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RESEARCH ARTICLE

EVALUATION OF GARLIC (*Allium sativum*) AND ONION (*Allium cepa*) EXTRACTS FOR THE MANAGEMENT OF FALL ARMYWORM (*Spodoptera frugiperda*) ON BABY CORN (*Zea mays* L) UNDER GREENHOUSE CONDITIONS

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ABSTRACT

The effects of *Allium sativum* and *Allium cepa* extracts on the Fall Armyworm and growth of Babycorn (Variety SG 18) under greenhouse conditions were evaluated. Treatments were laid out in a Randomized Complete Block Design (RCBD) with 3 replications. The factors included solvents used: Dichloromethane (DCM), Methanol (Me), Distilled water (Di) and type of plant used: *Allium sativum* and *Allium cepa*. The positive and negative controls were Coragen SC 200 (Co) and distilled water (Di), respectively. Data collection and analysis was done using appropriate procedures. The extraction yield was highest with Methanol for *A cepa* and distilled water for *A sativum*, while DCM yielded the least for both plants. Saponins, glycosides, alkaloid and tannins were present in all the plant species, but their presence was influenced by the solvent type. Flavoniods were only present in DCM-*A sativum* extract whose content was 5.2378 ± 0.1094 mg/mL. DCM and Methanolic extracts of *A cepa* and *A sativum* were as effective as Coragen SC 200 against FAW larvae as opposed to distilled water extract. No significant differences were noted for plant height and leaf numbers. Further evaluation should be done towards making commercially available and effective insecticide for integrated FAW management.

KEYWORDS

Variety SG 18, Fall armyworm, Alliaceae, botanicals.

1. INTRODUCTION

Baby corn is typically a maize (*Zea mays* L.) ear produced from regular corn plants which are harvested earlier, particularly when the silks have the size of 1-3 cm (Thavanprakash et al., 2005). The production of fresh vegetables including baby corn has increased from 239.7-279.7 million tonnes from 2003-2013 (FAO, 2015). It is one of the safest vegetable to eat (Kawatra and Sehgal, 2007). It is a good source of minerals such as phosphorus, potassium, calcium, zinc, iron and nutrients than other vegetables such as tomatoes, cucumber cauliflower and cabbages (Yodpetch, 1979). Baby corn is also rich in fibrous proteins that are easy to digest (Mishra et al., 2018). Similar to ordinary corn, baby corn productivity is reduced by pests and diseases. Fall armyworm (FAW)(*Spodoptera frugiperda*) is one of the problematic pest of maize. Its destruction was first reported in Georgia (Speir, 2012).

The need to combat the effects of this pest using synthetic pesticides, cultural, biological and mechanical methods have not been effective because each of these methods has its own unique limitations. For example, the use of chemical pesticides has not been effective in the management of FAW because the farmers fail to secure commercial chemicals due to their high cost, unavailability in local shops and ignorance of the chemical control methods (Turner and Chivinge, 1999). There is therefore need to seek for alternative methods of managing this pest using natural pesticides which are safer and eco-friendly. They reduce artificial interface and cut-down the use of synthetic pesticides (Stoll, 1988). Research findings have been documented on low-risk FAW management approaches using biopesticides based on biochemical, microbial or microbial pest management products for affected countries in Africa (Bateman et al., 2018).

FAO further emphasized the use of microbials and their extracts, botanicals, semiochemicals, inorganic biochemical, predators and parasitoids (FAO, 2018). Many of these have not been evaluated in Africa. Garlic contains volatile compounds that are mainly non-polar compounds and the bioactivity or repellence induced by these compounds could be related to higher volatility and the presence of functional groups that are capable of reacting with sensory receptors involved (Gaddaguti et al., 2014). It has been reported that DCM extract of garlic showed repellent activities against hard tick (*Hyalomma rufipes* (Acari: Ixodidae) (Nchu et al., 2016). Garlic essential oil, diallyl disulphide and diallyl sulphide oil induced symptoms of intoxication and necrosis in the larva, pupa and adult of *Tenebrio molitor* (Plata-Rueda et al., 2017).

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Garlic essential oil was also shown to possess insecticidal activity against *Blattella germanica*, *Reticulitermes speratus*, *Lycoriella ingenua*, *Ephestia kuehniella*, *Sitophilus oryzae*, *Sitophilus zeamais* and *Tribolium castaneum* (Tunaz et al., 2009; Park and Shin, 2005; Park et al., 2006; Huang et al., 2006; Mikhaiel, 2011; Mobki et al., 2014). Being in the same family, *A. cepa* has also been shown to possess insecticidal activity against insect pests. Several authors have supported the foregoing statement (Mann et al., 2011; Saidi et al., Itulya, 2006; Stoll, 2000; Usman et al., 2018; Salifu et al., 2019). These reports points out to limited research on the use of *A. sativum* and *A. cepa* for the management of FAW despite this plants showing insecticidal potential. This study therefore aimed at evaluating the effectiveness of *A. sativum* and *A. cepa* against FAW under local conditions for inclusions in the small-scale farmer FAW management schemes.

2. Materials and Methods

2.1 Experimental Site Description

The study was conducted at the University of Kabianga farm in Kericho County, Kenya. The site lies at an altitude of 2163 M above sea level and is within latitude 0° 49'0 N and longitude 35° 49'60 E. The average rainfall is 900-1200 mm per annum distributed mainly between the months of March and December with two distinct peaks in May and October. The soils at the site are Nitisols which are acidic, moderately deep and well drained (Jaetzold and Schmidt, 1982).

2.2 Experimental Materials

2.2.1 Plant Material

2.2.1.1 Baby corn

The plant material used was Baby corn; variety SG 18. It was planted in plastic pots (25 cm height x 30 cm width and 50 cm length) in a greenhouse at the University of Kabianga, School of Agriculture and Biotechnology Farm. The plants were watered as required. Fifteen days after seedling emergence, eight (8) 3rd instar larvae per plant were released onto the baby corn a modification of what used (Silva et al., 2017). The larvae were obtained from unsprayed infested maize farm in Kabianga and identified as such by an entomologist.

2.2.1.2 Allium sativum and Allium cepa

Fresh *A. sativum* cloves were purchased from the open market. The garlic cloves were peeled and surface sterilized using ethanol (99.9%). The garlic cloves were dried in the open shaded area. 300 g of garlic were weighed out and crushed in a blender. One litre of dichloromethane was added to the crushed garlic, 50% garlic extract mixture was obtained and placed into a glass container and extracted overnight at room temperature. The extract was filtered using a thin layer of cotton wool. The extract was subjected to a rotary evaporator (at 60-80 ^{0}C) to remove the dichloromethane thus leaving only a semisolid extract solution. The extract was then stored until the time of spraying. The same procedure was successively repeated for the other solvents (methanol and distilled water) for both 300 g of *A sativum* and 500g of *A cepa*.

2.2.2 Fall Armyworm

FAW larvae were obtained from the unsprayed maize farm at Kabianga and identified as such by an entomologist. The FAW were then infested to baby corns three weeks after emergence of the baby corn. Treatments were applied one week after infestation.

2.3 Experimental Design and Treatments

Treatments were laid out in a Randomized Complete Block Design (RCBD) with 3 replications. The factors included solvent used in extraction and the type of plant used. The Dichloromethane (DCM), Methanol extract (Me), Distilled water extract (Aq), positive control-Coragen SC 200 (Co) treated and negative control-Distilled water treated (Di). The plant type factor consisted of *A. sativum* (As) and *A. cepa* (Ac). The FAW infestation was done manually by infesting the whorls of all maize plants in experimental pots. Rate of infestation was eight 3rd instar larvae per plant following the protocol previously described by Davies et al. (1996) but slightly modifying the number of larvae and growth stage of the larvae. The bio-insecticides were applied using a hand sprayer. Each insecticide was thoroughly mixed with water following rate of application for 5-10 minutes. Each of the 500 ml solution was added to the hand sprayer and sprayed to each treatment. Plants treated with distilled water and Coragen SC 200 constituted the negative control and the positive control, respectively. Coragen SC 200 was applied at the recommended rate (Binias et al., 2017).

2.4 Data Collection

Data was collected on the following parameters.

2.4.1 Determination of the extraction yield

The extraction yield (%) was calculated as follows:
Extraction yield (%) =Weight of the extract after evaporating the solvent x 100/Dry weight of the sample.

2.4.2 Charaterization of phytochemical composition of A. sativum and A. cepa.

1 g and 0.5 g of crude extract was measured for both *A sativum* and *A cepa* respectively and constituted in 10ml of DCM, 10 ml methanol and 10 ml distilled water in its respective concentration. 1ml of the *A. sativum*- and *A. cepa*-dichloromethane, methanol and distilled filtrate was added to vails. The vails were labelled with the phytochemicals under analysis as saponins, flavonoids, glycosides, alkaloids and tannins respectively for each organic solvent used. The respective phytochemicals were tested as per the procedures described by [28-30] indicated here below (Sofowora, 1993; Trease and Evans, 1989; Harboene, 1973):

a. Saponins: Adding 2ml of distilled water in the extract then shaking gently; soapy characteristics indicated the presence of saponins.

b. Flavonoids: Into 1 ml of the sample, 2 ml of ammonium hydroxide solution was added. Two to three drops of concentrated sulphuric acid was then added; presence of yellow coloration indicated the presence of flavonoids.

c. Glycosides: In 1 ml of the plant extract addition of 2ml of acetic acid followed by 5% ferric chloride, and 2-3 drops of concentrated sulphuric acid were made; a reddish brown ring indicated the presence of glycosides.

d. Alkaloids: In 1 ml of the plant extract, 1 ml of Wagner's reagent (i.e. potassium iodide iodine) and 3 drops of concentrated sulphuric acid were added; deep brown appearance indicated presence of alkaloids.

e. Tannins: To 1 ml of the plant extract, 2 ml of ferric chloride was added; distinct layer indicates presence of tannins.

2.4.3 Quantification of Flavonoids in garlic-DCM extract

The total flavonoid content of DCM extract of *A sativum* was estimated by the method described with some modifications (Odhiambo et al., 2017). 1.0 mL of the extract was mixed with 4.0 mL of distilled water and subsequently with 0.30 mL of 10% $NaNo_2$ solution. After 5 minutes, 0.30 mL of 10% $AlCl_3$ solution followed by 2.0 mL of 1% NaOH solution were added to the mixture. Immediately, the mixture was thoroughly mixed, and absorbance determined at 510 nm versus the blank. A standard curve of catechin was prepared (0-1.25 mL/mL) and the flavonoid concentrartion expressed as catechin equivalents (mg catechin/g dried sample).

2.4.4 Larvae mortality

The data on effectiveness of the tested botanicals on the FAW were collected one week after treatment application. Data on the presence of live larvae on the baby corn plants indicated that a treatment was not effective (as given by a negative sign) while absence of FAW larvae on the plants indicated effectiveness of the treatment against the FAW (as given by a positive sign).

2.4.5 Plant height and leaf numbers

Plant height was determined using a meter rule while leaf numbers were determined by counting the fully expanded baby corn leaves. This was done at 60 days after planting.

2.5 Data Analysis

The data collected on plant height and leaf numbers were subjected to Analysis of Variance (ANOVA) of SAS statistical software. Data on phytochemical screening were qualitatively analysed and the results presented in form of tables.

3. RESULTS AND DISCUSSION

3.1 Determination of the extraction yield

Table 1: Effects of solvents on extraction yield of *A sativum* and *A cepa*

	Extraction % yield (w/w)		
Plant	**DCM**	**METHANOL**	**DISTILLED WATER**
A cepa	0.68	10.56	8.75
A sativum	0.53	0.77	9.45

Extraction yield is a measure of solvent and method's efficiency to extract out specific components from the plant matrix (Gurnani et al., 2016). It depends on the extraction method and the solvent used for extraction (Do et al., 2014). In the present study, bulbs of *A sativum* and *A cepa* were extracted with three solvents; DCM, methanol and distilled water. The extraction yields are presented on Table 1. *A cepa* extract was highest with methanol followed by water and least with DCM. On the other hand, *A sativum* extract was highest with distilled water followed by that of methanol and DCM yielded the least. It has been reported that the extract yield of *A cepa* and *A sativum* methanolic extracts was 6.8% and 7.0%, respectively, (Bhanot and Shri, 2010). Further, reported that *A cepa* distilled water yielded highest (16.76) while methanol gave the least (5.53) (Singh et al., 2017). It has been reported that absolute methanol to be more effective than water for extracting polyphenols from agricultural wastes (Lolita et al., 2012). Similarly, working with *Datura metel*, reported that methanol was the best extraction solvent (yielding 85.36%) followed by distilled water at 78% (Dhawan and Gupta, 2017).

Although *A sativum* and *A cepa* belong to the same genus, Allium; they gave different extract yields possibly because they are not of the same species. Even within the same species, varietal differences in extract yield have been reported (De Valle et al., 2008). A very important point has been fronted who indicated that there is no single solvent, which may be considered standard because it is usually different for different plant matrices (Al-Farsi and Lee, 2008). This is so because of the presence of various phytochemical compounds with different chemical characteristics and polarities, which may or may not be soluble in a particular solvent (Turkmen et al., 2006). Therefore, the recovery of polyphenols from plant materials is influenced by many other factors such as their solubility in the extraction solvent, the type of solvent, the degree of polymerization of phenols, the interaction of phenols with other plant constituents and the formation of insoluble complexes (Galvez et al., 2005).

3.2 Charaterization of phytochemical composition of A sativum and A cepa

Many secondary metabolites present in plants have been associated with biological activity including pesticidal activity. The phytochemical screening revealed that *A. sativum* and *A cepa* extarcts contain various biologically active compounds; saponins, flavonoids, glycosides, alkaloids and tannins. However, the availability of these compounds was influenced by the extraction solvent used.

3.2.1 Saponins

Saponins were only present in *A. sativum* distilled water extract while they were present in both methanolic and distilled water *A. cepa* extracts. Saponins are high molecular weight glycosylated plant secondary metabolites consisting of a sugar moiety linked to a triterpene or steroid aglycone (Price et al., 2987). Pesticidal attributes of saponins have been reported for other plants (Dorsaz et al., 1988; Sindambiwe et al., 1998). However, a group researcher reported that *A. sativum* did not contain saponosides (Kambou and Guissou, 2011)

3.2.2 Flavonoids

Flavonoids were only present in the DCM-*A sativum* extract. Flavonoids play an important role in the protection of plants against plant feeding insects and herbivores (Acheuk and Doumandji, 2013). Quercetin is a flavonoid found in onion (Behling et al., 2008). However, the flavonoids in onions are present as glycosides or aglycones as is clearly indicated by the presence of glycosides in the *A cepa* extracts (Corzo-Martinez et al., 2007). Flavonoids have very low solubility in water (Boumendjel et al., 2003; Mikaye et al., 1991). Flavonoids can inhibit enzymatic activity and prevent the growth of larvae of different insect species (Kim et al., 2000). Some flavonoids interfere in the process of moulting and reproduction of several insects through inhibition of the formation of the juvenile hormone (Oberdorster et al., 2001). Some have ovicidal effect, effects on oviposition, fecundity, mortality, weight reduction and emergence of adults (Salunke et al., 2005; Golawska et al., 2014).

3.2.3 Glycosides

Glycosides were present in all the plants extracts except in the methanolic extract of *A. sativum*. Cyanogenic glucosides present in plant species are considered to have an important role in plant defence against herbivores (Zagrobelny et al., 2004).

3.2.4 Alkaloids

Alkaloids were present in all the *A. sativum* and *A cepa* extracts regardless of the type of extraction solvent used. Alkaloids are the most important natural substances playing an important role in insecticidal role (Balandrin et al., 1985; Rattan, 2010). Furocoumarin and quinolone alkaloids extracted from *Ruta chalepensis* leaves showed larvicidal and antifeedant activities against the larvae of *Spodoptera littoralis* (Emam et al., 2009).

3.2.5 Tannins

Tannins were present in all the the *A. sativum* and *A cepa* extracts regardless of the type of extraction solvent used. Tannins have a strong deleterious effect on phytophagous insects and affect the insect growth and development by binding to the proteins, reducing nutrient absorption efficiency, and causing midgut lesions (Sharma et al., 2009; Barbehenn and Constabel, 2011).

Table 2: Phytochemical composition of *A sativum* and *A cepa*

	SOLVENTS	**Saponins**	**Flavonoids**	**Glycosides**	**Alkaloids**	**Tannins**
Plant						
Onion	DCM	-	-	+	+	+
	Methanol	-	-	+	+	+
	Distilled water	+	-	+	+	+
Garlic	DCM	-	+	+	+	+
	Methanol	+	-	-	+	+
	Distilled water	+	-	+	+	+

Notes: + present; - Absent

3.2.6 Quantification of Flavonoids in garlic-DCM extract

DCM-*A sativum* extract whose content was 5.2378 ± 0.1094 mg/mL.

3.2.7 Larvae Mortality

DCM and methanolic extracts of both *A sativum* and *A cepa* were as effective against FAW as the commercial insecticide (Coragen SC 200) as opposed to the distilled water extracts of both plants and the control where plants were sprayed with distilled water alone (Table 3). This study recorded a negative test for flavonoids with distilled water for the two plants. This could be one possible explanation for non-effectiveness against FAW larvae. It is also possible that despite testing possible for saponins, glycosides, alkaloids and tannins, these compounds were at low concentrations as reported for *Severinia buxifolia* [Truong et al., 2019). Contrary to this, it has been reported that effectiveness of a natural plant extract increase with decreasing polarity of the solvent (Denloye et al., 2000; Ojewole et al., 2000).

Allium cepa has been tried and proven effective in controlling diamond back moth (Ahmad and Ansari, 2010). A similar research was done where Dichloromethane extract of *Cedrela salvadorensis* and *Cedrela dugessi* afforded a photogedunin and cedrelanolide (Cespedes-Acuna et al., 2000). These compounds and photogedunin epimeric acetate 3 and 4 at 23-OH position were evaluated against *Spodoptera frugiperda.* When tested for the activity of on neonate larvae into the non-choice bioassays, gedunin, photogedunin epimeric mixture, and photogedunin acetates mixture caused significant larval mortality with LC_{50} of 39.0, 10.0, and 8.0 ppm at 7 days, respectively, as well as growth reduction.

Table 3: Effects of *A. sativum* and *A. cepa* botanical extracts on FAW

	SOLVENTS	EFFECT
Plant		
Onion	DCM	+
	Methanol	+
	Distilled water (extract)	-
Garlic	DCM	+
	Methanol	+
	Distilled water (Extract)	+
	Distilled water (-ve control)	-
	Coragen SC 200 (+ control)	+

KEY+: Effective against FAW larvae (No live larvae present); - Not effective against FAW larvae (Live larvae present).

3.2.8 Effects of A. sativum and A. cepa botanical extracts on Plant height and Leaf numbers

There was no significance differences between plant height and leaf numbers. Similar results were reported by (Sisay et al., 2019).

Table 4: Effects of *A. sativum* and *A. cepa* botanical extracts on Plant height and Leaf numbers

Treatment	Height	Leaf no
AsDCM	**32.8**	**7.5**
AcDCM	**32.1**	**8.2**
AsMet	**31.9**	**8.0**
AcMet	**32.9**	**8.0**
AsAq	**30.6**	**7.4**
AcAq	**31.5**	**7.8**
Di	**32.2**	**7.9**
Co	**32.4**	**7.4**
LSD 0.05%	**2.7**	**1.1**

4. Conclusions

Methanol and distilled water are the best solvents for *Allium sativum* and *Allium cepa* extract preparation. Such extracts are endowed with an array of phytochemicals associated with insecticidal activity as indicated by FAW larvae mortality. This proves that they can form an important IPM tool in sustainable farming systems to reduce that amount of synthetic pesticides used for the management of FAW. Further evaluation should be done towards making commercially available, effective, naturally derived insecticide for integrated FAW management.

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