

RESEARCH ARTICLE

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Effects of butachlor and pendimethalin herbicides on seed germination and early seedling growth of two species of cowpea

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ABSTRACT

Effects of butachlor and pendimethalin herbicides on seed germination and early seedling growth of Vigna sinensis and Vigna unguiculata were investigated in both the laboratory and field conditions. Seeds of both V. sinensis and V. unguiculata germinated at 5 mg/ml of the two herbicides, but at 50 and 100 mg/ml, germination was inhibited. The root and shoot lengths were significantly inhibited by the herbicides (P<0.05). The herbicides significantly reduced fresh and dry weights (P<0.05) on root and shoot biomass. Pigmentation pattern of the seedlings varied from yellowish and green in pendimethalin treated seedlings to dark green with patchy appearance on butachlor treated seedlings. From the results, seeds of the two species tolerated the two types of herbicides especially at low doses. Thus, application of butachlor and pendimethalin at low doses could be helpful in seed germination and proper growth and development of Vigna seedlings.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) belongs to the family Papillionacea [3]. It is an important crop and a major food crop of millions of people especially in the developing countries of the world. Cowpea provides cheap and nutritious food for relatively poor urban communities. Cowpea also has potential for multiple contributions not only to household food security, but also as cash crop. It also brings nitrogen into farming system through nitrogen fixation [14]. Nigeria accounts for 70% of world cowpea production, the bulk of which is grown in the drier region of Northern Nigeria [1, 7]. Nigeria and Niger are ahead in world production of cowpea. Cowpea is widely grown as an intercrop with cereals in the marginal land and through improved breeding lines and agronomic practices, there is potential for increased productivity despite the prevailing production challenges especially of declining soil fertility, unreliable rainfall, pests and diseases [10].

There are a number of constraints to cowpea production in Nigeria which include attack by insect pests, infestation by disease pathogens, soil infertility, poor weather condition in addition to weeds, all of which contribute to low yield [18, 4, and 17]. The first 3-4 weeks of cowpea growth are critical periods of weeds competition [19, 12]. Cowpea is highly susceptible to destructive weeds such as *Euporbia heteophylla*, *Calapogonium mucunoides* and *Rottboella cochichinensis*. Nigerian farmers carry out weed control manually at 3 and 5 weeks after planting. This is however associated with drudgery and high cost of labour [5]. Studies shows that recommended herbicides had not totally solved the problem of weeds on farmer's field due to inadequate selectivity of herbicides in action.

Cowpea varieties differ in many characteristics amongst which are responses to herbicides treatments. There was noticeable adoption of chemical weed control option to a large extent, by many farmers. However, not much of those farmers were aware that cowpea performance also depends much on spraying the agrochemicals with appropriate doses. Symptoms of chemical injury on weeds include rapid wilting, scorching and desiccation of the treated foliage. This research aimed at investigating the effects of Butachlor and Pendimethalin herbicides on seed germination and early seedling growth of two *Vigna* species.

MATERIALS AND METHODS

Two species of cowpea (*Vigna sinensis* and *V. unguiculata*) were collected from Kwatarkwashi village in Zamfara state, Nigeria. They were identified and authenticated by the taxonomist in the Department of Biological Sciences of Usmanu Danfodiyo University, Sokoto where voucher specimens were deposited in the Herbarium. Two synthetic herbicides namely, pendimethalin (3, 4-Dimelhyl-2,6-Dinito-N-(1-ethylpropyl)-benzanamine) and Butachlor (N-Butoxy methyl 1-2-chloro-2,6-Diethyl acetanilide) were purchased from the Sokoto Central Market: Three varying concentrations of each herbicides were prepared according to manufacturers' specifications.

Seeds of uniform sizes were selected and sown on two layers of Whatman No. 1 filter paper in Petri-dishes and in the field. All the Petri-dishes and filter papers were sterilized with 5 ml of formalin at 80^oC for one hour. In each, ten seeds were sown. Prior to sowing the seeds were treated with Pendimethalin and Butachlor at concentrations of 5, 50 and 100 mg/ml, separately. Each of the treatments was replicated three times; similarly, a set of three replicates were served as control. Observations of the germination were recorded on daily basis. Three weeks after sowing in the field, measurements of root and shoot lengths as well as counting the number of leaves were carried out by cutting the root of each seedling with a cleaned razor blade and measuring with tape rule and the shoot length of each seedling was measured by the use of tape rule. Subsequent measurement and counting was done at one-week intervals. The dry weight of the seedlings was determined by collection of three treated seedlings and blotted to remove moisture on them, they were put in envelops and dried at 50^oC. The data obtained were subjected to analysis of variance Bonferroni compare all columns, graphed instant Software (San Diego, USA).

RESULTS AND DISCUSSION

Effect of Butachlor on seed germination and seedling growth on V. sinensis and V. unguiculata

High percentage germination of seed was observed in seeds of *V. sinensis* with significant difference (P < 0.05) at 5 mg/ml of Butachlor than *V. unguiculata* at the same concentration. At 50 mg/ml and 100 mg/ml, no germination was observed in both varieties (Table 1). The first week measurements indicated that Butachlor affected the root and shoot lengths of *V. unguiculata* than those of *V. sinensis* at all levels of treatment with no significant difference (P < 0.05) at 5 mg/ml and 50 mg/ml (Tables 3 and 5). The fresh weight of root and shoot of *V. sinensis* were not inhibited by Butachlor herbicides. In *V. unguiculata* at all levels of treatment, fresh weight was not significantly affected (P < 0.05), whereas *V. sinensis* produced higher root; shoot dry weight than *V. unguiculata* and the root, shoot dry weight was not significantly different in *V. unguiculata* and significantly different in *V. sinensis* (P < 0.05).

Defoliation of leaves was observed in *V. unguiculata*, the leaves appeared yellowish in colour at all levels of treatment and it was significantly different (P < 0.05) but there was no colour change observed due to Butachlor on leaves of *V. sinensis* (Tables 3 and 5). The second week measurements of root, shoot length showed that there was inhibition in length of root and shoot of *V. sinensis* at 5 mg/ml with significant difference at (P < 0.05), but at 50 and 100 mg/ml, *V. unguiculata* was highly inhibited by Butachlor with no significant difference (P < 0.05) Tables 3 and 5). The root, shoot fresh weights of *V. sinensis* were also affected at 5 mg/ml but at 50 mg/ml and 100 mg/ml *V. sinensis* showed higher root and shoot fresh weight than *V. unguiculata*. Higher root, shoot dry weight was observed at 5 mg/ml and 50 mg/ml in *V. unguiculata*. At 100 mg/ml, *V. sinensis* showed higher root and shoot dry weight but with no significant difference (P < 0.05). The leaves of both species were not affected at 5 mg/ml with significant difference (P < 0.05) and *V. unguiculata* showed higher number of leaves than *V. sinensis*, and at 50 mg/ml the number of leaves become close to each other, at 10 mg/ml total defoliation was observed in *V. unguiculata* with no significant difference (P < 0.05, Tables 3 and 5). The third week measurement also indicated that *V. unguiculata* had longer roots and shoot at 5 mg/ml but at 50 mg/ml *V. sinensis* had longer root while *V. unguiculata* and shoot of *V. sinensis* was affected by Butachlor than *V. unguiculata* and shoot of *V. unguiculata* and shoot of *V. sinensis* was affected by Butachlor than *V. unguiculata* and shoot of *V. unguiculata* and shoot of *V. sinensis* was affected by Butachlor than *V. unguiculata* and shoot of *V. unguiculata* was affected than *V. sinensis* had longer root while *V. unguiculata* and shoot of *V. unguiculata* was affected than *V. sinensis* was affected by Butachlor than *V. unguiculata* and shoot of *V. unguiculata* was

The roots, shoot fresh weights of *V. sinensis* were affected by Butachlor at all levels of treatment except at 100 mg/ml whereas *V. sinensis* showed higher shoot fresh weight than *V. unguiculata*. The root dry weight of both varieties was the same at 5 mg/ml but at 50 mg/ml and 100 mg/ml *V. sinensis* showed higher root weight than *V. unguiculata* and shoot dry weight of *V. sinensis* was affected at all level of treatment but it is with significant difference (P < 0.05). The leaves of both varieties were affected by Butachlor at 5 mg/ml but *V. sinensis* showed higher number of leaves than *V. unguiculata* (Table 3) and at 50 mg/ml, there was more inhibition in the leaves of *V. sinensis* while at 100 mg/ml in the leaves of *V. unguiculata*.

	Treatment (mg/ml)	Day 1	Day 2	Day 3
	Butachlor			
	0	4.67 ^a	7.00^{a}	7.33 ^a
	5	1.33 ^b	3.00 ^b	3.00 ^b
	50	0.00 ^c	0.00°	0.00 ^c
	100	0.00 ^c	0.00°	0.00°
	SE <u>+</u>	1.10	1.66	1.73
	Pendimethalin			
	0	4.67 ^a	7.00^{a}	7.33 ^a
	5	2.67 ^b	4.67 ^b	4.67 ^b
	50	0.00 ^c	0.00°	0.00°
	100	0.00 ^c	0.00°	0.00 ^c
_	SE <u>+</u>	1.13	1.75	1.82
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Table 1: Effect of butachlor and pendimethalin on seed germination of V. sinensis.

Means followed by the same letters are not significantly different (P < 0.05).

Table 2: Effect of butachlor and pendimethalin of Seed Germination of V. unguiculata

Treatment (mg/ml)	Day 1	Day 2	Day 3
Butachlor			
0	5.33 ^a	6.67 ^a	7.00^{a}
5	0.00^{b}	0.67^{b}	0.67 ^b
50	0.00^{b}	0.00^{b}	0.00^{b}
100	0.00^{b}	0.00^{b}	0.00^{b}
SE <u>+</u>	1.33	1.62	1.70
Pendimethalin			
0	5.33 ^a	6.67 ^a	7.00^{a}
5	0.00^{b}	1.00^{b}	1.00^{b}
50	0.00^{b}	0.00^{b}	0.00^{b}
100	0.00^{b}	0.00^{b}	0.00^{b}
SE <u>+</u>	1.33	1.60	1.66

Means followed by the same letters are not significantly different (P < 0.05).

Table 3: Lengths of shoot, root and number of leaves of V. sinensis as affected by different concentration of butachlor and pendimethalin

Treatment (mg/ml)		Week 1			Week 2		Week 3				
Butachlor	Shoot Length	Root length	Leaf Number	Shoot Length	Root length	Leaf Number	Shoot Length	Root length	Leaf Number		
5	17.8 ^a	18.2 ^a	14.3 ^a	15.7 ^a	17.9 ^a	14.0^{a}	9.5 ^a	19.3ª	7.3 ^a		
50	17.1 ^a	17.7 ^a	23.0 ^b	10.4 ^b	16.4 ^b	5.0 ^b	12.1 ^b	22.3ª	4.6 ^b		
100	12.0 ^b	26.5 ^b	10.6 ^c	13.5 ^c	16.7 ^b	6.0^{b}	17.0 ^c	6.0 ^b	7.0 ^b		
SE <u>+</u>	1.83	2.85	3.68	1.54	0.46	2.85	2.19	5.01	0.85		
Pendimethalin											
5	21.9ª	13.2ª	30.0 ^a	14.7 ^a	12.6 ^a	8.6 ^b	16.2 ^a	20.0 ^c	6.6 ^a		
50	13.3 ^b	14.8 ^b	26.6 ^b	16.1 ^b	11.4 ^b	8.6^{b}	15.1 ^b	22.6 ^c	12.0 ^b		
100	20.8°	16.0 ^c	25.6 ^b	18.9 ^c	16.0 ^c	21.3 ^c	9.4°	5.6	1.3 ^c		
SE <u>+</u>	3.12	0.81	1.33	1.24	1.38	4.23	2.11	5.29	3.09		

Means followed by the same letters are not significantly different (P < 0.05).

Effects of Pendimentalin on seed germination and seedling growth on V. unguiculata and V. sinensis

Higher percentage of seed germination was observed on pendimethalin treatment on *V. sinensis* with significant difference (P < 0.05) at 5 mg/ml than *V. unguiculata*, but at 50 mg/ml and 100 mg/ml no germination was observed in both varieties (Table 2). The first week of root, shoot length measurements showed that pendimenthalin did not affect the root and shoot length of both varieties. Thus *V. unguiculata* showed longer root and *V. sinensis* have longer shoot at 5 mg/ml (Table 3) and at 50 mg/ml and 100 mg/ml *V. ungiculata* longer root and shoot length than *V. sinensis* and it is significantly different (P < 0.05) (Tables 4 and 6). The root fresh weight of both varieties appeared the same at 5 mg/ml with significant difference (P < 0.05) Table 3) while shoot fresh weight of *V. unguiculata* was higher than *V. sinensis* at higher concentration. The root dry weight of *V. unguiculata* was higher at 5 mg/ml, whereas the shoot dry weight of *V. sinensis* was higher at same concentration (Table 3) but at 50 mg/ml, *V. sinensis* had higher root, shoot dry weights than *V. unguiculata* and with significant difference (P < 0.05, Tables 4 and 6). The leaves of both varieties were affected at all levels of treatment but it was more pronounced in *V. unguiculata*. The second week measurement showed that there was significant different (P < 0.05) at 5 mg/ml the root length of *V. unguiculata* was affected by pendimethalin treatment than *V. sinensis* (Tables 3 and 5) while at 50 mg/ml and 100 mg/ml, complete inhibition was observed in *V. sinensis* than *V. unguiculata* while the shoot lengths, root and shoot fresh weight leaves of both species were affected at all levels of treatment with a significant

difference (P<0.05). The third week measurement also showed that the root and shoot lengths of *V. unguiculata* were affected at 5 mg/ml (Table 5) than those of *V. sinensis*, but at 50 mg/ml and 100 mg/ml, complete inhibition was observed on *V. sinensis* than on *V. unguiculata*. The roots, shoot fresh weights of *V. unguiculata* were highly inhibited at 5 mg/ml and 50 mg/ml (Table 6) while at 100 mg/ml, total inhibition was observed in *V. sinensis*. The root, shoot dry weights as well as the leaves of both species were affected at all levels of treatment with significant difference (P<0.05).

Table 4. Fresh and dry weights of root and shoot	of Viana cinancic as affected	by varying concentration of h	utachlar and nondimethalin
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Treatment (mg/ml)		Wee	ek 1		Week 2				Week 3			
Butachlor	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight
5	18.7 ^a	1.0 ^a	3.9ª	5.3 ^a	9.2 ^a	1.2 ^a	2.3 ^a	0.5 ^a	2.9 ^a	0.6 ^a	0.6 ^a	0.4 ^a
50	18.7^{a}	0.8^{b}	3.1 ^a	0.6^{b}	2.0^{b}	1.2 ^a	0.4^{b}	0.9^{b}	2.4 ^b	1.2 ^b	0.8^{b}	0.4^{a}
100	5.0 ^b	1.0 ^a	1.1 ^c	0.2 ^b	2.4 ^b	1.3 ^a	0.9^{b}	0.3 ^a	2.5 ^b	0.6^{a}	0.6 ^a	0.2 ^b
SE <u>+</u>	4.57	0.06	0.83	1.64	2.34	0.03	0.57	0.18	0.15	0.20	0.06	0.06
Pendimethalin												
5	29.0^{a}	0.9^{a}	4.2^{a}	0.15^{a}	6.4^{a}	1.4^{a}	4.7^{a}	1.0^{a}	9.5 ^a	1.2 ^a	3.6 ^a	0.9^{a}
50	14.3 ^b	1.0^{b}	3.7 ^b	0.4^{b}	2.5 ^b	0.4^{b}	0.5 ^b	0.1^{b}	6.5 ^b	0.7^{b}	1.9^{b}	0.1 ^b
100	26.0 ^c	2.0 ^c	6.1 ^c	1.0 ^c	16.2 ^c	2.4 ^c	4.2 ^a	0.7°	0.3°	0.1 ^c	0.1 ^c	0.03 ^c
SE <u>+</u>	4.48	0.35	0.73	0.25	4.08	0.57	1.32	0.26	2.71	0.32	1.01	0.28

Means followed by the same letters are not significantly different (P < 0.05).

Table 5:	Effect of herbicides on growth of V. unguiculata as at different concentrations
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Treatment (mg/ml)		Week 1			Week 2			Week 3			
Butachlor	Shoot length	Root length	Leaf number	Shoot length	Root length	Leaf number	Shoot length	Root length	Leaf number		
5	11.3 ^a	9.2ª	7.0^{a}	17.7 ^a	20.5 ^a	16.3 ^a	11.1 ^a	26.5 ^a	5.6 ^a		
50	5.9 ^b	15.1 ^b	8.0^{a}	10.4 ^b	9.2 ^b	4.6 ^b	14.0 ^b	16.8 ^b	11.6 ^b		
100	8.7°	11.6 ^c	1.6 ^b	11.7 ^c	7.6 ^b	1.6 ^b	9.6 ^c	10.3 ^c	3.6 ^a		
SE +	1.56	1.71	1.99	2.25	4.06	4.48	1.29	4.70	2.40		
Pendimethalin											
5	13.3ª	16.5 ^a	17.3 ^a	16.2 ^a	4.0^{a}	31.0 ^a	9.6 ^a	16.3ª	9.0 ^a		
50	14.8 ^b	17.3 ^b	33.6 ^b	13.5 ^b	26.2 ^b	9.0 ^b	10.3 ^a	20.0 ^b	7.0 ^b		
100	12.8 ^c	13.6 ^c	6.6 ^c	15.2 ^c	17.2 ^c	21.6 ^c	14.0 ^b	20.9 ^b	13.0 ^c		
SE +	0.60	1.12	7.85	0.78	6.45	6.37	1.37	1.41	1.76		

Means followed by the same letters are not significantly different (P < 0.05).

Table 6: Effects of varying concentrations of Butachlor and pendimethalin on fresh and dry weights of root and shoot of Vigna unguiculata.

Treatment		Wee	ek 1			Week 2				Week 3			
(mg/ml)	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	
Butachlor 5 50 100 SE <u>+</u>	0.3 ^a 0.3 ^a 0.04 0.08	0.06 ^a 0.07 0.04 ^c 0.01	0.2 ^a 1.3 0.03 ^c 0.39	0.02 ^a 0.06 0.01 ^c 0.01	13.9 ^a 0.9 0.2 4.45	3.2 ^a 0.2 0.01 ^c 1.03	4.7 ^a 0.2 0.01 ^c 1.53	1.0 ^a 0.07 0.01 0.32	4.6 ^a 12.4 0.7 ^c 3.44	0.9 ^a 3.1 0.1 0.89	0.9 ^a 2.7 0.1 ^c 0.77	0.4 ^a 0.5 0.03 0.14	
Pendimethalin 5 50 100 SE <u>+</u>	6.9 ^a 17.8 ^b 3.5 ^a 4.31	0.9 ^a 2.0 ^b 0.5 ^c 0.45	1.3 ^a 3.2 ^b 0.8 ^c 0.73	0.3 ^a 0.3 ^a 0.2 ^b 0.03	16.1 ^a 6.8 ^b 17.9 ^a 3.44	1.8 ^a 1.2 ^b 1.6 ^c 0.18	4.4 ^a 1.2 ^b 3.9 ^a 0.99	1.3 ^a 0.2 ^b 0.5 ^b 0.33	2.3 ^a 3.0 ^b 8.0 ^c 1.79	0.2 ^a 0.4 ^a 1.0 ^c 0.24	0.9 ^a 0.5 ^a 1.8 ^b 0.38	0.6 ^a 0.05 ^b 0.2 ^c 0.16	

Means followed by the same letters are not significantly different (P < 0.05).

High percentage germination of *V. sinensis* than that of *V. unguiculata* indicated that *V. unguiculata* was affected most. However, higher concentration of all herbicides showed inhibitory effect on germination. A good number of

studies revealed the effects of herbicides on seed germination of many plant species; for instance, [15] reported that Alachlor and Propachlor herbicides severely reduced seed germination of barley by interfering with metabolic processes related to it. Similar inhibitory effect of nitralin on secondary root development of cotton seedling was reported by [6]. Nitralin equally resulted in a multi neucleolate condition in Zea mays root tips, which implied chromosome duplication and irregularity in size of nuclei and suggested unequal separation in terms of numbers and the reduction in shoot and root weight of both V. sinensis and V. unguiculata [16, 11]. There was also similar report that pendimethalin residue reduced the root and shoot dry weights of Sorghum after 200 days of application [11], also reported on the adverse effects of pendimethalin on dry weight of succeeding crop viz: maize, soybean, cucumber and puddle seeded rice. Butachlor treated seedlings of V. sinensis and V. unguiculata at 50 mg/ml and 100 mg/ml showed chlorosis at the leaf margins. The results in this study are in agreement with the report [9, 17] for bean plant treated with Pyrazon. The pigmentation in V. unguiculata leaves was affected by butachlor, with dark green pigmentations while on V. sinensis, the epidermal cell of leaves showed symptoms such as curling of leaves. In V. unguiculata, lower percentage of seed germination was observed than on V. sinensis. The result also showed that V. unguiculata seedlings were inhibited in growth and development than V. sinensis. This showed that V. unguiculata was more susceptible to the studied herbicides than V. sinensis, Butachlor had higher effect on the root length, shoot length and the number of leaves of V. unguiculata than on V. sinensis unlike pendimethalin that also affected the root length, shoot length and number of leaves of both V. sinensis and V. unguiculata, For efficient seed germination and growth of seedlings, use of herbicides to control unwanted plants shall be at low concentrations with extra caution to ensure crop productivity.

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