts of some *Mentha spp* namely
296 *M. piperita*, *M. pulegium*, and *M. spicata* exhibited significant nematicidal activity
297 against *Meloidogyne incognita*, with the *M. spicata* water extracts exhibiting an EC50
298 value of 300 mg/L after 72 hours of exposure of *Meloidogyne incognita* to the extract
299 [24].

300 This observation may be attributed to the possibility of the abundant phytochemicals
301 i.e active components present in the water extract. Since polyphenols and tannins
302 have many polar hydroxyl groups, the water extracts should have given the highest
303 concentration. However, this was not the case in our study. According to Tiwari et al.,
304 [16], the decrease in the phenolic and tannin content of the water extract may be at-
305 tributed to the activity of the enzyme, polyphenol oxidase, which degrades poly-
306 phenols in aqueous media.

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

322 5. Conclusions

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

330 Disclaimer

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 333 [As per journal policy, pre-print article can be published as a journal article, provided it is not published in
 334 any other journal]

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