



Phytotoxicity of Cypermethrin Pesticide on Seed Germination, Growth and Yield Parameters of Cowpea (*Vigna unguiculata*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author SMO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors III and AYY managed the analyses of the study. Author UIH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The experiment was carried out at 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. Cypermethrin is popularly used by farmers as a means of treating seeds before planting to prevent insect, pest and birds attack. Randomized Complete Block Design (RCBD) involving 5 treatments T_0 as control (No cypermethrin used), T_1 with 0.25% cypermethrin (0.25ml of cypermethrin in 99.75ml of water), T_2 with 0.50% cypermethrin (0.50ml of cypermethrin in 99.50ml of water), T_3 with 0.75% cypermethrin (0.75ml of cypermethrin in 99.25ml of water) and T_4 contained 1.00% cypermethrin (1.0ml of cypermethrin in 99.00ml water). 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 T_1

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has the highest mean value. The stem girth shows a significance difference with T_0 having the highest mean value (7.32) at $P<0.05$. The result for the number of branches and the number of leaves shows significance difference with T_0 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 T_0 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. Alpha amylase enzyme activity was observed to be higher at lower concentration of the cypermethrin (T_1) on day 2 (2.75) but the increase in the enzyme activity tilted towards the highest concentration (T_4) on day 3 and day 4 with mean value 2.70 and 3.10 at 5% level of significance.

Keywords: *Phytotoxicity; cypermethrin; pesticide; seed germination; cowpea.*

1. 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 [1]. Results show that insecticides are the most effective control measures against pests and majority of the farmers rely heavily on the use of synthetic insecticides in the management of their cowpea pests [2].

Cowpea (*Vigna unguiculata*) (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 [3] and cowpea has appropriately been called "poor man's meat" [4]. 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 [5]. 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 [6] largely contribute to low yield and good grains cannot be obtained in farms without any form of control on insect pests [7]. The major/key insect pests include the foliage beetle, cowpea aphid (*Aphis craccivora*), the legume pod borer, *Maruca vitrata* (Fab) and a complex of pod sucking insects which include *Clavigralla tomentosicollis*, *Anoplocnemis curvipes*, 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. 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 [6]. However, abandonment of insecticides in favor of other control measures does not provide solution because this would worsen the present food situation [8]. 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 [9]. The use of pesticides in agriculture has been steadily increasing in the last 40 years [10]. 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 [11].

Germination and seedling establishment are critical stages in the plants life cycle [12]. In crop production, stand establishment determines plant density, uniformity and management options. Poor germination and crop establishment result in significant reduction in economic yield [13].

Although seed treatments have important benefits, they also pose certain risks if application rate and dose of these chemicals 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 [14]. The indiscriminate and unskillful use of pesticides affects the growth of plant and animal, increases pest resistant to pesticides, accumulates residue in fruits and vegetables, causes biodiversity losses and declines natural habitats [12]. Pesticides application can also lead to demolition of micro-fauna and flora of soil and water [16,17]. 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.

Starch is the essential polysaccharide of plant reserves. It is constituted by a chain of molecules of α -glucose, and present in two forms: amylose and amylopectin [18]. Plants degrade their starch-based reserves by using amylase, when monosaccharides and disaccharides are required for their growth and development. The α -amylase (α -1,4-glucan-4-glucanohydrolase) is a hydrolytic enzyme, catalyzing the hydrolysis of α -1,4-glycosidic connections of polysaccharides internal chains. It's a key enzyme of the saccharidic metabolism [19]. Certain substances have been reported to induce stress in crop plants and bring about delay in the internal metabolism of such plants. These substances are categorized as xenobiotics to plants and they include a host of substances like salts [20] insecticides [21] and crude oil [22] etc. Therefore, this study was carried out to examine the phytotoxicity of pesticide (cypermethrin) on seed germination, growth and yield parameters of cowpea (*Vigna unguiculata*).

2. MATERIALS AND METHODS

The experiment was carried out at Federal College of Forestry Jos, in Jos North Local Government Area of Plateau State. Jos is located between latitude 7-11° North and longitude 7-8° East. Temperature ranges between 10-32°C and main annual rainfall is about 1340mm, with an average elevation of 1200mm above sea level [23].

2.1 Materials

2.1.1 Source of materials

The materials used include insecticide (Cypermethrin) for seed dressing, certified seed of cowpea obtained from Plateau Agricultural Development Program (PADP), Dogon Dutse, in Jos North local government area of Plateau State. Petri-dish, whatman No. 1 filter paper, centrifuge, incubator. NaHSO₄ buffer, pH meter, 2% casein solution, Oven, 10% TCA, Spectrophotometer, DNS (dinitrosalicylic acid) reagent, KHPO₄ buffer, Starch solution and other laboratory reagents and equipments were obtained from the biology laboratory, Federal College of Forestry.

2.2 Method

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

α -amylase enzyme activity was carried out for four days for each of the pesticide treated germinating seed. The α -amylase activity revealed the amount of sugar liberated from the starch contained in the germinating seed.

2.3 Procedure for Enzymes Activities

The alpha amylase activity was carried out following the procedure described by Bernfeld [24], with some modifications. The procedure is as listed below:

2.3.1 Alpha amylase

- i. The enzymes were extracted by grinding the wet mash seed sample in 1ml of KHPO_4 buffer at pH 6.5.
- ii. The suspension was then centrifuged at 4°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°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.
- 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 thread was measured out using a metre rule.
- vi. Number of Seeds/Pod: Readings were taken from each of the treatments by counting the number of seeds per pod for each of the experimental units. The average of these readings were taken and the result used for further analysis
- vii. 100 Seed Weight: after harvesting the yield, 100 seeds were picked at random from 3 plants for each of the treatments and their replicates. The seeds were weighed using an electric weighing balance and readings taken in grams. Their average was thereafter taken accordingly.

2.4 Experimental Design

The experimental design used was Randomized Complete Block Design (RCBD) involving 5 treatments;

- i. T_0 = control (Water only),
- ii. T_1 = 0.25ml of Cypermethrin in 99.75ml water 100ml of solution,
- iii. T_2 = 0.50ml of cypermethrin in 99.50ml water 100ml of solution,
- iv. T_3 = 0.75ml of cypermethrin in 99.25ml water 100ml of solution and
- v. T_4 = 1.0ml of cypermethrin in 99.00ml water 100ml of solution.

The treatments were replicated four times.

2.5 Parameters Collected

- i. Radicle Length: The radicle length was measured with a thread and the actual length checked on a measuring tape at 3, 4, 5, and 6 days after germination. The average of the readings were taken and the analyzed
- ii. Plumule Length: The length of the plumule (shoot) was measured using meter rule at 4, 6, 8, 10 and 12 days after germination.

- viii. Pod Length: 5 pods were selected from three plants for each of the treatments and replicates and their length measured using a thread. The length of the thread was later measured out from meter rule and the average recorded.
- ix. Total Dry Matter: After the pods were removed, the plants were uprooted and dried. The weight of the dry matter for the treatments and their replicates was taken as the total dry matter.

2.6 Data 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. RESULTS AND DISCUSSION

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 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 effect of the insecticide begins to manifest. This is clearly shown in Table 1 as T_4 has the lowest mean value in terms of radicle length. Many hypotheses could explain this delay of growth in treated plants and seedlings. Firstly, insecticides could induce damages in the meristematic cells; in this way, Fayez and Kirsten [25] showed that chlorosulfuron has an obvious influence on the cellular structure of root caps of *Pisum sativum*, *Phaseolus vulgaris* and *Vicia faba*, and induce a reduction of radicle cell division, delaying the root growth [26].

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 T_4 . However, there was no significance difference between three of the groups (T_1 , T_2 and T_3), even though, there is slight changes in their mean values (7.22, 7.14 and 7.11) respectively. The three treatment groups are however statistically similar to T_4 which is the group with the highest concentration of cypermethrin (T_4) 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 [21] which states that cypermethrin 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 T_0 (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 T_2 , T_3 and T_4 with mean values as found in Table (1). In comparison, T_2 is significantly different from the mean value of T_3 , which in turn is also significantly different from T_4 . 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. Parween et al. [27] stated in his findings that chlorpyrifos brought about an increase in number of branches when applied at the rate of 1.5mM but further increase in the concentration of the organophosphate pesticide gave a negative effect by reducing the number of branches of *Vigna radiate*.

3.4 Number of Leaves

Plants manufacture their food in form of glucose through the use of carbon dioxide 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 photosynthesis taking place based on individual plants. The result of the cypermethrin treated cowpea can be seen (Table 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 T_2 , T_3 and T_4 groups. The lowest mean value was found in T_4 (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. [28] as he stated that insecticides could affect the photosynthetic system by the inhibition of photo system II and chain electron transport activities, as reported in *Vigna unguiculata* when treated by dimethoate. Pesticides could also lead to a delay in photosynthetic pigments rates such as chlorophylls [28].

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 significant difference between all the treatments. The highest mean value occurs in T_0 (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 T_1 and T_2 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 T_3 and T_4 . This implies that the plant's internal metabolic process can degrade the insecticide and reduce the effect until the effect is irresistible. Glover-Amengor and

Table 1. Phytotoxicity of Cypermethrin pesticide on the germination and growth parameters of cowpea (*Vigna unguiculata*) treated seed

Treatments	Radicle Length (cm)	Mean Collar Girth (cm)	Number of Branches	Number of Leaves
T ₀	3.25 ^{ab}	7.32 ^b	39.15 ^d	101.65 ^d
T ₁	4.34 ^c	7.22 ^{ab}	37.85 ^d	95.20 ^{cd}
T ₂	4.10 ^b	7.14 ^{ab}	35.67 ^c	84.16 ^{ab}
T ₃	3.65 ^{ab}	7.11 ^{ab}	31.40 ^b	88.60 ^{bc}
T ₄	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,

* = Significant at 0.05, SE = Standard Error

Table 2. Phytotoxicity of cypermethrin pesticide on the yield components of cowpea seed (*Vigna unguiculata*)

Treatments	Number of seeds/pod	100 seed weight (g)	Pod length (cm)	Dry Matter (g/m ²)
T ₀	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,

* = Significant at 0.05, SE = Standard Error

Tetteh [29] reported that pesticide's application may decline growth and yield of vegetables by affecting the beneficial microflora of soil.

3.6 100 Seed Weight

It will be noticed in Table 2 that at low concentration of cypermethrin, the effect was not significantly different from the control at ($P < 0.05$). The treatments T₂, T₃ and T₄ 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 significant effect on 100 seed weight. The seed of cowpea is made up of water and carbohydrate, including protein and some other components in small amount. 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. This can be related to the work of Dzemo et al. [30] in which the spray of dimethoate + deltamethrin gave a significance reduction in the 1000 seed weight of cowpea seeds.

3.7 Pod Length

The pod length of the yield obtained (Table 2) after analysis shows a significant difference among the different treatments considered. Treatment T₀ with the highest mean value (16.35) is seen to be significantly different from T₂, T₃ and T₄. The lowest mean value is observed to be in T₄ (15.83) which is not significantly different from T₂ with mean value 15.84. These two treatments are significantly different from T₀ and T₁. 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. [30] which states that singular spray of deltamethrin + dimethoate combination at the dose of 30 + 250 g a.i/l showed a significant

effect on the pod length of cowpea (*Vigna unguiculata*).

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. T₀ is seen to have the highest mean value (68.35), but not significantly different from T₁ with mean value 68.19. These two treatments are seen to be statistically similar to T₂, with mean value 67.50. The two treatments are however seen to be significantly different from T₃ and T₄ with mean values 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 bring about an increase in crop fibre weight. The presence of this pesticide must have led to changes in the metabolism of the crop responsible for the increase in fibre content. 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 Shakir et al. [31] in which a significant reduction in shoot fresh and dry weight was observed in tomato (*Lycopersicon esculentum*) treated with emamectin, benzoate, cypermethrin, lambda-cyhalothrin and imidacloprid pesticides.

3.9 Alpha Amylase Activity

Starch is quantitatively the most abundant storage material in all plant seeds according to

Chungh and Sawhney [32]. Alpha amylase enzyme catalyze the degradation of α -1,4-glucosidic connections of polysaccharide chains [21]. The amylase activity for the germinating seeds show a significance difference for day 2, 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 disaccharide gets degraded to its monosaccharide units which are glucose and fructose, which later undergo glycolysis in order to generate energy for the germinating seeds. The result (Table 3) shows that the rate at which this enzyme degrades the stored starch is greatly influenced by the presence of the cypermethrin contained in it. On day 2, the highest mean value for the enzyme activity can be seen in T₁. This shows that at lower concentration of cypermethrin, the activity of the enzyme is enhanced, even at the very high concentration of it (T₄). On day 3, the activity of the enzyme is seen to be reduced, even though it is significantly different from the control group T₀. On day 4, the activity of the enzyme is also significantly different for each of the group, with the highest mean value in T₄. 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. Generally, the activity of the enzyme decreases with increase in the concentration of the cypermethrin pesticide.

Table 3. Effect of Cypermethrin on the Alpha Amylase Activity ($\mu\text{mol maltose/ml/min}$) of Cowpea Treated Seeds

Treatments	Day 2	Day 3	Day 4
T ₀	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,

* = Significant at 0.05, SE = Standard Error

This result is similar with the work of Karim et al. [21] tomato and Uvere et al. [33] Sorghum, in which changes occur in the alpha amylase activity of crops during their germination. Uriyo [34] has also reported same results in the case of cowpea in which he found that germination had a highly significant effect ($P < 0.05$) on cowpea α -amylase activity. This result could be as a result of the cypermethrin, serving as a xenobiotic which inhibits the activity of alpha amylase enzyme in breaking-down the stored starch in the cowpea seeds. The stored starch were not degraded into their disaccharide and monosaccharide units which led to the lack of energy to enhance germination in the seeds. This can also be related to the reason the germination percentage reduced in T₂, T₃ and T₄.

4. CONCLUSION

The use of insecticides, cypermethrin inclusive, for the treatment of seeds before planting is a normal practice by farmers. These chemicals enter into the seeds through the seed membrane and percolate the cells, which likely change the genetic make-up of the seeds through gene mutation. This study examined some growth parameters, yield parameters, germination effect as well as alpha amylase enzyme activity of cowpea (*Vigna unguiculata*) seeds. It was observed that moderate usage of this pesticide stimulates early germination and hence, improves seedling vigor and growth parameters as well as the yield obtained. Higher concentration of it inhibits early germination and also affects the growth parameters of the crop. The insecticide also has higher concentration of alpha-amylase activity at the early days of the experiment at lower concentration, which reduces at later days. At higher concentration, the alpha-amylase activity increases with the days. This implies that higher concentration of cypermethrin reduces the rate of break-down of stored starch in germinating seeds. More studies are however encouraged by others researchers, especially in the area of genetics.

COMPETING INTERESTS

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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