Phytochemical Analysis and Efficacy Of Rosemary (*Rosmarinus Officinalis*) and Mint (Mentha Spicata) Extracts Against Fall Armyworm (*Spodoptera Frugiperda*) on Baby Corn (*Zea Mays*)

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Abstract:
Fall armyworm (Spodoptera frugiperda, J.E. Smith) is a pest with devasting effects on maize. A laboratory biassay was conducted to analyse the phytochemicals and determine the efficacy of M. spicata and R. officinalis extracts on FAW. Treatments were laid out in a Completely Randomized Design (CRD) with 3 replications. The factors included solvent [Methanol (Me), dichloromethane (DCM), distilled water (Di)] and the plant species (M. spicata and R. officinalis). Coragen SC 200 (Co) and Distilled water (Di) were the positive and negative controls, respectively. FAW rearing, plant extract preparation and phytochemical screening were done using standard procedure. Data collection and analysis was done using standard procedures. The extract yield was highest for R. officinalis regardless of the solvent used. Me-R. officinalis and Di-M. spicata extracts yielded the highest. Saponins, glycosides, alkaloid, flavonoids and tannins. Flavonoid contents were 7.9036 mg/mL and 6.0073 ± 0.6117 mg/mL in methanolic extract of M. spicata and R. officinalis, respectively. M. spicatha and R. officinalis extracts caused 100% mortality to 3rd instar larvae. Based on the findings, both M. spicata and R. officinalis have several secondary metabolites that confer insecticidal activity of the plants against FAW, hence should be evaluated under field conditions.

Keywords: Variety SG 18, Fall Armyworm, lamiaceae, botanicals.

1.0. Introduction

Baby corn (Zea may L.) a graminae refers to young corn ears [1] harvested right after silk emergence and consumed fresh or as preserves. It is considered the safest vegetable that is rich in proteins, carbohydrates, iron, phosphorus, β-carotenes and ascorbic acid [2]. In Kenya, baby corn is mainly produced for export by both small scale and large scale farmers. In 2014, it was grown on 567 Ha producing 4784 MT which had a value of KES 110 millions [3]. It has been shown that for successful baby corn production relies on among others, good cultural management which includes pest and disease control [4]. Like grain maize, it is attacked by many pests like beet armyworm (Spodoptera exigue) [5], corn stem borer (Ostrinia furnacallis)[6] and now the recently introduced and most problematic Fall armyworm (Spodoptera frugiperda) (FAW) [7].

A native to the Americas, the FAW was first reported in Kenya in March, 2016 [7]. It plays host to 186 species from 42 families [8]. This pest causes a big food security threat to Kenya because it is a pest of staple crops like maize, sorghum and millet as emphasized by [9]. There are efforts by stakeholders to manage this pest using various methods; chemical pesticides, cultural methods, biological control and use of botanicals. None of these methods will be effective against FAW since each one of them has been shown to have deficiencies. [9] reported that the preference should be given to a sustainable, cost-effective method that causes minimal risk to humans and the environment by using a combination of control methods in an integrated pest management (IPM) strategy. However, because of the magnitude of the damage by FAW, most farmers have found chemical control their best method for managing this pest. This has been not sustainable because most farmers have limited access to pesticides hence the need to search for cheap, accessible and sustainable alternatives such as the use of extracts based on locally available plants like Mint (Mentha spicata and Rosemary (Rosemarinus officinalis).

Mint essential oil has been used against various insect pests; peach-potato aphid (Myzus persicae) [10]; mosquitoes [11- 12]; black bean aphid (Aphis fabae) larvae [13]. Similarly some studies were conducted on rosemary essential oils for pest management; spidermite (Tetranychus urticae)[14-15], pests of stored products [16-17], onion thrips (Thrips tabaci) [18], Pseudaletia unipuncta, cabbage looper (Trichoplusia ni [19], Trogoderma granarium and Tribolium castaneum [20]; Oryzaephilus surinamensis and T. castaneum [21]. There is a clear indication that there is limited literature on the use of leaf extracts of these plants for the management of crop pests and specifically FAW. This study therefore sought to evaluate the efficacy of M spicata and R officinalis for the management of FAW under laboratory conditions.

2.0. Materials and Methods

2.1. Experimental Site Description

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. Four weeks after seedling emergence, the plants were used as a source of treated leaves for the bioassay.

2.1.2. Rosmarinus officinalis and Mentha spicata

Leaves of R. officinalis and M. spicata were collected from farms in Nakuru, Kenya. The plants were identified as such by a taxonomist. The collected materials were washed, shade dried under room temperature and powdered using an electric blender. Five hundred grams (500 g) were macerated with 1.5 litres of dichloromethane, methanol and distilled water sequentially for a period of 72 hours each and then filtered by Whatman No. a filter paper. The extracts were concentrated at reduced temperature on a rotary evaporator and stored until use. The same procedure was successively repeated for the other solvents (methanol and distilled water). The yield of each was determined under each solvent used for extraction.

2.2. Fall Armyworm

Fall Armyworm starter colony was obtained from unsprayed maize farm in Litein and identified as such by an entomologist. About 100 fourth instar larvae were collected and placed in plastic containers and placed in the laboratory and fed them with maize leaves collected from 15-30 days maize plants. The pupae were collected and placed in a moistened Petri-dish in a net cage. Sterile cotton soaked in a sugar solution was placed in a petri-dish inside the net cage as a food source for the emerging adults. The walls of the net cage were lined with wax paper as an oviposition media [22-24]. Eggs were monitored daily for hatching; as soon as the first instars emerged, they were provided with tender and fresh baby corn leaves [22]. The insects were reared as described above until sufficient population was maintained to run the experiment. The rearing was done at room temperature 24-26 0C and 40-50 % RH. Second generation (F2) larvae was used for the study.

2.3. Experimental Design and Treatments

Treatments were laid out in a Completely Randomized Design (CRD) with 3 replications. The factors included solvent used in extraction and the type of plant used. The solvent of extraction factor consisted of Methanol extract (Me), dichloromethane extract (DCM), distilled water extract (Di), positive control (Co) and Negative control (Di). The plant type factor consisted of R. officinalis (R) and M. spicata (M). Maize shoots were obtained from the 4 weeks old seedlings grown in the farm as described above. The shoots were cut 2-3 cm length and weighed to 3 g. The pieces were placed in a petri-dish and sprayed

with 3 ml of each of the prepared botanical extracts and the controls. Five third-instar larvae were released into the petri-dish containing the treated leaves five minutes after leaves were treated. Leaves treated with Coragen 200 SC constituted the positive control and those treated with distilled water constituted the negative control.

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 M. spicata and R. officinalis
1 g and 0.5 g of crude extract was measured for both M. spicata and R. officinalis respectively and constituted in 10ml of DCM, 10 ml methanol and 10 ml distilled water in its respective concentration. 1ml of the M. spicata - and R. officinalis -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 standard procedures with slight modifications as described by [25-27] as indicated here below:
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 1ml 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 1ml of the plant extract, 1ml 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, 2ml of ferric chloride was added; distinct layer indicates presence of tannins.

2.4.3. Quantification of Flavonoids in M spicata and Me-R officinalis-Methanol
The total flavonoid content of M spicata and R officinalis-Methanolic extracts was estimated by the method described by [28] with some modifications. 1.0 mL of the extracts was mixed with 4.0 mL of distilled water and subsequently with 0.30mL 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
Larvae mortality was assessed after 24 hours and 48 hours (when all the larvae had died) after treatment application. A larvae was considered dead if it could not move itself when placed on its dorsal surface. Data on the presence of live larvae petri-dish indicated that a treatment was not effective (as given by a negative sign) while presence of dead FAW larvae on the petri-dish indicated effectiveness of the treatment against the FAW (as given by a positive sign).

2.5. Data Analysis
Data on extraction yield, phytochemical screening and larvae mortality were qualitatively analysed and the results presented in form of tables.

3.0. Results and Discussion

3.1. Extract Yield
Different solvents yielded different amounts of extracts. R officinalis had the highest extraction yield across the solvents used. However, methanol yielded the highest (13.18%) extract with R officinalis while distilled water gave the highest (5.76%) mint extract. DCM yield the least (0.86%) and (2.04%) for M spicata and R officinalis, respectively (Table 1).

Table 1: Effects of solvents on extraction yield of M spicatha and R officinalis

	Extraction % yield (w/w)		
Plant	**DCM**	**METHANOL**	**DISTILLED WATER**
M spicatha	0.86	1.07	5.76
R officinalis	2.04	13.18	7.43

3.2. Charaterization of phytochemical composition of M. spicata and R. officinalis
Plants synthesize a wide array of secondary metabolism compounds that are generally thought to be involved in plant-insect interactions. Water extracts of Mentha piperita have been shown to have alkaloids, saponins, glycosides, tannins and flavonoids [29]. On the other hand, several phytocompounds have been isolated from essential oils and extracts of R. officinalis [30]. The current study also reports the presence of saponins in R. officinalis only, alkaloids, glycosides, tannins and flavonoids in both plant species. There were slight variations in availability based on the solvent used for extraction.

3.2.1. Saponins
Saponins are widely distributed plant glycosides divided into triterpenoid and steroidal saponins that contribute to plant defense mechanisms against herbivores [31]. Studies have shown the presence of saponins in methanolic extract of R officinalis leaves [32]. However, there are contradicting reports on the presence of saponins in mint. The current study revealed that saponins were absent in M. spicata regardless of the solvent used as was reported by [33] for Mentha longifolia methanolic extracts. On the other hand, other mint species have been shown to be rich in saponins; for example, M. spicata [34], propanol extracts of M piperita [35], water extract of M. arvensis, M. piperita [29], methanolic and chloroform extract of M. piperita, [36] ethano-water extract of M. spicata, methanol extract of M. arvensis [37].

3.2.2. Flavonoids
Flavonoids were present in all the plants regardless of the extract used for extraction. Flavonoids are polyphenolic compounds that constitute a large group of secondary metabolites in plants [38]. They could be useful in a pest management strategy because both flavonoids and isoflavonoids protect the plant against insect pests by influencing their behavior, growth and development [39]. Mentha plants are rich in flavonoids particularly flavones and flavanones [41]. For example, the presence of flavonoids has been reported in M. spicata [34], Mentha longifolia [42, 33], wild mint species [43-44], Mentha piperita [33, 45-46], M. spicata [47] and Mentha arvensis [37]. R. officinalis has been shown to contain flavonoids [33, 48-51]. Flavonoid insecticidal effects have

been reported for Aedes aegypti, Drosophila melanogaster and Manduca sexta [52], Heliothis zea and Trichoplusia ni [53-54], Planococcus citri [55] and S. frugiperda [56]. Methanolic extract of M. spicatha had flavonoid content of 7.9036 mg/mL while that of R. officinalis was 6.0073± 0.6117 mg/mL.

3.2.3. Glycosides

Glycosides were present in all the plants extracts except in the methanolic extract of R. officinalis and DCM-extract of M spicata. Glycosides are compounds that result from binding of a special type of organic matter resulting from metabolism with one or more simple sugars [57]. The presence of glycosides have been reported in mint species like in M. spicata [34], M. longifolia subsp. longifolia [57]. The same has also been reported in R. officinalis by [57-62]. Insecticidal effects of glycosides have been reported by several researchers [63-69].

3.2.4. Alkaloids

All the solvents used were effective in extracting alkaloids of M spicatha and R officinalis. Presence of alkaloids in mint species such as M. spicata [34], M. longifolia [33], M. arvensis [37], M. piperita [29, 36]. R. officinalis has also been shown to contain alkaloids [33, 70]. Alkaloids are the same important group of natural substances playing an important role in insecticidal activity [71-72]. Researchers have reported this activity [68, 73-75].

3.2.5. Tannins

Tannins were present in all the plant extracts except the DCM- M spicata extract. Tannins are a group of water-soluble polyphenols of intermediate to high molecular weight [76]. They have been shown to be present in mint species such as M. spicata ([34], M. piperita [77-78]. They are also present in R. officinalis [33, 48, 62, 79-81].

Table 2: Charaterization of phytochemical composition of M. spicata and R. officinalis

	SOLVENTS	**Saponins**	**Flavonoids**	**Glycosides**	**Alkaloids**	**Tannins**
Plant						
M spicatha	DCM	-	+	-	+	+
	Methanol	-	+	+	+	-
	Distilled water	-	+	+	+	+
R officinalis	DCM	+	+	+	+	+
	Methanol	+	+	-	+	+
	Distilled water	+	+	+	+	+

Notes: + present; - Absent

3.3. Effects of M. spicatha and R. officinalis botanical extracts on FAW larvae

M. spicata and R. officinalis botanical extracts caused 100% mortality to 3rd instar larvae of FAW 48 hours of exposure to the treatments as was the case for the positive control (Coragen 200 SC). These findings indicate that that the studied plant species are more effective than the species reported by [82-83] in Ethiopia and Malawi, respectively.

Table 3: Effects of M spicatha and R officinalis botanical extracts on FAW larvae

	TIME	**24 HOURS**	**48 HOURS**
Plant			
M spicatha	DCM	+	+
	Methanol	-	+
	Distilled water	-	+
R officinalis	DCM	-	+
	Methanol	-	+
	Distilled water	-	-
	Coragen Sc 200	+	+
	Distilled Water	-	-

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

4.0. Conclusion

Based on the findings, it can be concluded that methanol and distilled water can be used to prepare the crude extracts of R. officinalis and M. spicata, respectively. Saponins, glycosides, alkaloid, flavonoids and tannins were present in both plant extracts. M. spicata and R. officinalis botanical extracts caused 100% mortality to 3rd instar larvae of FAW within 48 hours of exposure. Based on the findings, both M. spicata and R. officinalis have several secondary metabolites that confer insecticidal activity of the plants against FAW, hence should be evaluated under field conditions.

5.0. Acknowledgement

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6.0. References

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