Original Research Article

PHYTOTOXICITY OF CYPERMETHRIN PESTICIDE ON SEED GERMINATION, GROWTH AND YIELD PARAMETERS OF COWPEA *(Vignaunguiculata)*

ABSTRACT

The experiment was carried out in Federal College of Forestry Jos, in Jos North Local Government Area of Plateau State to determine the phytotoxicity of cypermethrin pesticide on seed germination, growth and yield parameters of cowpea. Randomized Complete Block Design (RCBD) involving 5 treatments control (Water only), 0.25ml of Cypermethrin in 99.75ml water 100ml of solution, 0.50ml of Cypermethrin in 99.50ml water 100ml of solution, 0.75ml of Cypermethrin in 99.25ml water 100ml of solution and 1.0ml of Cypermethrin in 99.00ml water 100ml of solution.Data was collected on radicle Length, plumule Length, number of Leaves, number of branches, stem girth, number of seeds/pod, 100 seed weight, pod length and dry matter. Data collected was subjected to analysis of variance (ANOVA) at 5% level of significance using SPSS 23 and where significance was declared, Duncan Multiple Range Test (DMRT) was used to separate the means. The result of the research indicates that significance difference occurs in the radicle length ($P<0.05$) in which T1 has the highest mean value. The stem girth shows a significance difference with To having the highest mean value (7.32) at $P<0.05$. The result of the number of branches and the number of leaves shows significance difference with To having the highest mean values 39.15 and 101.65 respectively at $P<0.05$ level of significance. The yield parameters shows a significance difference for number of seeds/pod, 100 seed weight, pod length, as well as the total dry matter. The highest mean values for the yield parameters are observed in To with mean values 18.52, 18.53, 16.35 and 68.35 for number of seeds/pod, 100 seed weight, pod length and total dry mass respectively. Amylase enzyme activity was observed to be higher at lower concentration of the cypermethrin on day 2 (2.45) but the increase in the enzyme activity tilted towards the highest concentration on day 3 and day 4 with mean value 2.70 and 3.10 at 5% level of significance.

Keywords: Phytotoxicity, Cypermetrin, Pesticide, Seed Germination, Cowpea

1.0 INTRODUCTION

The use of high-quality seeds enhances the probability of success of a crop. Applications (seed dressing) of fungicides, inoculants, insecticides and micronutrients on seeds are practices mostly used by farmers for several years. These products have provided more favorable conditions to crop's growth as well as its development. Chemical treatment on seeds have been one of the most common techniques in use on current farming due to its low-cost technology, low-environmental impact, and, in general, a significant effect on yield (Zambolim, 2005). High quality seed is one of the essential prerequisites to achieve higher crop productivity.

Cowpea (*Vignaunguiculata)* (L.) Walp) is a legume crop which several people in African countries depend upon for several purposes: its dry grains are source of plant protein for those that are unable to afford meat, fish and egg protein. Cowpea is their hope for cheap protein (Afun., *et al* 1991) and cowpea has appropriately been called “poor man's meat” (Alabi, *et al* 2003). The abundance of vitamins, mineral salts and fats and oils in cowpea has further highly endeared the crop to man. Moreover, its usefulness as fodder crop in livestock is well recognized.

Cowpea cultivation is mainly a business in the tropical and sub-tropical regions where the crop grows in various soil types and climatic conditions (Degri, *et al.,* 2000). In Nigeria, it is cultivated mainly in the drier zones of Northern region, particularly the Sudan savannah. The cultivation lately has been adopted by farmers in Southern Nigeria and it is being successfully grown in the West and East. Nigeria produces the largest quantity of cowpea in the world and this comes mainly from Northern Nigeria. Yields can be high if production constraints are adequately addressed. Production constraints which include attacks and damages to the crops by insect pests (Egho, 2010) largely contribute to low yield and good grains cannot be obtained in farms without any form of control on insect pests (Emosairue, *et al*). The major/key insect pests include the foliage beetle, cowpea aphid Aphis craccivora Koch, the legume pod borer, *Marucavitrata* Fab and a complex of pod sucking pods which include *Clavigrallatomentosicollis*, *Anoplocnemiscurvipes*, etc. Various control measures to suppress insect species include host plant resistance (HPR), biological control, cultural control and the use of synthetic insecticides. The application of synthetic insecticides in insect pest control is an ancient method which all through the years has proved more reliable and effective than other control measures. Triple yields have been recorded in farms which received insecticide sprays (FOS 1995). The market today is heavy with various insecticides under different trademarks and new ones are being developed. This is against the outcry that chemicals, though useful constitute danger to crops, users, consumers and environment especially pollution. In Uganda farmers during the growing season spray their crops from 8 to 10 times (Egho. 2010). However, abandonment of insecticides in favor of other control measures does not provide solution because this would worsen the present food situation (Isubikalu 1998). The recommendation is that insecticides should be judiciously used to minimize the number of sprays and often incorporate other control methods.

Pesticide has been extensively used in agricultural Practice (Cserhati*et al.,* 2004). The use of pesticides in agriculture has been steadily increasing in the last 40years (Bohn *et al*., 2011). In recent years concern over problems associated with pesticide use has often been discussed. Although protection of seeds and seedlings from pests and disease organisms is the prime aim of seed treatment, secondary effects on the germination and growth are more likely to occur from seed treatments as well as from accumulated residues resulting from repeated use of pesticides (Wahengban*et al*., 2013).

Germination and seedling establishment are critical stages in the plants life cycle (Mohammed, 2015). In crop production, stand establishment determines plant density, uniformity and management options. Poor germination and crop establishment result in significant reduction in economic yield (Abro *et al.,* 2009).

Although seed treatments have important benefit, they also pose certain risks if application rate and dose of these chemical are not carefully controlled like, reduction in the shelf life of seed, residual problem of pesticides and phytotoxicity to plant i.e. lower germination rate or stunting and reduction in length of sprout, hence affecting the choice of planting depth.

(Akoto *et al* 2013). The risk of phytotoxicity response to seed treatment is affected not just by the active chemical being applied but also by factors such as, exposure period, conditions and crop species.Therefore, this study was carried out to examine the phytotoxicity of pesticide (cypermethrin) on seed germination and growth parameters of cowpea (*Vignaunguiculata)*

2.0 MATERIALS AND METHODS

The experiment was carried out in Federal College of Forestry Jos, in Jos North Local Government Area of Plateau State. Jos is located between latitude 7-11^{o} North and longitude 7-8^{o} east. Temperature ranges between 10-32^{o}C and main annual rainfall is about 1340mm, with an average elevation of 1200mm above sea level (Plateau State Information Communication Agency, 2015).

2.1 Materials

2.1.1 Source of Materials

The materials used include insecticide (Cypermethrin) for seed dressing, certified seed of cowpeaobtained from Plateau Agricultural Development Program (PADP), Dogon Dutse, in Jos North local government area of Plateau State. Petri-dish, whatman, filter paper, centrifuge, incubator. $NaHS0_4$ buffer, pH meter, 2% casein solution, Oven, 10% TCA, Spectrophotometer, DNS (dinitrosalicylic acid) reagent, $KHP0_4$ buffer, Starch solution andother laboratory reagents and equipment were obtained from the biology laboratory, Federal College of Forestry.

2.2 Method

20g of the cowpea seed was soaked in various concentrations of *Cypermthrin* pesticide (0.25%, 0.50%, 0.75% and 1.0%) for different number of hours as 6hrs, 12hrs and 24hrs. The *Cypermethrin* treated seeds was then germinated in Petri-dishes in the laboratory at suitable weather conditions. The percentage germinated seed was calculated for each concentration of the *Cypermethrin* treated seeds.

Protease and α amylase enzyme activity was carried out for four days for each of the pesticide treated geminating seed. The α amylase activity revealed the amount of sugar liberated from the starch contained in the germinating seed. The protease activity showed the total amino acid released from the liberated protein contained in the germinating seeds.

2.3 Procedure for Enzymes Activities

2.3.1 Alpha Amylase

i. The enzymes were extracted by grinding the wet mash seed sample in 1ml of $KHP0_4$ buffer at pH 6.5.
ii. The suspension was then centrifuged at 4^{0}c for 30 minutes at 5000rpm.
iii. The supernatant for enzymes assay was then collected and 2ml of the extract above was added to 1ml of 15% freshly part and starch solution and mixed.
iv. The mixture was incubated at 40^{0}c for 1 hour and the reaction was terminated by adding 3ml DNS reagent.
v. The mixture was then boiled in water bath for 5 minute, cool rapidly and diluted with 18ml of water.

vi. The optical density was measured at 550nm.

vii. The blank was also treated the same way but a DNS (3, 5-dinitrosalicylic acid) was added before the starch solution.

viii. A Standard curve was then prepared from a known concentration of maltose and from it, the amount of reducing sugars was calculated.

2.3.2 Protease Analysis

i. The protease enzymes was extracted with 0.1ml of $NaHS0_4$ buffer with pH of 6.5 then centrifuged at 5000rpm for 30minutes at 4^0c.

ii. 5ml of the supernatant was collected for assay and 10ml of 2% casein solution was added to it.

iii. The solution was then incubated for 30minute at 35^0c and the reaction was terminated by adding 10ml of 10% trichloro acetic acid solution.

iv. The solution was then filtered through WhatmanNo. 1 paper and the optical density of the filtrate was determined at 275nm.

2.4 Experimental Design

The experimental design used was Randomized Complete Block Design (RCBD) involving 5 treatments control (Water only), 0.25ml of Cypermethrin in 99.75ml water 100ml of solution, 0.50ml of Cypermethrin in 99.50ml water 100ml of solution, 0.75ml of Cypermethrin in 99.25ml water 100ml of solution and 1.0ml of Cypermethrin in 99.00ml water 100ml of solution. The treatments were replicated four times.

2.5 Parameters collected

i**.** RadicleLength: The radicle length will be measured with a thread and the actual length checked on a measuring tape at 3, 4, 5, and 6 days after germination.
ii. Plumule Length**:**The length of the plumule was measured using meter rule at 4, 6, 8, 10 and 12 days after germination.
iii. Number of Leaves:The number of leaves was also counted at two weeks interval and the average of each was recorded per replicate.
iv.Number of Branches:The number of branches was also counted at two weeks interval and the average of each was recorded per replicate.
v.Stem Girth:Thread was used to measure the girth (circumference) of the stem from five selected tagged plants after which the length of the threat was measured out using a metre rule.

2.6Data Analysis

Data collected was subjected to analysis of variance (ANOVA) at 5% level of significance using SPSS 23 and where significance was declared, Duncan Multiple Range Test (DMRT) was used to separate the means.

3.0 RESULT AND DISCUSSION

Table 1: Phytotoxicity of CypermethrinPesticide on Seed Germination and Growth Parameters of Cowpea

Treatments	Radicle Length(cm)	Mean Collar Girth (cm)	Number of Branches	Number of Leaves
To	3.25^{ab}	7.32^{b}	39.15^{d}	101.65^{d}
T1	4.34^{c}	7.22^{ab}	37.85^{d}	95.20^{cd}
T2	4.10^{b}	7.14^{ab}	35.67^{c}	84.16^{ab}
T3	3.65^{ab}	7.11^{ab}	31.40^{b}	88.60^{bc}
T4	3.10^{a}	7.01^{a}	29.20^{a}	75.25^{a}
S.E±	0.30	0.08	0.66	2.70
LS	*	*	*	*

Means within a column having same letters are not significantly different at $P \leq 0.05$.
LS = level of significance
* = Significantat 0.05
SE = Standard Error

3.1 Radicle Length: The result of the radicle length from Table 1 shows a significance difference among the different treatments used. The highest mean value can be seen in the T_1 (4.34), which is significantly different from the control. This result implies that at low concentration, cypermethrin induces the growth of the radicle length but as the concentration increases, the phytotoxic nature of the insecticide begins to manifest. This is clearly shown in Table 1 as T4 has the lowest mean value in terms of radicle length. Many hypotheses could explain this delay of growth in treated plants and seedling. Firstly, insecticides could induce damages in the meristematic cells; in this way, Fayez and Kirsten (1996) showed that chlorosulfuron has an obvious influence on the cellular structure of root caps of *Pisumsativum, Phaseolus vulgaris* and *Viciafaba*, and induce a reduction of radicle cell division, delaying the root growth (Ray *et al*,.1996).

3.2 Collar Girth: Table 1 shows the significance difference in the collar girth of cypermethrin treated cowpea. The highest mean value was observed in the untreated control group (7.32), which is significantly different from T1, T2, T3 and T4. However, there was no significance difference between three of the groups (T1, T2 and T3), even though, there is slight changes in their mean values (7.22, 7.14 and 7.11) respectively. The three treatment groups are however significantly different from the group with the highest concentration of cypermethrin (T4) with mean value of (7.01).The decrease in the mean value of the collar girth across the group could be as a result of the cypermethrin effect on the growth attribute in respect to the expansion on the stem. This corresponds to the findings of (Karim, *et al.,* 2013) which state thatcypermethrin induced a delay in germination and growth processes.

3.3 Number of Branches: The various mean values obtained for the number of branches in Table 1 shows a significance difference in the different concentrations of the cypermethrin treated cowpea. The highest mean value occurs in To (39.15), which is not significantly different from T_1 (37.85). These two treatments however have mean values which are significantly higher and different from T2, T3 and T4 with mean values as found in Table (1). In comparism, T2 is significantly different from the mean value of T3, which in turn is also significantly different from T4. This result shows that at a low concentration of this insecticide, the effect is negligible in term of the number of branches established by the plant. In contrast as the concentration increases, the effect begins to manifest so much so that subsequent treatments are significantly different from the successive ones. …..reported that the use of chemicals has a significant effect in the number of branches which is in line with this finding.

3.4 Number of Leaves: Plant manufacture their food in form of glucose through the use of carbondioxide and water in the presence of chlorophyll found mostly in the green leaves. The significance of leaves in plants growth and development cannot be over emphasized. The more the number of leaves and wider area, the more the photosynthetic taking place. The result of the cypermethrin treated cowpea can be seen (Tabale 1) to be significantly different in term of the number of leaves. The untreated group has the highest mean value and it is significantly different from the rest of the groups. The lowest mean value was found in T4 (75.25). This result shows that at higher concentration of the insecticide, the number of leaves per plant reduces. This result can be compared with that of Mishra et al. (2008) as he stated that insecticides could affect thephotosynthetic system by the inhibition of photo system IIand chain electron transport activities, as reported in *Vignaunguiculata*whentreated by dimethoate. Pesticides could also lead to a delayin photosynthetic pigments rates such as chlorophylls(Mishra *et al.,* 2008).

Table 2: Phytotoxicity of CypermethrinPesticide on Seed YieldParameters of Cowpea

Treatments	**Number of seeds/pod**	**100 seed weight (g)**	**Pod length (cm)**	**Dry Matter (g/m^2)**
T_o	18.52^d	18.53^d	16.35^c	68.35^c
T_1	17.49^c	18.40^d	16.28^{bc}	68.19^c
T_2	17.83^c	17.25^c	15.84^a	67.50^{bc}
T_3	16.84^b	15.85^b	16.05^{ab}	66.56^b
T_4	16.13^a	13.78^a	15.83^a	65.07^a
S.E±	0.24	0.26	0.08	**0.37**
LS	*	*	*	*

Means within a column having same letters are not significantly different at $P \leq 0.05$.
LS = level of significance
* = Significantat 0.05
SE = Standard Error

3.5 Number of Seeds/Pod: Number of seeds per pod is one of the important yield parameters which determines the yield outcome of the crop. The analysis for the number of seeds/pod (Table 2) shows a significance difference between all the treatments. The highest mean value occurs in T_o (18.52) which is significantly different from the mean values obtained in T_1 (17.49), T_2 (17.83, T_3 (16.84) and T_4 (16.13). Treatments T1 and T2 have mean values 17.49 and 17.83 and are seen not to be significantly different from each other. The result shows that at low concentration of cypermethrin, the effect is minute until the concentration increases further as seen in T3 and T4. This implies that the plant internal metabolic process can degrade the insecticide and reduce the effect until the effect is irresisitible. Glover-Amengor and Tetteh (2008) reported that pesticides application may decline growth and yield of vegetables by affecting the beneficial microflora of soil.

3.6 100 Seeds Weight: It will be noticed in Table 2 above that at low concentration of cypermethrin, the effect was not significantly different from the control at ($P<0.05$). The treatments T_2, T_3 and T_4 are seen to be significantly different from one another, with mean values 17.25, 15.85 and 13.78 respectively. The result shows that at very low concentration, cypermethrin has no significance effect on 100 seed weight. The seed of cowpeais made up of water and carbohydrate, including protein. The treatments with high cypermethrin concentration has low mean value for the 100 seed weight. This could be due to the stress the crop went through in reducing the cypermethrin effect.

3.7 Pod Length: The pod length of the yield obtained (Table 2) after analysis shows a significance difference among the different treatments considered. Treatment To with the highest mean value (16.35) is seen to be significantly different from T1, T2, T3 and T4. The lowest mean value is seen in T_4 15.83 which is not significantly different from T_2 with mean value (15.84). These two treatments are significantly different from To, T1 and T3. This result implies that cypermethrin has a significant effect on the pod length of cowpea, even at a concentration of 25%. This can be compared with the work of Dzemo et al. (2010) which states that singular spray of deltamethrin + dimethoate combinations at the dose of 30 + 250 g a.i/ l showed a significance effect on the pod length of cowpea (*Vignaunguiculata*).

3.8 Total Dry Matter: Table 2 shows the differences in the mean value for each of the different concentrations of cypermethrin treated cowpea seeds. To is seen to have the highest mean value (68.35), but not significantly different from T_1 with mean value (68.190). The two treatments are however seen to be significantly different from T_2, T_3 and T_4 with mean value 67.50, 66.56 and 65.07 respectively.Table 2 shows how the effect of the cypermethrin had led to the reduction in the dry matter of the cowpea. Crop plants make use of sunlight, carbon dioxide and water which they use to photosynthesize and brings about an increase in crop fibre weight. The presence of this pesticide must have led to changes in the metabolism of the crop. This led to the reduction of the total dry matter of the crops as the concentration of the pesticide increases. This can be related to the work published by Ajeigbe*et al*., (2012)

Table 3: Effect of Cypermethrin on the Alpha Amylase Activity (μmol maltose/ml/min) of Cowpea Treated Seeds

Treatments	Day 2	Day 3	Day 4
To	2.45^{d}	1.15^{a}	0.75^{a}
T_1	2.75^{e}	1.80^{b}	2.40^{c}
T_2	1.05^{b}	1.15^{a}	2.10^{b}
T_3	0.75^{a}	1.25^{a}	2.28^{c}
T_4	2.20^{c}	2.70^{c}	3.10^{d}
SE	0.02	0.04	0.05
LS	*	*	*

Means within a column having same letters are not significantly different at $P \leq 0.05$.
LS = level of significance
* = Significantat 0.05
SE = Standard Error

3.9 Alpha Amylase Activity: The amylase activity for the germinating seeds show a significance difference for day2, Day 3 and Day 4 as shown in Table 3. Germinating seeds have amylase enzyme which degrade the stored starch to give smaller unit of the carbohydrate in the form of maltose. The result above shows that the rate at which this enzyme degrades the stored starch is greatly influenced by the presence of the cypermethrin in it. On day 2, the highest mean value for the enzyme activity can be seen in $T_{1.}$ This shows that at lower concentration of cypermethrin, the activity of the enzyme is enhanced, even at the very high concentration of it (T_4). On day 3, the activity of the enzyme is seen to be reduced, even though it is significantly different from the control group To. On day 4, the activity of the enzyme is also significantly different for each of the group, with the highest mean value in T_4. This result indicates that the activity of the enzyme increases at the initial days for lower concentration of the pesticide but increases at later days for the groups with higher concentration of the pesticide in the later days as observed in T1 and T4 respectively at ($P<0.05$) level of significance. This result is in line with the work ofMishra *et al.* (2008) which states that insecticides could affect the photosynthetic system by the inhibition of photo system II and chain electron transport activities in *Vignaunguiculata*when treated by dimethoate. Pesticides could also lead to a delay in photosynthetic pigments rates such as chlorophylls (Mishra *et al.*, 2008).

4.0 CONCLUSION

The use of insecticides, cypermethrin inclusive, for the treatment of seeds before planting is a normal practice byfarmers. These chemicals enter into the seeds through the seed membrane and percolate the cells, which likely change the genetic make-up of the seeds. This study examined some growth parameters, yield parameters, germination effect as well as alpha amylase enzyme activity of cowpea (*Vignaunguiculata*) seeds. More studies are however encouraged by others researchers, especially in the area of genetics.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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