Chemical composition of essential oils from Citrus species peels and its insecticidal potential effect on cowpea.

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ABSTRACT

Volatile oils from the peels of sweet orange (Citrus sinensis) lemon (Citrus limon) and grape fruit (Ctrus paradiisi) were comparatively evaluated on cowpea plant at flowering and at pod stage weekly four weeks.the volatile oil were extracted by GC/MS,the main compound of the peels essential oil were oleic acid, benzene 1-ethyl -2methyl,decane and undecane which constitute the bulk of the three oil in these percentages (22.56%, 23.19%, and 24.26%, 3.6%, 10.32% and 7.90%, 15.24%, 16.28% and 13.64%, 9.42%, 15.74% and 12.34%) for sweet Orange, Grape, and Lemon fruits. These oils were used for the growing of cowpea plant, the citrus peels had insecticidal properties.

Keyword: Fruit, Citrus, Extract, GC/MS, Oil.

Introduction

Citrus essential oils are obtained from various *Citrus* species found within the family Rutaceae and the subfamily Aurantioideae; these include about 17 species distributed throughout the tropical and temperate regions [1] they are evergreen trees that give fruits of different forms and sizes (from round to oblong), which are full of Fragrances, flavour and juice. The citrus genus includes various species; Oranges (*Citrus sinensis*), Lime (*Citrus aurantifolia*), Tangerine (*Citrus reticulate*), Lemons (*Citrus Limon*) and Grapefruit (*Citrus paradisi*). Citrus is one of the most important Citrus is widely grown in Nigeria and many other tropical and subtropical regions [2]. Commercially-grown crops in the world Cowpea (*Vigna unguiculata* (L) walp) is an annual herb with varying growth forms. It may be erect, trailing, climbing or bushy, usually in determinate under favorable conditions. Cowpea (*Vigna unguiculata* (L) walp) is one of the most ancient human food sources and has probably been used as a crop plant since Neolithic times. A lack of archaeological evidence has resulted in contradicting views supporting Africa, Asia and South America as origin. Some literature indicates that cowpea was introduced from Africa to the Indian subcontinent approximately 2 000 to 3 500 years ago, at the same time as the introduction of sorghum and millet, while others state that before 300 BC, cowpeas had reached Europe and possibly North Africa from Asia. Nigeria is the world's leading cowpea producing country, followed by Brazil. Other countries in Africa, e.g.

West Africa, are Nigeria, Senegal, Ghana, Mali and Burkina Faso. Ghana, Niger, and Cameroon are significant producers. Although Nigeria is the largest producer of cowpea in the world producing about 56 percent of the world production, it is also the largest consumer of cowpea in the world [3].

This research was aimed at determining the potentials of the peels of Citrus species to control the pest of cowpea both in the field and storage.

Materials and Methods

Study area

The research was carried out in the Laboratory of the Department of Biological Sciences, Nigerian Defence Academy Kaduna.

Methodology

Fully ripened fresh fruits of Sweet orange (*Citrus sinensis)*, Lemon (*Citrus limon*), Grapefruit (*Citrus paradiise*) were source sourced from station market Kaduna. While the seed of Cowpea (*Vigna unguiculata* (L) *walp*) variety Sampea 12 were obtained from the Institute of Agricultural Research (IAR) ABU Zaria.

Experimental design

This study adopted the complete randomized design, having five different treatments in three replica.

Treatment 1: Sweet orange
Treatment 2: Lemon
Treatment 3: Grapefruit
Treatment 4: Standard insecticides
And control (distilled water)

Extraction of oil

The fruit of the three different citrus species were washed properly with water before being peeled; the peels were washed again and dried at room temperature for about 4 weeks the peels were pounded using mortar and pestle to a powdered form. About 100g of each of the three citrus peels powder were packed into a Soxhlet apparatus and extracted exhaustively with 750 ml of Hexane for 3h. The solvent was recovered using a Rotary evaporator. The oils were made moisture free by filtering through the anhydrous sodium sulphate. The oils was transferred into brown sample bottles and stored in the refrigerator until they were ready for analysis.

Gas chromatography–mass spectrometry analysis.

Compound within the peels of citrus species was detected by GC-MS analysis in NARICT Zaria based on the method described by [4] with some modification. The identification of peaks were based on computer matching of the spectra with the national institute of standards technology (NIST 08 and NIST 08s) library and by direct comparism with published data

Qualitative and quantitative analysis of the compounds

The compounds in each sample were been identified by comparison of their mass spectral pattern and their linear retention indices based on a homologous series of even normal alkanes (C_8–C_{24}) with those of authentic references 29 and the Wiley 257 mass spectra database. The percentage of each compound was calculated from peak area obtained by FID.

Phytochemical test of peels extract

Phytochemical screening for alkaloids, steroid, flavonoids, tannins, terpenoids and saponins were carried out as described below.

Test for Tannins

About 2ml of the aqueous extract was stirred with 2ml of distilled water and few drops of $FeCl_3$ solution (5% w/v) were added. The formation of a green precipitate indicated the presence of tannins.

Test of Saponins

To 5ml of aqueous extract was added 5ml of distilled water in a test tube the mixture was then warmed to a degree of a honey comb-shaped, stable form. Was taken as an indication for presence of saponins.

Test for Flavonoids

To 1ml of aqueous extract, 1ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for flavonoid.

Tests for Steroids

A greenish colour when 2 ml of the aqueous extract was dissolved in 2 ml of chloroform and was treated with sulphuric and acetic acids indicated the presence of steroids.

Test for Alkaloids

To 3ml of aqueous extract was stirred with 3ml of 1% HCl on a steam bath. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate will be taken as evidence for the presence of alkaloid

Test for Terpenoids

2ml of Chloroform and 3ml of concentrated H_2SO_4 were carefully added to 5ml of each extract to form a layer. A reddish-brown colour at the interface indicated the presence of terpenoids

Investigation of insecticidal of the Citrus species peels

The oils extracted from the peels of the three citrus species were each applied at different growth stage of cowpea plant

Design

5 Groups and 3 replicates
Treatment 1: sweet orange
Treatment 2: grape fruit
Treatment 3: lemon
Treatment 4: standard insecticide (perfect killer® chlorpyriphos 20% EC)
Treatment 5: control

The Flowering Stage

About 15ml of the citrus species oil were mixed with a litre of water, and sprayed on the plant weekly at the flowering stage for four weeks with the need to control flowering pests known as *Megalurothrips sjostedti* ,these are small shiny black insect that are most readily observe in flowers.

The pod stage

About 15mls of the citrus species oil were mixed with a litre of water, and was sprayed on the plant weekly at the pod stage for four weeks in order to control insects that attack the pod borer is known *Maruca Vitrata.*

Result

Phytochemical screening of the Citrus species peels

Tanins, flavonoid, steroid and terpenoids were present in all the three citrus species, saponins was present in sweet orange but absent in grape fruit and lemon fruit while alkaloids were absent in sweet orange and lemon but present in grape fruit. This result is presented in table 1

Chemical composition of oil extracted from peels of Citrus species

Results of the chemical composition of the three Citrus species are presented in tables 2 to 5.

Sweet Orange:

Nineteen compounds were isolated from the peels of sweet orange species. The compound with the least percentage was hexadecanoic acid 2,3-dihydroxypropyl ester with 0.57% and the highest oleic acid with 22.56%

Grape Fruit:

Thirteen compounds were isolated from the peels of the citrus species the compound with the least was pentadecanoic acid with 1.04% and the highest oleic acid with 23.19 %

Lemon

Eighteen compounds were isolated from the peels of the citrus species the compound with the least was hexadecanoic acid with 0.18% and the highest oleic acid with 24.26 %

Chemical composition of the three citrus species

Some of the compounds were present in the three and two of the citrus species, with highest percentage in oleic acid 24.26%

Table 1: *Qualitative analysis of the three citrus species peels*

Secondary metabolite	Sweet orange	Grape fruit	Lemon
Tanins	+	+	+
Saponins	+	-	-
Flavonoids	+	+	+
Steroid	+	+	+
Alkaloids	-	+	-
Terpenoid	+	+	+

Keys: + Present
- Absent

Table 2: *Chemical composition of oil extracted from peels of sweet orange*

Peak	Compound	Molecular weight	Percentage (%)
1	Octyl Bromide	142	3.90
2	Benzene 1-ethyl-2-methyl	140	3.69
3	Decane	142	15.24
4	Heptane 5-ethyl-2-methyl	156	6.36
5	Undecane	156	9.42
6	Hexadecanoic acid	200	2.57
7	Tetredecanoic acid	266	1.82
8	Hexadecanoic acid 2,3-dihydroxypropyl ester	280	0.57
9	Octadecanoic acid	256	10.16
10	Oleic acid	282	22.56
11	n-Hexadecanoic acid	84	7.37
12	1,E-11,Z-13 octadecatriene	276	5.15
13	9,12-octadecadienoic acid,methyl ester	280	1.91
14	Hexadecanoic acid 2,3-dihydroxypropyl ester	283	1.22
15	3,11-Tetradecadien-1-ol	339	2.52
16	Hexadecanoic acid	339	0.76
17	Pentadecanoic acid,2 hydroxyl	299	1.59
18	Octane,2-bromo	339	0.80
19	13-oxabicyclo	341	2.38

Table 3: *Chemical composition of oil extracted from peels grape fruit*

Peak	Compound name	Molecular weight	Percentage (%)
1	Octane3,6-dimethyl	142	4.84
2	Benzene 1-ethyl-2-methyl	140	10.32
3	Decane	142	16.28
4	Heptane 5-ethyl-2 methyl	156	7.69
5	Undecane	156	15.74
6	Hexadecanoic acid	256	9.31
7	Oleic acid	282	23.19
8	Pentadecanoic acid	284	4.69
9	9,12-octadecanoic acid, methyl ester	280	2.35
10	2-Butanone 3-methyl-1-phenyl	260	1.90
11	Pentadecanoic acid,10-undecenyl ester	295	1.04

12	1,2 Benzenedicarboxylic acid diisooctyl ester	279	1.20
13	1,5-Heptadiene2,6-dimethyl	343	1.45

Table 4: *Chemical composition of oil extracted from peels Lemon oil*

Peak	Compound	Molecular weight	Percentage (%)
1	Nonane 3-methyl	142	4.09
2	Benzene,1-ethyl-2-methyl	139	7.90
3	Decane	142	13.64
4	Decane 4-methyl	156	6.66
5	Undecane	156	12.34
6	Dodecanoic acid	200	1.27
7	Tetradecanoic acid	228	1.28
8	Hexadecanoic acid	270	0.18
9	Octadcanoic acid	256	12.18
10	Oleic acid	282	24.26
11	n-Hexadecanoic acid	284	5.18
12	1 E-11-Z-13 Octadecatriene	280	3.85
13	9,12-Octadecadienoic acid methyl ester	280	1.70
14	Hexadecanoic acid,2,3-dihydroxypropyl ester	283	0.57
15	7-Nonenamide	320	1.10
16	Pentanoic acid,10-undecenyl ester	295	1.10
17	1,2Benzenedicarboxylic acid,diisooctyl ester	279	0.98
18	10-undecenal	339	1.74

Table 5. *Chemical composition of the oil extracted from the peels of three citrus species*

Compound	Sweet Orange (%)	Grape Fruit (%)	Lemon (%)
Benzen1-ethyl-2-methyl	3.69	10.32	7.90
Decane	15.24	16.28	13.64
Undecane	9.42	15.74	12.34
Oleic acid	22.56	23.19	24.26
Heptane 5-ethyl-2-methyl	6.36	7.69	Nill
Hexadecanoic acid	0.76	9.31	Nill
Tetradecanoic acid	1.82	Nill	1.28
Octadecanoic acid	10.16	Nill	12.18
n-Hexadecanoic acid	7.37	Nill	5.18
9-12-Octadecadienoic acid methyl ester	1.91	Nill	1.70
1,E-11-Z-13 Octadecatriene	5.15		3.85
1,2 Benzenedicarboxylic acid diisooctyl ester	Nill	1.20	0.98

Discussion

Result of phytochemical analysis carried out revealed the presence of tannins, flavonoid, terpenoid and steroid in all the three citrus fruits (Table 1) these result is in agreement with [5] and [6] who also found similar phytochemicals in addition to other compounds. In the three citrus species studied the presence of saponins in sweet orange may be responsible for the sweet taste of orange. While the bitter taste of grape fruit may be attributed to the presence of alkaloids. In this study, the percentage yields recovered from the extracts ranged from 0.10– 24.26%. This may be considered low. The reasons for these may be due to the solvent systems used for extraction, mode of extraction and time of extraction. The authors recommend carrying out the extraction with a range of solvents and extraction protocols to optimize the yield of extraction. Currently, citrus fruit peels are being exploited for their essential oils deposits [7]

The chemical constituents of these oil were analysed using gas chromatography coupled with mass spectrometry. It revealed the presence of oleic acid in these percentages 22.56% 23.19%, 24.26% Benzene 1-ethyl-2methyl in these percentages 3.6%, 10.32% and 7.90% Decane 15.24%, 16.28%, and 13.64%, and Undecane 9.42%,15.74% and 12.34% for sweet orange, grape fruit and lemon respectively. Plants in general have the ability to synthesise compounds that, while having no apparent function in primary metabolism, are physiologically active in insects and other organisms, so providing plants with one of their most important defense mechanisms. During recent years, increasing political and consumer pressures to reduce the use of synthetic insecticides, and the decreasing efficacy of them due to the development of resistance, have fuelled the search among plants for potentially useful Insecticides. Plant-derived insecticides are likely to be more rapidly degradable and consequently more environmentally and toxicologically safe and more selective. *Citrus* species are known to contain chemicals that exhibit different properties (toxicity, deterrence, feeding deterrence) against insects. Studies undertaken so far on the insecticidal potency of *Citrus* species have mainly concentrated on *Citrus limon*, while no attention was given to *Citrus aurantium*. The result of my research reveals that peels of *Citrus limon* contain chemical with insecticidal properties it was observed to have the highest percentage of the entire chemical constituent followed by grape fruit and lastly sweet orange. The application of the oil on cowpea plant at different stages of the growth

had no significant effect on the growth of the plant and so seed stored with *citrus* extract can be planted without any effect. This result is in agreement with the result of [8] who found out that orange has (*Citrus sinensis*) has a promising insecticidal activity against mosquitoes and cockroach. The oil extracted from the three citrus species shows that Heptane5-ethyl-2methyl has 6.36% in sweet orange,7.69% grapefruit but absent in lemon. Hexadecanoic acid was present in sweet orange with 1.8% and lemon 1.28% but absent in grape fruit. The compound present in two of the *Citrus* species but absent in one may be, properly that the compound present in the citrus species has the ability to destroy pest in field and storage.

Citrus peels had insecticidal properties; however the extract from lemon peels extract was more effective in controlling storage pest of *Callosobruchus maculatus* from 1-3 days with concentration exposure time of 3-24hr. The present finding are similar to those of [9] who studied the effects of volatile component of *Citrus Paradisi*,*C.aurantum*,*C.limonium* and *C.sinensis* peels essential oils against cowpea adult bruchid *Callosobruchus maculatus*.their result showed that citrus oils have fumigant activity against adult beetles. They also reported that the mortality of 1-2day old adults increases with concentration of exposure time from 3-24hrs.the oil of *C. paradisi* was found to be more effective than those of *C. aurantum and C. limonium*, many scientist investigated [10] that essential oil of Artemisia species possess vapour toxicity and repellent activity against Coleopterans beetles (*Sitophilus* Sp, *T. castaneum* and *C. maculatus*). Review of work done on *Citrus* species by (Don-Pedro, 1985) investigated that fruit peels of some citrus species possess insecticidal properties against insect pests. With regard to my research, mortality rate of C. limon had a mortality rate of 62.8% at28days of treatments while the standard and control showed maximum mortality rate of 57.75%and 57.88% Similarly [11] proved that the essential oils of *Citrus* peels cause reduction in oviposition of larval emergence through parental adult mortality. Other studies shows that the plant materials, *Citrus sinensis* peel powder and *Azadiractha indica* seed powder were effective in reducing insect damage (for example the number of emergence holes) over three months period [12]. Similar outcomes to the present study have been reported that Citrus peels caused adult mortality in *C. maculatus* and deterred beetles from admixed cowpea [13] and [14] noted that the non-volatile fraction of lemon oil extracted from fresh peels of Eureka lemon (*citrus lemon L. Burm*) was effective against *C. maculatus.* [15] suggested that the reasons for seeking new natural insecticides included the continuing need to combat insects which had not yielded to effective control, to eliminate chemicals poisonous to man, to find substitutes for existing chemicals, and to provide a supply to meet insecticidal shortages. These reasons appear as valid today as they did then, despite the dominance of synthetic insecticides, many of which are now environmental hazards, however the use of natural product can be considered as important alternative for the control of stored product pest.

Conclusion

The results obtained in this study revealed that the essential oils of *C. limon C. sinensis* and *C. paradisi* have strong effect in protecting cowpea seeds from *C. maculatuss* damage. *Citrus* species extract and oil may therefore be Incorporated and adopted for the control of pulse pests, this could further reduce the use of synthetic chemical Pesticides.

Recommendations

1. The insecticidal activity of oleic acid benzene 1-ethyl-2metyl, Decane, undecane (the active compound of the citrus peels oils) should be investigated.
2. Further studies should be done on the individual components and their insecticidal activity against the pests for their possible application in the field or as natural preservatives.
3. There is the need to use biotech tools to come up with methods that will extract higher quantity of the oil so as to make economically valuable

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