Effect of powdered extract of peppermint (*Mentha piperita*) on adult cowpea bruchid (*Callosobruchus maculatus*) mortality during storage in Jos - Plateau

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ABSTRACT: The over dependence and continues use of synthetic insecticides to control storage pests have been associated with several demerits. This study was conducted to evaluate the insecticidal effect of peppermint (*Mentha piperita*) leaf powder on *Callosobruchus maculatus*. The peppermint plant was sourced locally and the *C. maculatus* were cultured to obtain the adult Bruchids. The peppermint was washed, dried under ambient temperature and pulverized. Complete Randomized Design was adopted for this trial with three replications of six treatment dosages of 2.5, 5.0, 7.5, 10.0, 12.5 and 0.0 g respectively of the plant material. The trial lasted for four weeks. High Bruchids mortality was observed after treatment with *Mentha piperita* leaf powder extract. Phytochemical screening of the test plant revealed presence of flavonoids, tannins, saponins, carbohydrates and cardiac glycosides. Descriptive statistic and analysis of variance (ANOVA) were used to analyze the data. Means (p≤0.05) were separated using Duncan Multiple Range Test (DMRT). Dosage rate and mean mortality values at the various doses were statistically significant after 48, 72 and 96 hours. The mortality could be attributed to high presence flavonoid in the *Mentha piperita* leaf powder. The results suggest that the leaf of peppermint has insecticidal property against adult *C. maculatus*. However, further trials are required to investigate effective dosage rates for other stored grains and to isolate and characterize the active flavonoids contained in the plant.

Keywords: Bruchid, cowpea, *Mentha piperita*, mortality, phytochemicals, storage pests.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L) Walp) is an important legume in many developing countries. It is grown mainly for its grains as a major source of vegetable protein in sub-Saharan Africa where it constitutes an important part of the local diet, culture and economy (Olakojo et al., 2007; Deshpande et al., 2011; Lephale et al., 2012). The seed is regarded as a completely balanced food that feeds millions of people in developing worlds with an annual world-wide production estimated around 4.5 metric tons on 12 to 14 million hectares (Diouf, 2011). According to IITA (2001), it was reported that Nigeria is the world leading producer of cowpea, producing about 272,672 metric tons annually.

Cowpea production in the country is faced with a wide range of biotic and abiotic constraints (Singh, 2005). In storage, *Callosobruchus maculatus*, is regarded as the most important and common pest of cowpea both in Africa...
and Asia (Jackai and Daoust, 1986; Deshpande et al., 2011). Estimates of 4 to 90% losses have been reported (IITA, 1989; Umeozor, 2005; Udo and Harry, 2013) due to perforations from newly hatched larvae which bore into the cowpea grains. Holes left in the seeds by the emerging adults, reduces the degree of usefulness and making the seeds unfit either for planting or human consumption (IITA, 1989). The initial infestation starts on the field and extends to the store.

Some synthetic insecticides available for the control of this key pest are Actellic (2%) or Actellic super and Phostoxin gas are very helpful to the farmer, but they are expensive and may not be available everywhere. Also, Phostoxin is a fumigant that can kill humans and animals (Joana and Gungula, 2010) and requiring training for their efficient use such that many cowpea growers cannot afford them, also farmers are not taught on how to apply them properly; they pose a health risk to the human population, killing of non-target species and destruction of natural enemies. Synthetic insecticides can cause pests resistance, resurgence and, development of resistance by insects etc. In the light of the above, alternative control consideration could be sourced from indigenous preservatives locked in plants as bio constituents (Yusuf et al., 2006; Mundi et al., 2012; Bamaiyi et al., 2006). Studies have shown that Green plants act as a reservoir for inexhaustible source of innocuous pesticides, which are non-toxic to mammals and easily biodegradable than synthetic chemicals (Joana and Gungula, 2010). Phytochemical constituents like anthraglycosides, phlobatanin, tannins, flavonoids, alkaloids, saponins, coumarins, phenols, carboxylic acids, terpenes etc., have conferred specific characteristics and properties to plants which could be exploited for use as insect pests antifeedant or repellents (Trease and Evans, 1989; Lee et al., 2003; Kumar et al., 2013)).

The peppermint plant (M. piperita, Lamiaceae) also known as Mentha balsamea Wild has been reported to be native to Europe, midle east but currently cultivated in different parts of the world (Behnam et al., 2006). The Leaves of peppermint are used as condiment in some food dishes and as flavoring for ice creams and liqueurs. Its essential oils and extracts of M. piperita leaves have been used as medicine to treat inflammation of the oral mucosa, cold, musculoskeletal pain, gastrointestinal and respiratory diseases (Mckay et al., 2006). The presence of secondary metabolites in its essential oils such as monoterpenoids and phenolic compounds have conferred this much desired ethnobotanical significance (Mimica-Dukic and Bozin, 2008). Diniz do Nascimento et al. (2020), reported that the chemical composition of peppermint essential oils may varies due to extraction method, environmental conditions, and geographical origin, but, in general, it contains as the major constituents: menthol, epoxycimene, linalool, menthone, eucalyptol, and neomenthol.

There are many high plant species in Nigeria whose phytochemical constituent potentials are still unexploited. However, this preliminary study was carried out to evaluate, amongst other objectives, the insecticidal efficacy of powdered of Mentha piperita in the control of the bruchid (Callosobruchus maculatus (L Walp) attacking cowpea (Vigna unguiculata (L Walp) seed in storage.

MATERIALS AND METHODS

Study area

The study was carried out in the Chemistry Laboratory of the Federal College of Jos, Plateau State, (09°.56’N; 08°.53’E at an altitude of 1,217 m), in the Northern Guinea Savanna agro-ecological zone of Nigeria (Kowal and Knabe, 1972).

Insect culture

Infested cowpea seeds were purchased from Katakro market in Jos, Plateau State. About 500 g of the infested cowpea seeds were weighed. The infested seeds containing the eggs was separated and put in a container. The containers used in rearing the insects were plastic containers measuring 17 cm and 17 cm diameter and depth, respectively. Each container was covered with 10 mm mesh sieve to allow free air circulation and also to prevent insects from escaping (Habib et al., 2013). This was carried out at ambient temperature of 28°C (±2) and relative humidity of between 70-75% for 21 days. The newly emerged bruchids of about 24 hours old were kept for the work.

Collection of plant material

The plant material Mentha piperita leaves were collected from Chinese garden Jos. The leaves were washed with distilled water and air dried for two weeks. The plant materials were pulverized into powder using a mortar and pestle and sieved to obtain uniform size. The powder was kept in air- tight containers labeled and stored ready to be used for the experiment.

Treatment application

The plant powder was added to each of the containers containing 100 g of the cowpea seeds in following dosages: 0.0 (control), 2.5, 5.0, 7.5, 10.0 and12.5 g. The cowpea grains and the plant powder were thoroughly mixed with the aid of glass rod and agitated for 5 to 10 minutes. Ten freshly emerged bruchids of about 24 hours’ old were introduced into each of the treatment. The containers were then arranged in the laboratory bench in a completely randomized design (CRD). All the treatments
were replicated three times. Mortality count was taken after 24, 48, 72 and 96 hours respectively. Mortality of adult bruchids was obtained by counting the number of dead bruchids in each of the translucent plastic after application of treatment and dead bruchids were removed after every count to avoid double-counting. The data were recorded after 24, 48, 72 and 96 hours according to Asawalam and Arukwe, (2004) and Jembere et al. (1995).

Data analyses

Data collected were analyzed using (ANOVA) to test for level of significance \((p≤0.05)\) on the effects of different dosages of the plant and mean separation was carried out using Duncan Multiple Range Test (DMRT).

Extraction methods of the plant material

100g of the pulverized leaves of peppermint was percolated in 200 ml of distilled water for 24 hours. The content was filtered using Whatman No1 filter paper and the filtrate was then concentrated on a bath to obtain the aqueous crude extract.

Phytochemical analysis

The determination of the bioactive constituents responsible for the insecticidal activity of peppermint \((Mentha piperita)\) was carried out using the method described by Rao et al. (2016). Qualitative phytochemical screening of alkaloids, tannins, saponins, flavonoids, carbohydrates, steroids, anthraquinones and cardiac glycosides were used for the analyses.

Test for alkaloids

About 2 ml of the aqueous extract of the plant sample was carefully measured into a test tube and 2 ml of Mayer’s reagent (potassium-mercuric iodide) was immediately added. The appearance of a yellow colour precipitate confirmed the presence of alkaloids.

Test for tannins

About 2 ml of the aqueous plant extract was added into 2 ml solution of 10% ferric chloride in a test tube. The observation of the presence of blue-black or greenish black precipitate confirmed the presence of tannins in the plant sample.

Test for flavonoids

About 1 ml of the extract was pipetted and added to 1 ml of 2N sodium hydroxide solution in a test tube. The appearance of yellow or orange colour indicated the presence of flavonoids in the sample.

Test for saponins

About 2 ml of the aqueous extract of the plant sample was added to 2 ml of distilled water in a graduated measuring cylinder. The content was then vigorously mixed by shaking. The formation of 1 cm layer of foam in the glass ware confirmed the presence of saponins.

Test for carbohydrates

To 2 ml of the extract sample, few drops of Molisch's reagent was added, followed by the addition of 1 ml of concentrated \(H_2SO_4\) to the side of the test tube. The mixture was then allowed to stand for 2 minutes after which 5 ml of distilled water was added. The formation of red or dull violet colour between the two layers indicated the presence of carbohydrate in the sample.

Liebermann-Burchard test for steroids

About 2 ml of the extract was added to 2 ml acetic anhydride in a test tube, after which few drops of concentrated sulphuric acid was added. The presence of blue-green ring confirmed the presence of steroids.

Test for anthraquinones

2 ml of the plant extract was added to 2 ml of dilute tetraoxosulphate (IV) acid. The content was placed in a water bath and then filtered. To the cold filtrate, equal amount of chloroform or benzene was added. The organic layer of benzene was then removed after which 5 ml of ammonia was added. The formation of red, pink or violet colour in the ammoniacaal lower layer indicated the presence of anthraquinones.

Test for cardiac glycosides

A few drops of glacial acetic acid were added to 2 ml of the aqueous extract. Drops of 10% ferric chloride and then concentrated tetraoxosulphate (IV) acid were then added. The formation of reddish brown colouration between the two layers showed the presence of cardiac glycosides.

RESULTS

Phytochemical screening

The results of the phytochemical screening of the powdered leaf extract of \(Mentha piperita\) leaves as presented in Table 1 indicated the presences of useful
Table 1. Phytochemical components of Mentha piperita leaf powder.

| Phytochemical       | Mentha piperita |
|---------------------|----------------|
| Alkaloids           | -              |
| Tannins             | ++             |
| Flavonoids          | +++            |
| Saponins            | +              |
| Carbohydrates       | +              |
| Steroids            | -              |
| Anthraquinones      | -              |
| Cardiac glycosides  | +              |

Note: - Negative; + = Mildly present; ++ = More Moderately present; +++ = Highly present.

Flavonoids were observed in the study. However, no significant traces of steroids, alkaloids and anthraquinones were detected in the crude plant leaf powdered extract (Table 1).

Figure 1. Effect of Mentha piperita powder on bruchid mortality after 48 hours under different dosages.

Figure 2. Effect of Mentha piperita powder on bruchid mortality after 72 hours under different dosages.

Figure 3. Effect of Mentha piperita powder on bruchid mortality after 96 hours under different dosages.

DISCUSSIONS

The phytochemical result of M. piperita leaves powder revealed the presence of some bioactive compounds such as saponins, tannins, carbohydrates and cardiac glycosides and flavonoids in varying amounts. The presence of these metabolites in this extract with known insecticidal properties (Rubabura et al., 2014) could be responsible for the mortality of the weevils. Although, this study did not set out to quantify the relative amount of the respective phytochemicals present in the extract or to isolate the compound believed to be responsible for the bruchid mortality. However, the mortality could be due to the presence of limonene present in the flavonoids fraction of the plant material. An independent study carried out by Hebeish et al. (2008) reported that limonene present in flavonoids of Mentha piperita were found responsible for...
the mortality of rice weevil, *Sitophilus oryzae*. Also, Jang 
et al. (2005) and Phillip et al. (2010) reported that limonene 
present in the flavonoids has been used as systemic and 
contact fumigants with toxicity that can cause up to 100% 
mortality in housefly, bruchids, saw-toothed grain beetle 
and German cockroach. Similarly, Kordali et al. (2006) 
reported about 100% mortality of the Colorado potato 
beetle during their studies as a result of limonene presence 
in flavonoids. Present mortality suggests diverse mode of 
action of the extract on the target pests. Results showed 
increased mortality of weevils with increased quantity of 
leaf powder extract applied over time. The percentage 
mortality of *Callosobruchus maculatus* at 48, 72 and 96 
hours showed a direct proportionality to applied dosages 
and time of exposure. The present study also agrees with 
Aniszewski (2007), who reported that some toxic 
secondary metabolites which can block inhibit enzymes 
activities, ionic channels, interfere with neurotransmission, 
loss of coordination, and death.

**Conclusion and Recommendation**

Data from this study indicate that mint plant leaf powder 
has potential for the preservation of cowpea seeds against 
*C. maculatus*. The study recommends that the leaf powder 
of peppermint could be used as an alternative control 
against adult *C. maculatus* during storage of cowpea 
seeds at 0.0125 kg powder of peppermint to 0.1 kg seeds. 
Also, further studies should be carried out to investigate 
effective dosage rates of the test plant extract for other 
stored agricultural grains.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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