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Effects of Bioinsecticides Made from Some Plant Leaves and Pepper Seeds on the Growth of Spinach

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Abstract
A laboratory-based experiment was conducted at the Federal University of Technology Owerri, Nigeria to determine the effect of bioinsecticides using curry leaves, scent leaves, pepper seeds and neem leaves on the growth of spinach. The experiment was laid out in a Completely Randomized Design and replicated four times. Data collected were germination percentage, plant height (cm), number of leaves, girth size, number of pests, number of punctures on leaves, number of flowers, and number of seeds produced. Analysis of variance (ANOVA) was used to analyze the results while the least significant difference was used to separate the means. Results showed the proximate composition of the botanicals and phytochemical contents such as Alkaloids, Tannins, Flavonoids, Saponins, Terpenoids, and Cardiac glycosides. Germination and growth parameters of spinach were influenced by different treatment combinations with treatment T_8CLE + SLE (82%) recording the highest germination percentage. There was a significant difference ($p<0.05$) between the values of the growth parameter and the control. This could be due to the high active ingredient found in the botanicals. The study recommends curry leaves, scent leaves, pepper seeds and neem leaves as alternatives to conventional synthetic insecticides.

Keywords: Bioinsecticides, Curry Leaf, Cent Leave, Pepper Seeds, Neem Leaves, Spinach.

Introduction

The term bioinsecticides refers to compounds that are used to manage agricultural pests using specific biological effects rather than as broader chemical pesticides. It refers to products containing biocontrol agents i.e., natural organisms or substances derived from natural materials (such as animals, plants, bacteria, or certain minerals), including their genes or metabolites, for controlling insects and pests. According to the FAO definition, bioinsecticides include those biocontrol agents that are passive, in contrast to biocontrol agents that actively seek out the pest, such as parasitoids, predators, and many species of entomopathogenic nematodes (Abbamondi et al., 2016). Based on the nature and origin of the active ingredients, bioinsecticides fall into several categories such as botanicals, antagonists, compost teas, growth promoters, predators and pheromones. Plants and microorganisms are the major sources of bioinsecticides due to the high components of bioactive compounds and antimicrobial agents. The active compounds in plants include phenols, quinones, alkaloids, steroids, terpenes, alcohols and saponins. Different plant families have varied antimicrobial bioactive compounds which include oil components such as α- and β-phillandrene, limonene, camphor, linalool, β-caryophyellene and linalyl acetate depending on the plant family(Alavanja et al., 2020). Microbial bioinsecticides include bacteria species such as *Pseudomonas*, *Bacillus*, *Xanthomonas*, *Rahnella* and *Serratia* or fungi such as *Trichoderma*, Verticillium and Beauveria species. Bioinsecticides exhibit different modes of action against pathogens such as hyperparasitism, competition, lysis and predation (Ali et al., 2018).

Over the years, conventional synthetic insecticides have been successfully utilized to control pests and boost crop production (Barrett, 2020). Researchers around the globe have attributed the increased, albeit insensitive use of pesticides in large-scale manufacturing processes to the increasing need for global food productivity. However, recently, pertinent issues related to human health, safety, and the environment are threatening the continued use of synthetic pesticides (Anger, 2015).

In the absence of effective alternative management options to tackle insects, smallholder farmers rely extensively on the indiscriminate application of synthetic pesticides (Birech et al., 2016). These synthetic pesticides are harmful to human health, detrimental to the environment and biodiversity, and lead to a rapid build-up of resistance in the target pests while decimating natural enemies of pests, resulting in secondary pest outbreaks (Apata et al., 2020). The chemical insecticides used in crop protection, to reduce the damage caused by pathogens and pests in agricultural fields, pose many long-term threats and risks to living beings due to their harmful side effects (Babanyara et al., 2010). They are known to cause cancers and foetal impairments and they persist in the environment for many years (i.e., they are nonbiodegradable). Based on their potential application and strong inhibitory activity against pests, these synthetic pesticides dominate the market and have a significant impact on the manufacture of products.

Materials and Methods

Investigations were carried out in the screen house at the Department of Biology, Federal University of Technology, Owerri located at latitude 5.3866° N, and longitude 6.9916° E. The region is in the tropical rainforest zone with about 2500mm annual rainfall, and an average daily temperature of 26ºC-30ºC. Materials used for the study were curry leaves, scent leaves, pepper seeds, neem leaves and spinach which were procured from the Agriculture Development Programme (ADP) in Imo State. Collected plant samples were authenticated by a plant taxonomist before usage. The experimental design used was Completely Randomized Design (CRD), replicated four times with four treatments given a total of sixteen plates of plant extracts in the laboratory. The plant samples were procured, washed and chopped into pieces, and was air dried for 2-3 weeks pieces and air dried for 2-3 weeks after which it was ground into powdery form and soaked in 1 litre of water with the various weights and left for 48 hours. The liquids were sieved with Muslim cloth to produce various concentrations of foliar spray of the botanicals respectively. Parameters measured were germination percentage, plant height (cm), number of leaves, girth size, number of pests, number of punctures on leaves, number of flowers, and number of seeds produced.

Seed germination percentage was recorded using the method of Agbogidi (2019), as follows:

- **Percentage germination** = $\frac{Number\ of\ seeds\ germinated}{Number\ of\ seeds\ sown}$ X 100

Plant height (cm) was measured with a measuring tape from the top soil level to the terminal bud four weeks after planting (WAP).

The number of leaves was determined by visually counting the number of leaves per seedling per polypot per treatment.

Stem girths were measured from soil level using Venire callipers.

Number of pests. This was visually counted and recorded per treatment. Total number of punctures was counted and recorded per treatment. The number of flowers produced was counted at the end of the experiment and recorded.

The number of seeds produced was counted at the end of the experiment and recorded. The analysis for alkaloids, tannins, flavonoids, saponins, terpenoids and cardiac glucosides was carried out according to the standard methods of Edeoga et al. (2006) and Obadoni and Ochuko (2001).

A few ml of plant sample extract was prepared, and two drops of Mayer's reagent were added along the side of the test tubes. 1.0ml portion was treated similarly with Dragenduff's reagent. The appearance of a white creamy precipitate indicates the presence of alkaloids. 0.5g of powdered sample of the plant parts were boiled in 20ml of distilled water in a test tube and 0.1% $FeCL_3$ was added to the filtered sample and observed for brownish green or a blue-black colouration which shows the presence of tannins. 20mg of the plant extracts were dissolved in 1 ml of distilled water. 0.5ml of dilute ammonia solution was added to it and concentrated sulphuric acid was added later. A yellow colour indicated the presence of flavonoids. The yellow colour disappeared allowing the solution to stand. 2g each of powered samples of the plant material was boiled together with 20 ml of distilled water in a water bath and 10 ml of the filtered sample was mixed with 5 ml of distilled water in test tubes and shaken vigorously to obtain a stable persistent forth. The frothing is then mixed with 3 drops of olive oil for the formation of emulsion which indicates the presence of saponins. 20mg of plant material was dissolved in 1 ml of chloroform and 1 ml of concentrated sulphuric acid was added to it.

A reddish-brown discolouration at the interface showed the presence of terpenoids. 20ml of the plant extract was dissolved in 1ml of glacial acetic acid and 1-2 drops of ferric chloride solution was added. 0.5 ml of concentrated sulphuric acid was slowly added along the side of the test tube. A brown ring at the interface indicated a deoxysugar characteristic of cardenolides or cardiac glycoside constituents. Data collected were subjected to statistical analysis using ANOVA (analysis of variance) and mean differences were determined using least significant difference (LSD) at 0.05% probability level by Gomez and Gomez (1984).

Results

Results of the proximate composition of Neem Leaf, Curry Leaf, Scent Leaf and Pepper Seed are presented in Table 1. Results obtained revealed the presence of Dry matter (%) in Neem (51.62), curry leaf (81.87), scent leaf (80.62) and pepper leaf (7.85). Crude protein (%) content varied as follows: Neem leaf (9.51), Curry leaf (9.62), Scent leaf (8.11) and Pepper seed(7.43). Crude protein (%) content was higher in Curry leaf (9.62) while the lowest value was obtained in Pepper seed (7.43). Other proximate contents observed in the leaves are Crude fibre (%), Ash (%), Ether extract (%), and Nitrogen free extracts (%) at various percentages.

Table 1: Proximate Composition of Neem Leaf, Curry Leaf, Scent Leaf and Pepper Seed

S/N	Constituents	Neem leaf	Curry leaf	Scent leaf	Pepper seed
1.	Dry matter (%)	51.62	81.87	80.62	7.85
2.	Crude protein (%)	9.51	9.62	8.11	7.43
3.	Crude fibre (%)	12.22	10.54	11.65	11.62
4.	Ash (%)	6.44	8.14	8.13	7.22
5.	Ether extract (%)	8.15	7.33	5.61	8.23
6.	Nitrogen free extracts (%)	49.10	42.40	48.92	36.11

Phytochemical contents of Neem Leaf, Curry Leaf, Scent Leaf and Pepper Seed

The phytochemical contents of Neem Leaf, Curry Leaf, Scent Leaf and Pepper Seed are shown in Table 2. The results showed the presence of Alkaloids, Tannins, Flavonoids, Saponins (except in pepper seed), Terpenoids and Cardiac glycosides in the samples. However, Terpenoids and Cardiac glycosides were not detected in the Curry leaf and Scent leaf.

Table 2: Phytochemical Analysis Of Neem Leaf, Curry Leaf, Scent Leaf And Pepper Seed

S/N	PARAMETERS	Neem leaf	Curry leaf	Scent leaf	Pepper seed
1.	Alkaloids	++	+	+	+
2.	Tannins	+	+	++	+
3.	Flavonoids	+	++	++	+
4.	Saponins	+	+	+	-
5.	Terpenoids	+	-	-	+
6.	Cardiac glycosides	+	-	-	+

Legend: + = Present
- = Absent
++ = Present in large quantity

Germination of spinach treated with different botanical insecticides

The germination percentage of spinach treated with different botanical insecticides is presented in Table 3. The results revealed that the different botanical insecticides influenced the germination percentage of spinach at different treatment combinations.

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Table 3: Germination of spinach treated with different botanical insecticides

Treatments (Foliar spray)	Germination (%)
T_0Control (no application)	81
T_1 Neem leaf extract (NLE)	82
T_2 Curry leaf extract (CLE)	81
T_3 Scent leaf extract (SLE)	80
T_4 Pepper leaf extract (PLE)	80
T_5NLE + CLE	81
T_6NLE + SLE	80
T_7NLE + PLE	80
T_8CLE + SLE	82
T_9CLE + PLE	81
T_{10}SLE + PLE	80

Mean parameters of spinach treated with different botanical insecticides at 4 WAP

Mean plant height (cm), number of leaves, girth size (cm), number of pests, and number of punctures on leaves of spinach treated with different botanical insecticides at 4 WAP are shown in Table 4. The results showed that the botanical insecticides significantly ($p<0.05$) influenced the growth parameters of spinach compared to the control.

Table 4: Mean parameters of spinach treated with different botanical insecticides at 4 WAP

Treatments (Foliar spray)	Mean plant height (cm)	The mean number of leaves	Mean girth size (cm)	The mean number of pests	The mean number of punctures on leaves
T_0Control (no application)	$12.2^{e}\pm2.1$	$24.3^{h}\pm2.4$	$2.1^{d}\pm0.2$	$37.6^{a}\pm2.6$	$42.8^{a}\pm6.3$
T_1 Neem leaf extract (NLE)	$14.3^{d}\pm2.3$	$27.4^{g}\pm2.6$	$2.3^{d}\pm0.3$	$9.2^{b}\pm0.9$	$12.3^{b}\pm2.5$
T_2 Curry leaf extract (CLE)	$14.8^{d}\pm3.2$	$32.0^{f}\pm2.7$	$2.4^{d}\pm0.4$	$8.7^{c}\pm0.7$	$10.4^{b}\pm2.2$
T_3 Scent leaf extract (SLE)	$16.4^{d}\pm3.7$	$32.2^{f}\pm2.9$	$2.7^{c}\pm0.6$	$8.5^{c}\pm0.5$	$8.6^{c}\pm1.8$
T_4 Pepper leaf extract (PLE)	$15.6^{d}\pm3.5$	$34.6^{e}\pm2.8$	$2.7^{c}\pm0.6$	$6.7^{cd}\pm0.4$	$6.3^{d}\pm1.5$
T_5 NLE + CLE	$17.1^{c}\pm3.6$	$36.1^{d}\pm3.1$	$2.8^{c}\pm0.7$	$5.2^{d}\pm0.3$	$5.7^{d}\pm1.4$
T_6 NLE + SLE	$18.4^{c}\pm3.9$	$36.3^{d}\pm3.3$	$3.2^{b}\pm0.9$	$5.0^{d}\pm0.3$	$4.5^{e}\pm1.3$
T_7 NLE + PLE	$17.3^{c}\pm3.8$	$38.5^{c}\pm3.5$	$3.4^{b}\pm1.1$	$5.1^{d}\pm0.3$	$2.2^{f}\pm0.7$
T_8 CLE + SLE	$19.5^{b}\pm4.1$	$39.4^{c}\pm3.7$	$3.6^{a}\pm1.3$	$3.4^{e}\pm0.2$	$2.2^{f}\pm0.5$
T_9 CLE + PLE	$20.3^{b}\pm4.2$	$42.3^{b}\pm3.9$	$3.6^{a}\pm1.3$	$3.2^{e}\pm0.2$	$0.0^{g}\pm0.0$
T_{10} SLE + PLE	$22.4^{a}\pm4.5$	$46.4^{a}\pm4.7$	$3.9^{a}\pm1.5$	$1.5^{f}\pm0.1$	$0.0^{g}\pm0.0$

This means having different superscripts of letters along the column differ significantly at p= 0.05 according to the Duncan Multiple Range Test.

Mean parameters of spinach treated with different botanical insecticides at 8 WAP

Mean plant height (cm), number of leaves, girth size (cm), number of pests, and number of punctures on leaves of spinach treated with different botanical insecticides at 8 WAP are shown in Table 5. The results showed that the botanical insecticides significantly ($p<0.05$) influenced the growth parameters of spinach compared to the control.

Table 5: Mean parameters of spinach treated with different botanical insecticides at 8 WAP

Treatments (Foliar spray)	Mean plant height (cm)	The mean number of leaves	Mean girth size (cm)	The mean number of pests	The mean number of punctures on leaves	The mean number of flowers	Mean weight of seeds (kg)
T_0 Control (no application)	$15.9^f \pm 3.1$	$36.6^h \pm 2.4$	$3.0^b \pm 0.3$	$63.2^a \pm 1.7$	$42.5^a \pm 7.7$	$12.2^f \pm 1.3$	$2.2^e \pm 0.2$
T_1 Neem leaf extract (NLE)	$19.2^e \pm 3.3$	$38.3^g \pm 2.6$	$3.2^b \pm 0.4$	$7.1^b \pm 0.7$	$6.4^b \pm 2.6$	$22.3^e \pm 2.1$	$3.2^d \pm 0.3$
T_2 Curry leaf extract (CLE)	$19.3^e \pm 4.2$	$41.6^f \pm 2.7$	$3.5^a \pm 0.6$	$6.7^b \pm 0.5$	$5.2^c \pm 1.1$	$21.4^e \pm 2.2$	$3.1^d \pm 0.3$
T_3 Scent leaf extract (SLE)	$23.6^d \pm 4.7$	$43.5^e \pm 2.9$	$3.5^a \pm 0.7$	$6.5^b \pm 0.4$	$4.3^d \pm 1.3$	$23.2^d \pm 2.7$	$3.2^d \pm 0.3$
T_4 Pepper leaf extract (PLE)	$25.2^d \pm 4.5$	$44.1^e \pm 2.8$	$3.8^a \pm 0.8$	$4.7^{cd} \pm 0.4$	$3.4^e \pm 1.4$	$31.4^c \pm 3.0$	$4.3^c \pm 1.2$
T_5 NLE + CLE	$26.8^c \pm 4.6$	$45.4^d \pm 3.1$	$3.8^a \pm 0.9$	$4.2^c \pm 0.4$	$3.5^e \pm 1.4$	$31.6^c \pm 3.0$	$5.1^b \pm 1.5$
T_6 NLE + SLE	$27.4^c \pm 4.9$	$46.4^d \pm 3.3$	$4.3^a \pm 1.4$	$3.0^d \pm 0.1$	$3.7^e \pm 1.5$	$31.3^c \pm 3.0$	$5.5^b \pm 1.7$
T_7 NLE + PLE	$29.6^c \pm 5.8$	$48.2^c \pm 3.5$	$4.1^a \pm 1.2$	$1.1^e \pm 0.2$	$1.4^f \pm 0.5$	$32.3^c \pm 3.1$	$5.4^b \pm 1.6$
T_8 CLE + SLE	$31.2^b \pm 6.1$	$48.3^c \pm 3.7$	$4.2^a \pm 1.3$	$1.4^e \pm 0.1$	$1.3^f \pm 0.3$	$32.4^c \pm 3.2$	$5.6^a \pm 1.5$
T_9 CLE + PLE	$32.4^b \pm 6.2$	$50.1^b \pm 3.9$	$4.3^a \pm 1.4$	$1.2^e \pm 0.1$	$0.0^g \pm 0.0$	$36.3^b \pm 3.3$	$6.3^a \pm 1.8$
T_{10} SLE + PLE	$39.1^a \pm 8.5$	$53.2^a \pm 4.7$	$4.3^a \pm 1.4$	$0.0^f \pm 0.0$	$0.0^g \pm 0.0$	$38.3^a \pm 3.7$	$6.6^a \pm 1.9$

This means having different superscripts of letters along the column differ significantly at $p = 0.05$ according to the Duncan Multiple Range Test.

Discussion

Over the last several years, plant-based extracts and essential oils have emerged as attractive alternatives to synthetic insecticides for insect pest management. These insecticides are naturally occurring insecticides as they are derived from plants and contain a range of bioactive chemicals. Botanical pesticides ("botanicals") are characterized by bioactive mixtures/extracts/compounds from plant materials, which serve as insecticides and repellents but also as bactericides, fungicides, herbicides and nematicides. Proximate analysis of the plant samples showed the presence of Dry matter (%), Crude protein (%), Crude fibre (%), Ash (%), Ether extract (%), and Nitrogen extracts (%). This is in line with the report of Nwachukwu et al. (2018), Gupta and Dikshit,(2010), and Compant et al. (2009). Phytochemical screening of the botanicals used in this study showed the presence of Alkaloids, Tannins, Flavonoids, Saponins, Terpenoids and Cardiac glycosides. A study by Chandler et al. (2011) recorded similar phytochemicals in botanical spices in their study on phytochemical screening of selected plant species as potential bioinsecticides. The study by Chandler et al. (2020) identified, examined, and highlighted the potential of several plant-based sources of pesticides in Nigeria. The authors demonstrated that the leaves, bark, seeds, roots, and fruits of over 30 plant species in Nigeria contain bioactive pesticide agents. The study revealed that the plant extracts influenced the germination percentage of spinach at different treatment combinations. This might be attributed to the phytochemicals in the plant extracts. Mean parameters of spinach treated with different botanical insecticides at 4 and 8 weeks after planting (WAP) were also observed in this study.

Conclusion

This study was carried out to determine the effects of bioinsecticides using curry leaves, scent leaves, pepper seeds and neem leaves on the growth of spinach. Based on the results obtained from the study, extracts of curry leaves, scent leaves, pepper seeds and neem leaves have been proven to be alternative insecticides for some insect pests of spinach.

Recommendations

1. Bioinsecticides should be replaced with conventional synthetic insecticides
2. Considerable research on biological control agents, including bioinsecticides, is required for the development of the biopesticide market in the future.
3. Farmers and society at large should benefit from the mixed and judicious use of both conventional chemical pesticides and biopesticides,
4. while it is imperative to emphasize the research in the area of biopesticides for reaping greater benefits from it in the future.

5. This information should be disseminated to the farmers nationwide through the agricultural extension agents.

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