



Impact of pesticides on soil microorganisms, physicochemical properties and cowpea (*Vigna unguiculata* L.) yield.

*¹Ilusanya, O. A. F¹; Adegboyega, T. T.²; Onajobi, I. B¹; Banjo, O. A¹; Oyeyipo, F. M¹ and Bankole, S. A¹

¹Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.

²Genetic Resources Center, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

E-mail: olusolapeilusanya@yahoo.co.uk

Received 2nd May, 2020

Accepted for publication August 17, 2020

Published November 20, 2020

Abstract

Cowpea (*Vigna unguiculata* L.) is an herbaceous annual crop mostly grown in the tropics in Latin America, Africa and South Asia with pesticides application to control associated pests. These chemicals are applied indiscriminately by peasant farmers in Nigeria without due consideration of their non-target effects. This study investigates the effects of lambda-cyhalothrin, and a mixture of cypermethrin and dimethoate applied at their recommended field application rates and twice the recommended rates on soil microorganisms, physicochemical properties and cowpea yields. Parameters measured include soil pH, organic carbon, nitrogen, phosphorus content and cation exchange capacity. Soil microorganisms were isolated and enumerated using dilution plate method while cowpea yields were determined by seeds weights. Results showed that the pesticides had no significant effects on soil pH, nitrogen, phosphorus and cation exchange capacity. The recommended rate of lambda-cyhalothrin significantly increased the soil organic carbon content at ($P < 0.05$). Both pesticides significantly reduced microbial counts with twice the recommended rates having higher inhibitory effects compared to the control soil (without chemical treatment). Reduction of predominant bacteria and fungi genera as well as the elimination of some genera was observed. The mixture of cypermethrin and dimethoate at twice the recommended rate had the highest cowpea grain yield of 4.5 kg/ha, while the control plot had the lowest grain yield of 1.6kg/ha. It may be concluded that soil microorganisms important to soil fertility were adversely affected by the application of insecticides. Efforts should be made to use alternate non-chemical approaches in ensuring food and environmental sustainability.

Keywords: Cowpea, insecticides, soil microorganisms, physicochemical, yield.

Introduction

Cowpea or black-eyed pea (*Vigna unguiculata* L.) is one of the economically preferred staple foods in Nigeria. It has now taken important role in the livelihood of several homes and communities that derive various nutrients thereby increasing food sources for humans, animals and also assist in income generation in diverse forms (Langyntuo *et al.*, 2003). The production of cowpea by the rural farming communities plays a great role in the Nigerian economy as about 58% of 8 million hectares of cowpea farmlands in the world is obtained from the country. Indeed, Kano, Sokoto, Zamfara, Yobe, Borno and Niger States produced about 80% of the total annual cowpea outputs (National Agricultural Extension Research,

2013). The outputs from these States have been integrated across markets within and outside the country, thus contributing to the international and domestic income (Abdusalam *et al.*, 2005).

Globally, the African region produces the largest quantity and consumption of cowpea particularly the West and Central African sub region. In the West African subregion, Nigeria produces more than 2 million metric tonnes of cowpea (FAOStat, 2017) while different food products are made in several in different ethnic groups in the country. After Nigeria Niger and Burkina-Faso are other countries in the subregion that also produces significantly higher quantities (Singh, 2002). Cowpea has been documented as a reputable drought tolerant crop and grown in areas with less than 300 mm of rainfall annually (Boukar *et al.*, 2019). It is majorly cultivated for the seeds which have been shown to contain high amount of proximate contents. In a report by Samireddypalle *et al.* (2017), the authors revealed that apart from the seeds, cowpea dried leaves and pods could also provide additional income for smallholder farmers and increase use in feeding livestock. Cowpea fodder could contain up to more than 19 g protein per 100 g dry weight (Duke, 1990). Consumer preferences are key as well as those in Northern Nigeria prefers the white colored seeds while in Southern Nigeria, the brown-seeds are very prominent and seen on popular markets and local variety stores. Not forgetting the fact that several sweet and delicious meals are prepared from cowpea in various states of the federation (Boukar *et al.*, 2019).

Insects and diseases constitute some of the production constraints. The productivity of cowpea in typical SSA farmers' fields is abysmally low at less than 600 kg/ha when compared with a potential grain yield of over 2,000 kg/ha. A number of abiotic and biotic factors are responsible for these low yields. The adverse effects of these yield limiting constraints can be reduced to their barest minimum through genetic improvement. Cowpea seedlings can be attacked and even killed by aphids (*Aphis craccivora*) if not controlled with insecticides or planting of resistant variety. In storage, the seeds are sometimes affected by weevils (*Callosobruchus maculatus*) thereby causing devastating damage that could lead to economic loss (Boukar *et al.*, 2019).

Cypermethrin is a synthetic, pyrethroid insecticide that has high insecticidal activity, and adequate stability in air and light (U.S.D.A., 1995) while Dimethoate [0-0-dimethyl S (N-methyl carbamoyl) phosphorodithioate] is an organophosphorus insecticide that has an efficiency for killing a wide range of insects on contact including aphids, thrips, planthoppers and whiteflies systemically (Hayes, 1997). A mixture of dimethoate and cypermethrin has been recommended for the control of cowpea pests (Karungi *et al.*, 2000). The use of lambda-cyhalothrin based insecticides against infestation of pests on the field in different countries has also been documented (Badii *et al.*, 2013). Due to the success accrued to pesticides usage, many peasant farmers in many developing countries especially in rural areas which constitute the main stay of economy now use high concentrations of pesticides with the premise of enhancing their production (Ayansina and Oso, 2006). It has been reported that only 2-3% of applied chemical pesticides are effectively used for preventing, controlling and killing pests while the rest get into the soil and persist in the soil (EPA, 2005). Most of the pesticides therefore reach the non-target parts of the agricultural ecosystem (Ayansina and Amushan, 2013) and compromise the quality of soils, air, continental and coastal water bodies (Surekha *et al.*, 2008). Soil microorganisms are the most abundant of all the biota in the soil and are responsible for nutrients cycling, and transformation as well as soil fertility (Vig *et al.*, 2006). The presence or absence of these organisms in the soil can be used as indicator of soil fertility and sustainability. Comparative studies of the numbers of bacteria and fungi in soil treated with pesticides in comparison with untreated soil are one of the fundamental approaches commonly used for the assessment of microbial response to environmental stress (Cycon and Piotrowska-Seget, 2007). This study is therefore aimed at assessing the impacts of insecticides applications to control cowpea pests at the manufacturer's field recommended and twice the recommended rates on soil physicochemical properties, microorganisms and cowpea grain yield.

Materials and Methods

Experimental Design

The study was conducted at the College of Agricultural Sciences, Olabisi Onabanjo University Project site situated in Ago-Iwoye, Ogun State, Nigeria with latitudes 6°55' and 7°00'N and between longitudes 3°45' and 4°05'E. Ago-Iwoye is only two kilometres from Oru and five kilometres from Ijebu-Igbo, which are the two other towns in Ijebu-North Local Government Area. The town is about 100 km south-east of Abeokuta, the Ogun State capital. The site lies to the south-west of Ago-Iwoye. It is approximately 3.50 km from centre of the town, and is bounded in the north by Ijebu-Igbo/Oru/Ago-Iwoye/Ijesa-Ijebu/Illisan road and on the east by Ago-Iwoye/Imodi-Imosan/Ijebu-Ode road.

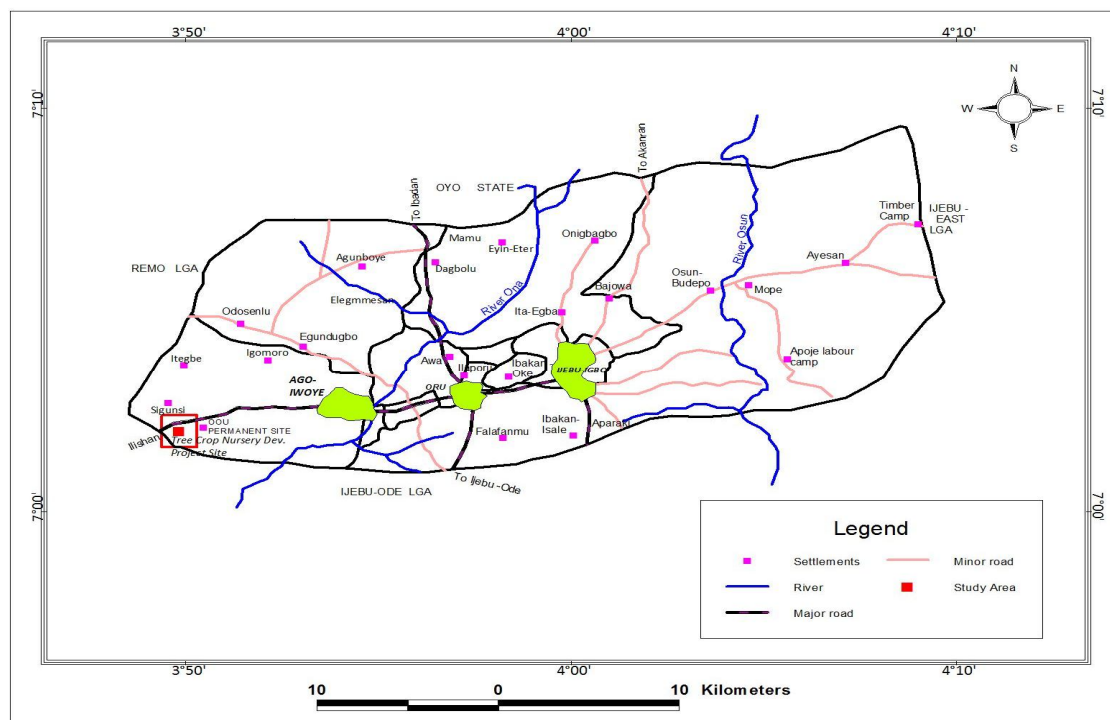


Figure 1: Map of Ijebu North Local Government showing the experimental site at Ago-Iwoye, Ogun State, Nigeria.

Source: Department of Geography, Olabisi Onabanjo University, Ago-Iwoye, Ogun State.

The daylight temperature of the area ranges between 27°C and 29°C. This low range accounts for its constantly high temperature. The mean annual sunshine recorded for the area is between 1,800 – 2,000 hours and the mean monthly record is between 100 – 200 hours. The mean monthly rainfall is between 230mm to 280mm. The mean annual rainfall is around 1350mm with about 80% of it being concentrated within the months of April and October. Ago-Iwoye has high records of relative humidity ranging from 80% to 87% for the morning, 60% to 80% for the afternoon and 100% during and immediately after rainfalls

Cowpea planting

Cowpea seeds (Ife brown) were collected from The Institute of Agricultural Research and Training (IAR&T), Obafemi Awolowo University, Ibadan, Oyo State, Nigeria and planted. Plant spacing was done based on the standard recommendation as described by Obuo *et al.* (1998), at a spacing of 60 cm between rows and 20 cm within row giving 6 rows per plot. Three seeds were planted per hole but after two weeks of growth, seedling was thinned to two plants per hill after germination in two planting seasons.

Insecticides used

The insecticides used were lambda cyhalothrin a synthetic pyrethroids available as an emulsifiable concentrate and marketed as Marshal Lambda– Cyhalotrin and contains 2.5 EC of the active ingredient (25g a.i./ha) and a mixture of cypermethrin, a contact pyrethroid insecticide and dimethoate, a systemic organophosphorus insecticide. The mixture was marketed as Uppercott at Cypermethrin 200 g a.i. and Dimethoate 200 g a.i./ha (Karungi *et al.*, 2000).

Treatments

The insecticides treatments were as follows: post emergence application of (i) Recommended rate of Lambda-cyhalothrin 2.5l/ha (SL), (ii) Twice the recommended rate of Lambda-cyhalothrin 5.0l/ha (DL), (iii), Recommended rate of the mixture of Cypermethrin and Dimethoate, 2.5l/ha. (SC+D) and (iv). Twice the Recommended rate of the mixture of Cypermethrin and Dimethoate, 5.0l/ha (DC+D) (v) Untreated control (CO) Each insecticide treatment was applied as a foliar spray to cowpea seedling at the pre-flowering, 50% flowering (10 to 14 days after first spray) and at podding (10 to 14 days after second spray) stages of the crop (Karungi *et al.*, 2000). Knapsack sprayer filtered with a 2.5 deflector nozzle was used for spraying

Soil sampling

Top soil samples (0-15 cm deep) were randomly collected using soil auger from 8-10 places per plot before cowpea planting at 1-week interval for a period of 6 weeks. The soil samples from different places for the same insecticide treatment were bulked and representative composite samples for each treatment taken to the laboratory of the Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State for analysis.

Analysis of soil physicochemical properties

The physicochemical properties of the soil samples: pH, organic carbon, total nitrogen, available phosphorus was analyzed using glass electrode pH meter method (Orion, USA), Walkley and Black's rapid titration method, macro Kjeldahl method and Olsen's method respectively (Jackson, 1958). Cation exchange capacity in the soil was determined by the procedure described by Black, (1965).

Analysis of microbial population and identification of microorganisms

Enumeration of the populations of bacteria and fungi in the soil was done using serial dilutions at 10^{-6} and 10^{-4} dilutions respectively, followed by pour plate methods. Nutrient agar (Oxoid UK®) was used for bacteria and incubated at 35°C for 24 - 48 h while Potato dextrose agar (Oxoid UK®) was used for fungi and incubated at 35°C for 72 h. Bacterial isolates were characterized to genus level based on morphological, cellular and biochemical characteristics as described by Bergey's manual of systematic bacteriology (1984), while fungi identification was carried out in accordance with descriptions in Barnett and Hunter, (1972) and Cappucino and Sherman, (2002).

Effect of insecticides usage on cowpea grain yield

Cowpea pods were harvested as they matured and threshed to obtain its grains. The grains obtained were sun dried to 12% moisture content. Cowpea grains from each plot were weighed and the results were extrapolated to kilograms per hectare (Kg/ha) for each treatment.

Statistical Analysis

Data generated from the study were subjected to One-way analysis of variance (ANOVA). Comparison of means was done with Duncan Multiple Range Test at $p=0.05$.

Results and Discussion

The effect of lambda-cyhalothrin and the mixture of cypermethrin and dimethoate applications on soil physicochemical properties is presented in Table 1. There was no significant impact of the insecticides on the pH, nitrogen, phosphorus, cation exchange capacity contents of the soils however, the recommended rate of lambda cythalothein significantly ($P < 0.05$) increased the organic carbon content of the soil. The increase or reduction observed in the pH values of the insecticides contaminated and the control soil still fell within critical levels of soil pH as described by Adeoye and Agboola (1985). Also, the non-significant effect of insecticides on soil pH corroborates the works of other researchers (Komal *et al.*, 2001; Ayansina and Amusan, 2013). Reports have suggested that soil pH is a very important factor in pesticides degradation (Wharton and Mathieson, 2005; Singh, 2008). The Insecticides had no significant impact on the nitrogen content of the soil which is in agreement with the work of Vig *et al.* (2006) who reported that available nitrogen levels were similar in both control and soil treated with insecticides on the field.

Table 1: The effect of insecticides on soil physicochemical properties

Soil Parameters	Treatments				
	CO	SC+D	DC+D	SL	DL
pH	6.29 ^a	6.22 ^a	6.07 ^a	6.22 ^a	6.16 ^a
N%	0.11 ^a	0.10 ^a	0.10 ^a	0.10 ^a	0.09 ^a
OC	0.58 ^a	0.56 ^a	0.55 ^a	0.71 ^b	0.58 ^a
P	5.39 ^a	5.10 ^a	4.84 ^a	3.84 ^a	8.55 ^a
CEC	3.95 ^{ab}	4.34 ^b	4.25 ^b	3.03 ^a	3.60 ^{ab}

Values are means compared by Duncan's Multiple Range Test at the 5% Level ($P=0.05$). Means sharing similar letters across the row are not significantly different from one another.

Key: SC+D-Recommended rate of cypermethrin and dimethoate, DC+D- Twice the commended rate of the mixture of cypermethrin and Dimethoate, SL- Recommended rate of Lambda cyhalothrin, DL- Twice the recommended rate of Lambda cyhalothrin, CO-control.

Effect of insecticides on soil microbial populations

Mean total bacterial count in the control soil was significantly higher than those in the insecticides- contaminated soil. There were no significant differences in bacterial populations obtained in all the insecticides contaminated soil. Applications of both insecticides significantly reduced the mean fungal counts when compared with the fungal counts in the control plot however, the mean fungal count was significantly higher in the recommended rate of lambda-cyhalothrin soil samples when compared to the other insecticides- treated soils (Table 2). This result corroborates the findings of earlier researchers of reduction in microbial counts as a result of pesticides applications (Hussain *et al.* 2001; Gupta, 2004; Ahmed and Ahmad, 2006; Ekundayo, 2006). Cycon and Piotrowska-Seget *et al.* (2009) found fenitrothion significantly decreasing the numbers of soil bacterial population with dosages that exceeded the recommended dosages, having more negative effect while Das *et al.* 2005 observed total bacterial population reduced as a result of the application of a carbamate insecticide carbofuran at its recommended field rate. Contrary to the findings in this work, Cyconet *al.* (2009), Singh and Singh, (2005) and Zabaloy (2008) observed increases in microbial populations after pesticides treatment.

Table 2: Comparative effects of insecticides on soil bacterial and fungal populations (cfu/g)

Microbial Group	Insecticides	Mean± S.E.
Bacteria	SC+D	122.13±5.881 ^a
	DC+D	112.48±5.670 ^a
	SL	123.94±4.749 ^a
	DL	113.33±4.193 ^a
	Control	185.54±4.173 ^b
Fungi	SC+D	8.79±0.566 ^a
	DC+D	9.31±0.666 ^a
	SL	12.06±0.346 ^b
	DL	10.00±0.426 ^a
	Control	16.29±0.341 ^c

Values are means compared by Duncan's Multiple Range Test at the 5% Level ($P=0.05$). Means with similar letters within the column are not significantly different from one another.

Key: SC+D-Recommended rate of the mixture of cypermethrin and dimethoate, DC+D- Twice the recommended rate of the mixture of cypermethrin and Dimethoate, SL-Recommended rate of Lambda cyhalothrin, DL- Twice the recommended rate of Lambdacyhalothrin, CO-control.

The insecticides at both concentrations had a marked reduction effect on the occurrence and distribution of the bacterial species in the soil with twice the recommended rate of the mixture of cypermethrin and dimethoate having more adverse effect (Table 3). *Bacillus* spp. and *Pseudomonas* spp. were the predominant bacterial species in both the control and treated soil samples. The occurrence of all fungal species in the insecticides contaminated soils were reduced when compared with the control soil. *Aspergillus* was the predominant organism followed by *Mucor* spp, *Fusarium* spp and *Penicillium* spp. *Rhizopus* was the least isolated spp (Table 4). Reduction of predominant bacterial and fungal genera as well as the elimination of some genera in the insecticide contaminated soils is an indication that some microorganisms were inhibited as a result of the toxic effects of the insecticides. This could be detrimental to sustaining soil fertility since beneficial soil bacteria like *Bacillus* and *Pseudomonas* species are useful in the biological control of plant pests and pathogens or used as plant growth promoters while fungi like *Aspergillus*, *Mucor* and *Rhizopus* contribute to soil aggregation and fertility. Their continuous reduction or suppression in soil could be detrimental to plant productivity (Singh and Singh, 2005)

Soil fertility is influenced by the presence of adequate nutrients and number of the diversity of soil micro flora. Once applied, agrochemicals may persist in the soil and have a negative impact on soil microbial flora (Araujo *et al.*, 2003), such as the killing and elimination of certain specific group(s) of microorganisms. A decrease in microbe number may disturb different processes in the food web performed by an individual or group leading to disruption in the components relying upon it. Reduced number of predominant bacteria and fungi genera as well as the elimination of some secondary genera in the soil as found in this research pointed out to the fact that the microorganisms were not able to use the insecticides and their degraded products for their metabolism and growth, rather some of them were badly affected by the toxic effect of

insecticides in the rhizosphere of cowpea. Comparison of the results obtained from the temperate regions and those obtained in the tropical regions from the literature shows that climate has a strong impact on the effect and action of pesticides on soil microorganisms because most of the results from the temperate regions reported stimulatory effects while more inhibitory effects of pesticides on soil micro flora were observed by researchers from the tropical regions. The higher inhibitory effect of the mixture of cypermethrin and dimethoate on soil microbes compared to that of lambda cyhalothrin could be attributed to the broad spectrum and the combined action of two insecticides (cypermethrin and dimethoate) which was found to be more effective in cowpea insects pests' control.

Table 3: Effect of insecticides application on the occurrence and distribution of genera of bacteria in soils

	Treatments				
	CO (%)	SC+D (%)	DC+D (%)	SL (%)	DL (%)
Bacteria	Number and frequencies	Number and frequencies	Number and frequencies	Number and frequencies	Number and frequencies
<i>Bacillus</i> spp.	15 (34.9)	9 (31.0)	7 (29.2)	8 (24.4)	8 (25.8)
<i>Pseudomonas</i> spp.	10 (23.3)	10 (34.5)	9 (37.5)	7 (21.2)	6 (19.4)
<i>Micrococcus</i> spp.	6 (13.9)	2 (6.9)	2 (8.3)	0 (0.0)	0 (0.0)
<i>Staphylococcus</i> spp.	7 (16.3)	5 (17.2)	4 (16.7)	7 (21.2)	5 (16.1)
<i>Streptococcus</i> spp.	1 (2.3)	1 (3.4)	1 (4.2)	5 (15.2)	6 (19.4)

Key: SC+D-Recommended rate of the mixture of cypermethrin and dimethoate, DC+D- Twice the recommended rate of the mixture of cypermethrin and Dimethoate, SL-Recommended rate of Lambda cyhalothrin, DL- Twice the recommended rate of Lambda cyalothrin, CO- control. All values in parenthesis are in percentage (%).

Table 4: Effect of insecticides application on the occurrence of genera of fungi in soils

	Treatments				
	CO (%)	SC+D (%)	DC+D (%)	SL (%)	DL (%)
Fungi	Number and frequencies	Number and frequencies	Number and frequencies	Number and frequencies	Number and frequencies
<i>Aspergillus</i> spp	19 (63.3)	8 (53.3)	6 (40.0)	14 (58.3)	13 (59.1)
<i>Penicillium</i>	3 (10.0)	2 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Mucor</i>	4 (13.3)	3 (20.0)	6 (40.0)	5 (20.8)	3 (13.6)
<i>Fusarium</i>	4 (13.3)	2 (13.3)	3 (20.0)	5 (20.8)	6 (27.3)
Total	30	15	15	24	22

Key: SC+D-Recommended rate of cypermethrin and dimethoate, DC+D- Twice the recommended rate of the mixture of cypermethrin and Dimethoate, SL-Recommended rate of Lambda cyhalothrin, DL- Twice the recommended rate of Lambdacyalothrin, CO- control. All values in parenthesis are in percentage (%).

The use of insecticides to control pests of cowpea increased the grain yields of cowpea (Table 5). The highest grain yield (4.56kg/ha) was obtained in plots treated with twice the recommended rate of the mixture of cypermethrin and dimethoate followed by 2.67kg/ha in recommended rates of the mixture of cypermethrin and dimethoate ,2.36kg/ha in the twice the recommended rate of lambda cyalothrin and 2.28kg/ha in recommended field application rate of lambda cyalothrin while the control (unsprayed plot) had the lowest grain yield of 1.61kg/ha. The increase in the grain yield of cowpea following the use of insecticides could be attributed to the effectiveness of these insecticides in controlling the insect's pests attacking the grain legumes on the field. Muthoni, (2001), had reported that yield losses obtained under different pesticides treatments was lower in dimethoate treated soil. A mixture of dimethoate and cypermethrin for the control of cowpea pests as recommended by Karungi *et al.*, (2000) proved to be more effective in cowpea pest control as seen by the highest grain yield obtained on the plots obtained from twice the recommended rate of the mixture of cypermethrin and dimethoate plot as practiced indiscriminately by farmers however this high yield obtained as results of the combination of two chemical groups is at the future detriment of the soil fertility. The indiscriminate use of high concentrations of pesticides with the hope to promote effective weed and insects' control (Mathews, 1992) might work for some years but will lead to loss of beneficial microorganisms as seen in this study which will invariably lead to loss of fertility in soil.

Table 5: Total grain yield of Cowpea grown on insecticide treated plots(kg/ha)

Insecticide	Grain Yield (kg/ha)
Control	1.61
SC+D	2.67
DC+D	4.56
SL	2.28
DL	2.36

Key: SC+D-Recommended rate of the mixture of cypermethrin and dimethoate, DC+D- Twice the recommended rate of the mixture of cypermethrin and Dimethoate, SL-Recommended rate of Lambda cyhalothrin, DL- Twice the recommended rate of Lambda cyhalothrin, CO- control.

Conclusion

Despite inherent drawbacks of pesticides usage, their use either on foliage or in soil cannot be totally dispensed with because of the benefits accrued to the usage such as seen by the high yields of cowpea obtained in insecticides plots and quick and easy benefits of weed control. Pesticides however should be used with caution because of the adverse effect on the environment especially soil microbes which play major part in soil fertility. Farmers should be educated on the proper use of pesticides so as to curtail the dangers associated with indiscriminate uses. Mixed farming in which leguminous crops are incorporated should be encouraged since it was observed to minimize the effect of insecticides on microbial populations.

Acknowledgements

The authors are grateful to everyone that has supported the experiments as well as the reviewers.

Conflict of Interests

The authors declare there was no conflict of interest in any form.

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