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Phytotoxic Effect of Aqueous Extracts of *Acalypha wilkesiana* Mull. Arg., *Centrosema pubescens* Benth and *Phyllanthus amarus* Schum. & Thonn on Germination and Growth of *Amaranthus cruentus* L. and *Corchorus olitorius* L.

Olasupo John Ilori [a*] and Tobi Dada [a]

[a] *Department of Biological Sciences, Anchor University, Lagos, Nigeria.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Allelopathy is a concept that is applied in weed management to minimize extensively the reliance on herbicide in agriculture. The allelopathic potential of *Acalypha wilkesiana, Centrosema pubescens* and *Phyllanthus amarus* on *Amaranthus cruentus* and *Corchorus olitorius* were investigated. Different concentrations of the leaf aqueous extracts of *A. wilkesiana, C. pubescens* and *P. amarus* were applied to determine their effect on *A. cruentus* and *C. olitorius* under laboratory conditions.

**Corresponding author: E-mail: oilori@aul.edu.ng, olasupoilori@yahoo.com;*

The profiling of phytochemicals in the extracts showed that glycosides, phenols, terpenoids, saponins and steroid were present in the extracts. The seed germination, radicle length, plumule length of test crops were significantly decreased by leaf extracts compared with control treatments. The results revealed that the inhibitory effect was proportional to the concentrations of the extracts, higher concentrations had the stronger inhibitory effect. The result suggested that the inhibitory or stimulatory effect may be due to the presence of allelochemicals like glycosides, phenols, terpenoids and steroids etc. in the aqueous leaf extracts of *A. wilkesiana, C. pubescens* and *P. amarus* which may cause allelopathic effects under field conditions. Therefore, these plants should be controlled were they grow with cultivated crops.

Keywords: Phytotoxic effect; Acalypha wilkesiana; phytochemicals; germination; allelochemicals.

1. INTRODUCTION

"Allelopathy is an age-old concept which is defined as all biochemical interactions, stimulatory or inhibitory among plants including microorganisms" [1]. "It is a biological process where plant interactions result in unleash of botanicals that are either deleterious or beneficial to one or the other's germination or growth, and recruitment of offspring" [2]. "Allelochemicals are differently liberated from roots, stems and leaves of plants by exudation, leaching and volatilization respectively, and residue decomposition" [3]. These allelochemicals affect the target organisms and community beneficially, e.g., for the management of agricultural activity like crop protection, control of weed, or crop restoration (positive allelopathy), or harmfully, e.g., autotoxicity, soil sickness, or biological infestation (negative allelopathy). "Once identified and characterised, allelochemicals may serve as templates for the development of new herbicides that are environmentally less injurious" [4]. "Research already promulgates the adoption of the allelopathic phenomenon in weed control in efforts to diminish reliance on chemical herbicides that usually exude great harm to the environment" [5].

The inhibition of one plant by another through the release of allelochemicals is well documented [6]. Muhammad et al*.* [7] stated that "aqueous extracts of *Eucalyptus camaldulensis* L. inhibited seed germination, fresh and dry weight of wheat seedlings"*.* "Aqueous leaf extract of *Acalypha indica* L. exhibited substantial allelopathic effect on local rice and maize varieties. The inhibitory effect of leaf extract measured by germination percentage, vigour index and speed of germination index revealed severe effect on maize while moderate on rice seeds" [8].

The plant *Acalypha wilkesiana* Muell-Arg is one of the most widely known and utilized of the family Euphorbiacaece. They are found worldwide mostly around the tropics of Africa, America and Asia. "*Centrosema pubescens* Benth is a legume in the family Fabaceae, subfamily Faboideae, and tribe Phaseolae. It is native to Central and South America and cultivated in other tropical areas as a forage for livestock" [9]. *phyllanthus amarus* is an herb that can grow to 30-60cm in height [10]. It contains flavonoids (quercetin-3-0-glucoside and ruin), tannins (geraniin, amariin and gallocatechin) and alkaloids (phyllantine, quinolizidine type, securinine, norsecurinine, isobubbialine and epibubbialine),

"*Amaranthus cruentus* L. is an annual herbaceous plant which reproduces only by seeds and has a short growing period: of 4–6 weeks" [11]. *Corchorus olitorius* L. is a leafy vegetable that belongs to Malvaceae family. It is native to tropical Africa, Asia, and now is spread out over the world. The objective of the study is to investigate the effects of aqueous extracts of *A. wilkesiana, C. pubescens* and *P. amarus* on *A. cruentus* and *C. olitorius*.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The seeds of the test crops *Amaranthus cruentus, Celocia argentea, Cochorus olitorius* were obtained from National Horticulture Research Institute (NIHORT) NIORT. *Acalypha wilkesiana, Centrosema pubescens* and *phyllanthus amarus* were collected from the campus of Anchor University Lagos, Nigeria. The plants were identified and authenticated by a botanist from the Department of Biological Sciences, Anchor University Lagos.

2.2 Preparation of Extracts for the Different Treatments

Fresh leaves (100 g/400 ml, 250 g /500 ml, 50 g/200 ml) of *Acalypha wilkesiana Centrosema*

pubescens and phyllanthus amarus respectively were extracted with water. The solution was filtered through cheese cloth to remove debris and then filtered through Whatman No 1 filter paper. These extract solutions (100%) were diluted appropriately with distilled water to give 75%, 50%, and 25% concentrations of the aqueous extracts while distilled water served as control

2.3 Phytochemical Screening of the Water Extracts of *Acalypha wilkesiana Centrosema pubescens and phyllanthus amarus*

Phytochemical screening for phenols, flavonoids, saponins, terpenoids, glycosides phlobatannins were carried out according to the methods of Sofowora [12] and Ghani [13].

2.4 Germination Experiment

"Petri-dishes were thoroughly washed and oven dried**.** The seeds of the different test plants were selected randomly on the basis of uniformity of size and the seeds were then soaked for five minutes separately in 5% sodium hypochlorite to prevent fungal infection. Thereafter they were rinsed for about five minutes in running tap water. Twenty of the seeds were placed in each of the clean oven dried Petri-dish which had been lined with a Whatman No 1 filter paper. The filter paper in each of the Petri-dishes allocated to the control was moistened with ten millilitres of distilled water while that of the Petri-dishes allocated to the other treatments were moistened with ten millilitres of the appropriate concentration of the extracts. The Petri-dishes were incubated at room temperature for two weeks. Emergence of one millimetre of the radicle was used as the criterion for germination. Daily measurements of the plumule and radicle lengths were taken using a metre ruler" [14].

2.5 Statistical Analysis

The data obtained were analysed by factorial Analysis of Variance (ANOVA) to determine significant ($P< 0.05$) effects. The significant differences between means were determined using Duncan's Multiple Range Test DMRT.

3. RESULTS AND DISCUSSION

The results of the phytochemical screening of the plants under study are shown in Table 1.

The germination of the seeds in the aqueous extracts regimes was lower than that of the control. In some cases, the germination of the 25% was almost equivalent to that of the control. The percentage germination of the seeds increased as the concentration of the extracts decreased. The radicle and plumule length of the control was significantly ($P<0.05$) higher than that of the treated seedlings in most of the extract regimes and these reduced with increase in the concentration of the extracts (Tables 2-7).

Table 1. Phytochemical screening of the plants under study

Secondary metabolites	*A. wilkesiana*	*C. pubescens*	*P. amarus*
Glycosides	+	-	+
Phenols	+	+	+
Terpenoids	+	+	+
Saponins	-	+	+
Steroids	+	+	+
Phlobatannins	_	_	_

Key: + = Present; - = Absent

Table 2. The effects of *Acalypha wilkesiana* aqueous extracts on germination and seedling growth of *Amaranthus cruentus*

Treatment with plant extract (%)	Germination (%)	Radicle length (cm.)	Plumule length (cm.)
Control	71.67 ± 3.33[a]	2.17 ± 0 .09[a]	3.87 ± 0 .03[a]
100	-	-	-
75	-	-	-
50	-	-	-
25	20.00 ± 5.00[b]	0.33 ± 0 .03[b]	1.70 ± 0.45[b]

Means followed by the same letter in a column are not significantly different at P< 0.05
(-) = Indicate the absence of germination, plumule length and radicle length

Table 3. Effects of *Acalypha wilkesiana* aqueous extracts on germination and seedling growth of *Corchorus olitorius*

Treatment with plant extract (%)	Germination (%)	Radicle length (cm.)	Plumule length (cm.)
Control	70.00 ± 5.77[a]	4.10 ± 0 .12[a]	3.60 ± 0.26[a]
100	-	-	-
75	-	-	-
50	-	-	-
25	73.33 ± 6.67[a]	0.47 ± 0 .07[b]	3.53 ± 0 .03[a]

Means followed by the same letter in a column are not significantly different at P< 0.05
(-) = Indicate the absence of germination, plumule length and radicle length

Table 4. Effects of *Centrosema pubescens* aqueous extracts on germination and seedling growth of *Amaranthus cruentus*

Treatment with plant extract (%)	Germination (%)	Radicle length (cm.)	Plumule length (cm.)
Control	63.33 ± 3.33[a]	1.70 ± 0.06[a]	2.47 ± 0.19[a]
100	-	-	-
75	-	-	-
50	41.67 ± 10.14[b]	0.73 ± 0.09[b]	1.53 ±0 .18[b]
25	70.00 ± 2.89[a]	1.37 ±0.32[a]	2.53 ± 0 .03[a]

Means followed by the same letter in a column are not significantly different at P< 0.05
(-) = Indicate the absence of germination, plumule length and radicle length

Table 5. Effects of *Centrosema pubescens* aqueous extracts on germination and seedling growth of *Corchorus olitorius*

Treatment with plant extract (%)	Germination (%)	Radicle length (cm.)	Plumule length (cm.)
Control	83.33 ±6.01[a]	2.6333 ± 0.03[a]	2.90 ± 0.12[a]
100	5.00 ± 0 .10[c]	.1333 ± 0.03[c]	1.73 ± 0.37[b]
75	30.00 ± 0.10[b]	.1667 ±0 .067[c]	1.50 ± 0.30[b]
50	75.00 ± 2.89[a]	.8667 ± 0.03[b]	1.73 ± 0.37[b]
25	81.67 ± 1.67[a]	2.5000 ± 0.10 [a]	2.90 ± 0.20 [a]

Means followed by the same letter in a column are not significantly different at P< 0.05
(-) = Indicate the absence of germination, plumule length and radicle length

Table 6. Effects of *Phyllanthus amarus* aqueous extracts on germination and seedling growth of *Amaranthus cruentus*

Treatment with plant extract	Germination (%)	Radicle length (cm.)	Plumule length (cm.)
Control	78.33 ± 3.33[a]	-	-
100	11.66 ± 1.66[c]	-	-
75	13.33 ±1.66[c]	-	-
50	-	-	-
25	58.33. ± 0.40[b]	-	-

Means followed by the same letter in a column are not significantly different at P< 0.05
(-) = Indicate the absence of germination, plumule length and radicle length

Table 7. Effects of *Phyllanthus amarus* aqueous extracts on germination and seedling growth of *Corchorus olitorius*

Treatment with plant extract (%)	Germination (%)	Radicle length (cm.)	Plumule length (cm.)
Control	76.67 ± 4.41 [a]	4.20 ± 0 .30 [a]	1.60 ± 0.20 [a]
100	5.00 ± 0.10 [e]	-	-
75	25.00 ± 5.00[d]	-	-
50	41.67 ±3.33 [c]	-	-
25	65.00 ±2.89 [b]	2.10 ± 0.40[b]	1.57 ± 0.19[a]

Means followed by the same letter in a column are not significantly different at P< 0.05
(-) = Indicate the absence of germination, plumule length and radicle length

In this study, *Amaranthus cruentus* and *Corchorus olitorius* were subjected to the same growth conditions. Since the variables were the extracts. It can be suggested that the observed differences in the germination and growth studied were probably due to the effect of the different extracts of *Acalypha wilkesiana, Centrosema pubescens* and *Phyllanthus amarus*.

The phytochemical screening of the water extracts of *Acalypha wilkesiana, Centrosema pubescens* and *Phyllanthus amarus* indicated the presence of glycosides, phenols, terpenoids, flavonoids and saponins in the extract while phlobatannins were absent in the water extract [15]. This was consistent with the work of Madziga, et al. [16] who reported the presence of tannins and flavonoid, saponins, alkaloids and cardiac glycosides, terpenes and steroids in the aqueous leaf extract of *A. wilkesiana.* From the reports of Imaobong and Uwakmfon [17], leaf extracts of *A. wilkesiana* revealed a high presence of tannins and glycoside, saponin, flavonoids, Phylobatanins and glycosides, alkaloids and cardiac glycosides. While Oladunmoye [18] reported the presence of saponins, tannins, anthraquinones and glycosides in the leaves of *Acalypha wilkesiana* recently Oyebisi et al. [19] reported the presence of glycoside, terpenoid, alkaloid, saponin, steroid, phenolic and eugenol in the extract of *Acalypha wilkesiana*. According to Tapsell et al. [20], there are more than a thousand known phytochemicals in *Phyllanthus amarus*. Secondary metabolites from *Phyllanthus amarus*. Secondary metabolites like alkaloids, tannins, flavonoids, saponins, anthocyanins, steroids, phenolics, coumarins, cardiac glycosides and cyanogenic glycosides have been identified in *Phyllanthus amarus* [21]. The findings of the present study are in agreement with the findings of Mariraj et al. [22] who asserted that the *C. pubescens* extracts contain primary and secondary metabolites, which include carbohydrates, proteins, amino acids, total phenolic compounds, flavonoids, saponins, flavones, and glycosides

Javed and Asghari [23] found that “the leaf extract of *Helianthus annus* inhibited the rate of germination of wheat seedlings”. A related work by Arshad (2011) showed that “the water and methanolic extracts of *Withania somnifera* markedly suppressed the germination, root and shoot growth of *Parthenium hysterophorus”*. The increase in phyotoxic effects on the target crops with increasing extract concentrations was consistent with the works of Muhammad et al. [7], [24] who reported that the inhibitory effects of aqueous extracts of *Eucalyptus camaldulensis L.* and *Croton bonplandianum* Baill. on wheat, *Melilotus alba* Medik., *Vicia sativa* L. and *Medicago hispida* Gaertn were concentration dependent.

4. CONCLUSIONS

The germination and growth of the test crops were reduced when subjected to aqueous extracts. The inhibitory effect of the extracts increases with concentration of the extracts. Therefore, some toxic allelochemicals may be present in *Acalypha wilkesiana, Centrosema pubescens* and *Phyllanthus amarus*. This then suggested that the water extracts of *A. wilkesiana, C. pubescens* and *P. amarus* contain such allelochemicals that could be isolated, identified and concentrated for further use as bio herbicide.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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