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EFFICACY OF *CHROMOLAENA ODORATA (L.) LEAF* AND *CITRUS SINENSIS (L.) Osbeck RIND* EXTRACTS IN THE MANAGEMENT OF PEPPER INSECT PESTS

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SUMMARY

Laboratory and field studies were conducted to evaluate the efficacy of methanol and aqueous extracts of *Chromolaena odorata* leaves and *Citrus sinensis* rind in the management of insect pests of pepper. Phytochemical analysis and contact toxicity test of the extracts were carried out using standard methodology. Phytochemical analysis of both solvent extracts revealed the presence of flavonoids, tannins, saponins, alkaloids, steroids, terpenes, cardiac glycosides, and anthraquinones. Within the 72 hours of application, methanol and aqueous extracts of *Chromolaena* and orange rind recorded high mortality (> 50%) on the test insects relative to the control in the laboratory; and also lowered the occurrence and severity of the insect pests in the field. The extracts of both plants gave higher agronomic and yield values on treated plants than the control. However, the methanol extract of orange rind recorded significantly (P>0.05) highest values of these parameters when compared to other extract treatments used in the study. Fruit damage (over 60%) by the insects was significantly higher in control plants. Methanol and aqueous extracts of *Chromolaena* and *Citrus* rind should be included as a sustainable component of integrated pest management in pepper production.

Keywords: Biopesticides, Orange Rind, *Chromolaena*, Phytochemical Analysis, Steroids, Insecticidal Activity, Pepper.

Pepper (*Capsicum annuum L.*) is an annual or perennial plant in the Solanaceae family () that originated from Central and South America (). The crop is grown for its edible fruits, mostly consumed as spice and characterized by pungent and special flavour. Pepper is rich in amino acids, minerals such as calcium, iron, magnesium, and potassium, as well as vitamins A, B, C, and E, which are essential for healthy human growth and development (,). It also contains phytochemical compounds such as phenolics and flavonoids that are important antioxidants that prevent degenerative and chronic diseases such as cancer, diabetes, cardiovascular diseases, and anemia (,). Globally, the demand for pepper and its products is on the rise due to its nutritive and medicinal value (,). However, its cultivation is hampered by both biotic (such as diseases and insect pest infestations) and environmental constraints from nursery to field throughout its lifespan resulting in significant reductions in marketable fruit yield (). A number of these insect pests that hamper pepper production include whitefly (*Bemisia tabaci Gennadius*), aphid (*Aphis gossypii Glover and Myzus persicae* Sulzer), variegated grasshopper (*Zonocerus variegatus L.*), potato beetle (*Leptinotarsa decemlineata Say*), leaf-miner (*Lyriomyza* spp), and thrips (*Thrips tabaci Lindeman*). These insect pests cause direct damage to pepper fruits, while some are vectors of many diseases (, ,).

In the management of insect pests on pepper, most farmers in Nigeria use conventional insecticides due to their effectiveness against a wide range of insect pests and ease of application (,). However, the problems of environmental pollution, accumulation of toxic residues beyond permissible tolerance in produce, secondary pest outbreaks, the evolution of resistance, and the resurgence of treated pest populations (, ,) have necessitated the need for environment-friendly alternatives. A lot of botanicals have been studied against diverse insect pests these days because they are easy to apply, biodegradable, reduce environmental contaminations, and help maintain the biological diversity of organisms in the ecosystem (). These studies have shown that many botanicals have antibacterial, insecticidal, repelling, and ovicidal effects on insect pests which makes them important natural chemicals (, , ,). Extracts of *Citrus sinensis* and *Chromolaena odorata* have been reported to contain bioactive compounds singly or synergistically which can mediate different biological activities on insects (,). This study, therefore, evaluated the efficacy of methanolic and aqueous extracts of *Citrus sinensis* and *Chromolaena odorata* in controlling insect pests of pepper.

MATERIALS AND METHODS

The study was conducted at the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria (Latitude 7° 25'N and Longitude 3° 52'E, 210 m above the sea level). The site is located in the rainforest zone of Southwestern Nigeria with an annual rainfall of 1821.88 mm. The site is characterized by a bimodal rainfall pattern that peaked in June and September with a dry spell in August.

Insect culture:

Preliminary studies carried out earlier on the pepper field highlighted six major insect pests: Mealybug (*Ferrisia virgata C*), *variegated grasshopper* (*Zonocerus variegatus* L), Aphids (*Aphis gossypii G,*), whitefly (*Bemisia tabaci G.*), flea beetle (*Podagrica* spp L.) and green stink bug (*Nezara viridula L,*). Each of the insect was collected from field infested pepper plants into separate collection bottles and introduced into 6 weeks pepper plants in different wooden cages measuring (65 cm × 65 cm × 70 cm) in the laboratory. The insect culture was maintained under ambient temperature 25° C - 28° C and relative humidity 75 - 90%. At 10 days after introduction (when oviposition would have taken place), the insects were removed and subsequent emerged adults were used for the study.

Plant collection and preparation

Fresh leaves of *Chromolaena odorata* and *Citrus sinensis* rind were collected and air-dried in the laboratory at room temperature (27± 2ºC). The materials were later ground into powder using a blender. From the powder obtained, 100 g of each was taken and added to 1000 ml methanol or distilled water (10% w/v) in separate containers. They were allowed to stay for 24 hours after which the extract was carefully decanted into collection bottles (**).** The methanol extracts were later left for 24 hours to evaporate the solvents while the aqueous extracts were dried in a water bath at 40°C. The slurry obtained after solvent evaporation was used for the experiments.

Phytochemical analysis

The extracts were subjected to

phytochemical studies using standard procedures as described by Chang *et al*., (), Brunner (), Harborne, (), and El-Olemy *et al*. ().

Contact toxicity of extracts by topical application in the laboratory

A 10% concentration of the slurry in 10 ml of distilled water for each plant extract was maintained. One drop of Teepol was added as a surfactant. Contact toxicity by topical application was carried out on five unsexed adults of each insect which were obtained from the laboratory culture and placed individually in Petri dishes lined with filter paper. The Petri dishes were then placed in the refrigerator for 2 minutes to reduce the movement of the insect. Each plant extract (1 ml) was applied separately to the dorsal surface of the thorax of each insect with a micro applicator. Five treatments per plant extract (methanol and aqueous) as well as cypermethrin and water (as control) with three replicates were maintained. The set-up was arranged in a Completely Randomised Design (CRD) on a flat surface in the laboratory. Mortality of the insects was observed at 72 hours after treatment by probing with a blunt object. Insects were considered dead when they did not respond to three probing. Percentage insect mortality was calculated using the formula according to Baba-Tierto Niber ().

$$\text{Mortality (\%)} = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

On the field

The pepper seeds were raised in sterilized soil in trays in the nursery and later transplanted to the field at 6 weeks after planting (WAP). Field area of 145 m^2 and plot size of 2 m x 1 m with a 1 m border was used while pepper plants were spaced at 0.5 m x 0.5 m apart and laid out in a randomized complete block design (RCBD) with three replicates. The treatments used were: CTR – Control (distilled water), CHR_1 – Chromolaena aqueous extract (1 ml extract slurry 10 ml distilled water) (10% vol/vol), CHR_2 – Chromolaena methanol extract (1 ml extract: 10 ml methanol) (10% vol/vol), OR_1 – Orange rind aqueous extract (1 ml extract: 10 ml distilled water) (10% vol/vol), OR_2 – Orange rind methanol extract (1 ml extract: 10 ml methanol) (10% vol/vol) and Cypermethrin (check)(1 ml cypermethrin: 100 ml distilled water) (1% vol/vol).

Treatment applications/Data collection

Three applications were made at 2 weeks after transplanting (WAT), 6 WAT, and 10 WAT using a hand sprayer. The pepper plants were examined daily between 7 and 8 a.m. when the insects were relatively inactive. Number of leaves produced, plant height, and stem diameter were measured on 5 tagged plants from 3 WAT once a week till fruiting. Insect count was done on 5 randomly selected plants at 3 WAT, 5 WAT, 7 WAT, 9 WAT, and 11 WAT. Yield-related data such as fruit length, width, and weight) were taken from 12 WAT, while marketable fruits were separated from unmarketable ones.

Statistical analysis

Data collected were subjected to Analysis of Variance (ANOVA), number of insects collected in the field experiment was transformed using square-root model $\sqrt{}$ (X + 0.5) before analysis of variance (ANOVA), agronomic parameters and yield data were subjected to one-way ANOVA at 5% probability level. Significant means were separated using the Studentized Newman-Keuls test (SNK) with the SAS 9.0 statistical package.

RESULTS

Phytochemical analysis

As shown in Table 1, the methanol and aqueous extracts of *Chromolaena odorata* leaves (COL) and *Citrus sinensis* rind (CSR) were found to contain the following classes of compounds; flavonoids, tannins, saponins, alkaloids, steroids, terpenes, cardiac glycosides, and anthraquinones. In addition, the methanol extracts of both COL and CSR had more of these secondary metabolites as compared to the aqueous extracts. Flavonoids and terpenes were found to be in large quantities in both extracts as compared to the other compounds.

Contact toxicity

Insect mortality ranged from 51.85% - 92.47% within 72 hours of application (Table 2). The methanol and aqueous extracts of *Chromolaena* leaves and orange rind recorded high mortality (< 50%) on the test insects relative to the control. The methanol extracts of orange rind recorded significantly higher mortality in all the test insects. No significant difference was observed in the degree of mortality caused by both the methanol extract of orange rind (OR2) and the check insecticide (Cypermethrin- CYP). Amongst the treatments, the lowest insect mortality was recorded in the aqueous extracts of *C. odorata* leaves while no mortality was recorded in the control treatment throughout the application period in the study.

On the field

Variegated grasshoppers and whitefly were observed on the pepper plants at 2 WAT, mealy bugs and aphids at 3 WAT while the flea beetles and green stink bugs were observed at the onset of the flowers (5 WAT).

The occurrence of whitefly, mealy bug, flea beetle, and stink bug was not significant relative to that of variegated grasshoppers and aphids which were on the field from the onset to the time of harvest. Amongst the extracts, pepper plants treated with methanol extracts of orange rind recorded lower incidence and mild severity of grasshopper and aphids, this was closely followed by methanol extract of orange rind while pepper plants treated with *Chromolaena* aqueous extract recorded the highest insect occurrence and severe infestation. No insect infestation was recorded on plants treated with cypermethrin which was the check used in the study. However, the control plants recorded the highest occurrence and severe infestation of grasshoppers and aphids.

Significantly higher number of leaves, plant height, and stem diameter were observed in pepper plants treated with methanol extracts of orange rind relative to other extract treatments. This was however not statistically different from what was obtained in pepper plants treated with cypermethrin. The control pepper plants recorded the lowest of these characters (Table 4).

Number of fruit and fruit weight was significantly higher in pepper plants treated with methanol extracts of orange rind than the other plant extracts and the control while having no significant difference from those treated with the synthetic insecticide (cypermethrin) (Table 5). Also, damage on the fruits by the insects was significantly higher in control plots (over 60%); while the pepper plants treated with methanol extract of orange rind recorded the lowest damage (8%) of all the plant extracts.

DISCUSSION

Several research works have reported plants to contain different phytochemicals that help them fight against pests and diseases; this has also been validated by the results from this study. Though the use of chemical inputs in agriculture is inevitable to meet

Table 1: Phytochemical analysis of aqueous and methanol extracts of *Chromolaena* leaves and orange rind

Treatment	Flavonoids (mg/100g)	Tannins (mg/100g)o	Saponins (mg/100g)	Alkaloids (%)	Steroids (mg/100g)	Terpenes (mg/100g)	Cardiac glycosides (mg/100g)	Anthraquinones (mg/100g)
Chromolaena (methanol)	2364.29a	1602.89a	250.45a	1.15a	212.13c	3318.16a	1027.96a	165.91a
Chromolaena (aqueous)	965.04d	560.89d	87.64d	0.56d	147.47d	1689.04d	419.58d	85.12d
Orange rind (methanol)	2033.56b	1233.78b	192.78b	1.01b	572.38a	3205.52b	884.15b	160.28b
Orange rind (aqueous)	1288.74c	873.11c	136.42c	0.68c	502.79b	2131.19c	560.32c	106.56c

Means in the same column with the different letters are significantly different ($p > 0.05$).

Table 2: Effect of topical application of plant extracts and synthetic insecticide on mortality of major insect pests of pepper after 72 hours of application

Treatments	Percentage mortality (%)					
	Mealybug	**Grasshopper**	**Aphids**	**Stink bug**	**Whitefly**	**Flea beetle**
CTR	0.00c	0.00c	0.00e	0.00d	0.00d	0.00d
CYP	80.20a	92.47a	81.55a	89.65a	87.35a	82.45a
CHR1	60.7b	51.85c	58.36d	60.10c	53.55c	56.46c
OR1	72.35b	75.76b	73.55b	76.71b	68.35b	70.52b
CHR2	68.45b	72.35b	65.82c	72.45b	64.15b	68.15b
OR2	78.4a	86.15a	78.35a	82.65a	80.57a	79.25a

Means in the same column with the similar letters are not significantly different ($p > 0.05$) using Studentised Newman Keuls (SNK). CTR – Control:, CYP – Cypermethrin:, CHR1 – Chromolaena aqueous , OR1 – Orange rind aqueous extract:, CHR2 – Chromelaena methanol extract, OR2 – Orange rind methanol extract.

Table 3: Occurrence and severity of insect pests on treated pepper plants in the field

Treatment	Incidence						Severity					
	Grasshopper			Aphids			Grasshopper			Aphids		
	7 WAT	9 WAT	11 WAT	7 WAT	9 WAT	11 WAT	7 WAT	9 WAT	11 WAT	7 WAT	9 WAT	11 WAT
CTR	15.24a	22.75a	30.10a	5.55a	6.10a	8.40a	3	3	3	4	4	4
CYP	0.00e	0.00f	0.00f	0.00d	0.00e	0.00e	0	0	0	0	0	0
CHR1	7.45b	10.45b	11.35b	2.65b	4.68b	5.10b	1	1	1	1	1	1
OR1	5.10c	6.2d	8.25d	2.26b	2.85c	3.10c	1	1	1	1	1	1
CHR2	5.75c	8.11c	10.25c	2.35b	4.25b	4.85b	1	1	1	1	1	1
OR2	3.26d	4.58e	6.25e	1.85c	2.1d	2.25d	1	1	1	1	1	1

Means in the same column with the similar letters are not significantly different ($p > 0.05$) using Studentised Newman Keuls (SNK)
CTR – Control:, CYP – Cypermethrin:, CHR1 – Chromolaena aqueous , OR1 – Orange rind aqueous extract:, CHR2 – Chromelaena methanol extract, OR2 – Orange rind methanol extract.

Table 4: Agronomic characters of treated pepper plants on the field after insect infestation

Treatment	Number of leaves			Plant height (cm)			Stem diameter (cm)		
	7 WAT	9 WAT	11 WAT	7 WAT	9 WAT	11 WAT	7 WAT	9 WAT	11 WAT
CTR	16.3d	20.5e	43.16e	10.50d	12.7d	15.2d	0.5a	0.60b	1.00b
CYP	43.6a	52.15a	65.62a	25.60a	30.4a	38.7a	0.8a	1.20a	1.60a
CHR1	20.3d	25.1d	39.7d	13.10c	15.9c	20.8c	0.6a	0.90b	1.00b
OR1	29.50c	38.50c	51.13b	20.50b	23.2b	26.56b	0.6a	0.90b	1.11b
CHR2	25.65c	32.50c	42.35c	19.50b	22.8b	25.60b	0.7a	0.90b	1.10b
OR2	38.72b	48.26b	60.32a	21.80b	28.50a	32.2a	0.7a	0.90b	1.21b

Means in the same column with the same letter arenot significantly different ($p > 0.05$) Studentised Newman Keuls (SNK). CTR – Control, CYP – Cypermethrin:, CHR1 – Chromolaena aqueous, OR1 – Orange rind aqueous extract:, CHR2 – Chromelaena methanol extract, OR2 – Orange rind methanol extract.

Table 5: Efficacy of extracts on pepper yield and damage by insect pests

Treatments	Number of fruit/plant	Average weight/fruit(g)	Damage fruit (%)
CTR	12.65e	3.92d	60.58a
CYP	35.48a	12.55a	1.85f
CHR1	18.60d	5.28c	28.67b
OR1	25.36b	8.15b	15.25d
CHR2	21.95c	7.95b	20.18c
OR2	32.56a	10.72a	8.76e

Means in the same column with the same letter are not significantly different ($p > 0.05$) Studentised Newman Keuls (SNK). CTR – Control, CYP – Cypermethrin:, CHR1 – Chromolaena aqueous, OR1 – Orange rind aqueous extract:, CHR2 – Chromelaena methanol extract, OR2 – Orange rind methanol extract.

the growing demand for food in the world, its arbitrary use can be reduced. Unlike synthetic pesticides, the biodegradability of the secondary metabolites contained in plant-based pesticides will reduce the incidence of pesticide residues and environmental pollution associated with synthetic pesticides. This study confirms the findings from earlier studies by Agaba and Fawole (); Agaba *et al*. (); Uyi and Igbinoba (); Gotmare and Gade (), who reported the presence of flavonoid, tannin, terpenoid, alkaloid and saponin in the plants studied. These compounds are naturally occurring, highly biodegradable; and have insecticidal properties (,). The presence of these compounds in the plant extracts used in this study may have been responsible for the mortality of the experimental insects. This study showed that terpenes and flavonoids were the most abundant compounds in the orange rind and *C. Odorata* leaves used. It has been reported that high dose of flavonoid alters the normal body functioning of insects (,). Flavonoids protect plants against insect pests by influencing their behaviour, growth, and development (,). Terpenes are known to act as irritants when applied either externally or consumed internally. Findings from this study agree with the findings of Simon-Oke and Akeju () who also reported high amounts of terpenes in *Citrus sinensis* rind. Hence, the high amount of terpenes and flavonoids contained in the plant extracts studied may have contributed to the high rate of insect mortality obtained in this experiment.

The high effectiveness of the botanical extract (methanol extract of orange rind) was comparable to that of Cypermethrin (when topically applied) as there was no significant difference between the degree of mortality caused by both, on the studied insects. Although, cypermethrin had a slightly higher effect on the insects' incidence and severity on the field. The comparable insecticidal action can also be seen from the yield obtained from pepper plants with these treatments as there was no significant difference in their yield. From the results, the methanol extract of Chromolaena had the highest concentration of all the secondary metabolites (except steroids) and these concentrations were significantly different from that of other treatments. This was closely followed by the methanol extract of orange rind which also had the highest concentration of steroids. The comparable effect of the orange rind methanol extract to the check insecticide may have resulted from the distinctly high steroid content contained in it. Plant steroids play important functional roles as plant hormones in plant development (); and also, as insect deterrents and chemical barriers against pathogens (). Soriano *et al*., () also suggested that phytoecdysteroids might be good candidates for the development of new and safer strategies for crop protection against insects. Steroids and cardiac glycosides have also been reported to have anti-inflammatory, sedative, insecticidal and cytotoxic activities. Loko *et al*, () reported that steroids (in addition to other phytoconstituents) contained in Khaya senegalensis leaf extract, was responsible for the mortality of *Dinoderus porcellus*.

CONCLUSION

In conclusion, Methanol and aqueous extracts of *C. Odorata* leaves and orange rind confirm mortality upon contact with the test insect pests of pepper, although at varying degrees, with methanol extract of orange rind having higher contact efficacy. This study shows that methanol and aqueous extracts of *Chromolaena odorata* leaves and *Citrus sinensis* rind could be considered and included as a sustainable component of integrated pest management in pepper production. The incidence and severity of the test insects on the field were greatly reduced by cypermethrin and also significantly different from the rest of the treatments. This suggests that more work has to be done to develop biopesticides with comparable and even better efficacy in the eradication of field crop pests. This study forms the basis for further research in elucidating the individual and synergistic effects of compounds that are responsible for this comparable insecticidal action in the plants studied. It also accentuates the potential of identified biological waste materials in the environment.

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