Phytotoxic effect of aqueous extracts of *Acalypha wilkesiana* Mull.Arg., *Centrosema pubescens* Benth and *Phyllanthus amarus* Schum. & Thonn on seed germination and seedling growth of *Amaranthus cruentus* L. and *Corchorus olitorius* L.

ABSTRACT

Allelopathy concept has been used in weed management to minimize extensively the reliance on herbicide in agriculture. The present study was conducted to investigate the allelopathic potential of *Acalypha wilkesiana, Centrosema pubescens* and *Phyllanthus amarus* on germination and growth of *Amaranthus cruentus* and *Corchorus olitorius*. Leaf aqueous extracts of *Acalypha wilkesiana, Centrosema pubescens* and *Phyllanthus amarus* at 100%, 75%, 50% and 25% concentration were applied to determine their effect on seed germination and seedling growth of *Amaranthus cruentus*. and *Corchorus olitorius* under laboratory conditions. Phytochemical screening indicated the presence of glycosides, phenols, terpenoids, saponins and steroid. Results indicated that seed germination, radicle length, plumule length of test crops were significantly decreased by leaf extracts compared with control treatments. The aqueous extracts of all donor plants also significantly affected the seedling growth of both test crops. 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.

INTRODUCTION

Rice (1984) defined allelopathy as the direct or indirect harmful or beneficial effect of one plant on another through the production of chemical compounds that escape into the environment. Allelopathy may also play an eminent role in intraspecific and interspecific competition and may determine the type of interspecific association. Allelopathy is an age-old concept which is defined as all biochemical interactions, stimulatory or inhibitory among plants including microorganisms (Novak *et al*., 2018). 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 (Kural and Özkan, 2020). Allelochemicals are differently liberated from roots, stems and leaves of plants by exudation, leaching and volatilization respectively, and residue decomposition (Kato-Noguchi, 2020). Organisms existing in the surrounding can be affected by these allelochemicals with factors like the germination, survival, growth, and reproduction (Meena *et al*. 2020; Banerjee *et al*. 2020, 2021; Wang, *et al.* 2022).

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). The allelochemicals are secondary metabolites not necessary for the allelopathic individual's metabolism (i.e., development, reproduction, and growth). Defense against herbivory of the plant is the major importance of allelochemicals with negative allelopathic effect (Stamp, 2003). Many factors affect the process of allelopathy like plant taxon, soil, and environment. Precipitation, season, temperature, air, and light intensity are the environmental factors affecting allelopathic procedure. Soil factors comprise microorganisms, pH, water, type of soil, and organic matter which affect allelopathy. Growth stage, plant species,

biochemistry, and physiology of plant are also responsible for process of allelopathy (Rice, 2012). once identified and characterised, allelochemicals may serve as templates for the development of new herbicides that are environmentally less injurious (Hussain *et al*. 2019). 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 (Chen *et al*. 2017).

The inhibition of one plant by another through the release of allelochemicals is well documented (Alagesaboopathi, 2011). A large number of plants impose inhibitory effects on the germination and growth of neighbouring or successional plants by releasing allelopathic chemicals into the soil, either as exudates from living tissues or by decomposition of plant residues. Muhammad *et al.* (2009) 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 (Gupta and Bandyopadhyay, 2013).

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. The leaves measure 4-8 Inch as heart-shaped with a combination of colours. The dense, much branched growth habit creates a full shape but plants occasionally need chopping to maintain a neat appearance. Its upright growth can reach 10-15 feet in height making it well suited to be used as accent in mixed shrubbery borders (Edward, 2011). *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 (Souza *et a*l., 2011). The plant is vigorously twining; slightly hairy and it naturally suppresses weeds by creating a dense ground cover and is fairly good at spreading naturally to cover a large surface area. *Phyllanthus amarus* Schum. & Thonn is an herb that can grow to 30-60cm in height (Itoro *et al*. 2013). Its thin branches spread out, and each branch has two rows of small, elliptic-oblong leaves of 5-10mm long that are arranged alternately. It grows well in soil of high moisture with light shade, and reaches maturity in 2-3 months (Samy and Manickam, 2005). *P. amarus* contains flavonoids (quercetin-3-0-glucoside and ruin), tannins (geraniin, amariin and gallocatechin) and alkaloids (phyllantine, quinolizidine type, securinine, norsecurinine, isobubbialine and epibubbialine),

Amaranthus species belong to the Caryophyllales order, Amaranthaceae family, Amaranthoideae subfamily, and Amaranthus genus (Montoya-Rodríguez *et al.* 2015; Mlakar *et al*. 2009). *Amaranthus cruentus* L. is an annual herbaceous plant which reproduces only by seeds and has a short growing period: of 4–6 weeks (Makinde *et al*. 2010). *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. Their leaves are consumed and largely used in folklore medicine, believing in their high nutritional and medicinal properties against as a series of ailments and diseases. It is used fresh or as dried leaves in soups and broths in many Arab and African regions (Alimi *et al.* 2018; Giro and Ferrante, 2016). Therefore, the objective of the study is to investigate the effects of aqueous extracts of fresh leaves of *Acalypha wilkesiana, Centrosema pubescens* and *Phyllanthus amarus* on the germination and growth of *Amaranthus cruentus* and *Corchorus olitorius*

2.0 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 (1982) and Ghani (1998).

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.

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.

3.0 RESULTS AND DISCUSSION

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

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 Key: + = Present; - = Absent

The percentage germination of the control was higher than that of the aqueous extracts regimes. 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. On carrying out an Analysis of Variance (ANOVA) significant reduction of the germination of the seeds by most of the extracts at $P < 0.05$ (Tables 2-7). Seedlings of control had radicle and plumule length that was higher than that of the seedlings in most of the extract regimes. The radicle and plumule lengths of the test crops reduced with increase in the concentration of the extracts. Significant difference was observed between the plumule and radicle length of the seedlings in the control and that of the seedlings in the extract regimes. There were significant differences among the extract concentration at ($P < 0.05$). (Tables 2-7)

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*.

Chemicals with allelopathic activity (allelochemicals) have been isolated and characterized from various plants and are present in many organs, including leaves, flowers, fruits and buds (Seigler, 1996). According to Eze and Gill (1992), *Chromolaena odorata.* L. a weed in the family Asteraceae contains a large amount of allelochemicals especially in leaves which inhibit the growth of many plants. It was observed that shoot extracts from the reproductive stage are more inhibitory than extracts from the vegetative stage under laboratory conditions (Hedge and Miller, 1992). 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. This was consistent with the work of Madziga, *et al*. (2010) 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 (2019), leaf extracts of *A. wilkesiana* revealed a high presence of tannins and glycoside, saponin, flavonoids, Phylobatanins and glycosides, alkaloids and cardiac glycosides. While Oladunmoye (2006) reported the presence of saponins, tannins, anthraquinones and glycosides in the leaves of *Acalypha wilkesiana* recently Oyebisi *et al*. (2022) reported the presence of glycoside, terpenoid, alkaloid, saponin, steroid, phenolic and eugenol in the extract of *Acalypha wilkesiana*. According to Tapsell *et al*. (2006), 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* (Lai and Roy, 2004). The findings of the present study are in agreement with the findings of Mariraj *et al.* (2020) 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

Preliminary investigations have revealed that the aqueous extract from the leaves of *T. diversifolia* retarded the germination and the radicle growth of *Oryza sativa, Amaranthus cruentus, Capsicum annum and Lycopersicon esculentum* (Ilori *et al.* 2007; Otusanya *et al*. 2007; Otusanya *et al.* 2008). Javed and Asghari (2008) also 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 retardation of the juvenile seedling growth of the target crops was observed to increase significantly with increasing extract concentrations. This was consistent with the work of Muhammad *et al.* (2009) who reported that the inhibitory effects of aqueous extracts of *Eucalyptus camaldulensis L.* on germination and seedling growth (fresh and dry weight) of wheat were increased as the extract concentration increased. A similar result was obtained by Swapnal and Badruzzaman (2010) on the allelopathic effect of *Croton bonplandianum* Baill. weed on seed germination and seedling growth of crop plants. They reported that the root length, shoot length of *Melilotus alba* Medik., *Vicia sativa* L. and *Medicago hispida* Gaertn. decreased progressively when the plants were exposed to increasing concentration of the extract of *Croton bonplandianum* Baill.

CONCLUSIONS

The percentage of seedling germination, plumule and radical growth of the test crops were reduced by the application of the aqueous extracts. From these findings, it was suggested that the inhibitory effect increases with increase in concentration of the extracts. From this study, it is concluded that 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.

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