Nigerian Journal of Ecology (2022) 18(1):45-52

ISSN: 1116-753X (Print); E-ISSN: 2955-084X (Online)

Allelopathic effects of aqueous extracts of *Olax subscorpioidea* Oliv. on seed germination and growth of Okra (*Abelmoschus esculentus* (L.) Moench) in Ibadan, Nigeria

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(Accepted 28 June, 2022)

ABSTRACT

Allelochemicals are being explored as environmentally friendly options in weed management and crop yield improvement as opposed to use of synthetic pesticides which have serious environmental damages. Allelopathic effects of aqueous extracts of different parts of *Olax subscorpioidea* on Okra seed germination in Petri-dish bioassays and okra performance in pot experiment were conducted in the Department of Crop Protection and Environmental Biology, University of Ibadan using a complete randomized design (CRD) and with treatments replicated five times. Ten okra seeds in three replicates were treated in separate petri-dishes with 2 ml water extracts of stem, leaf, fruit, bark and root of *Olax subscorpioidea*, and 2 ml distilled water (control) in two trials. Pot trials was carried out using okra seedlings with treated with 100 ml extracts at seven days intervals. Data were collected on number of germinated seeds daily and lengths of five randomly selected plumules and radicles at 7 days after sowing (DAS). Number of leaves, plant height, root and shoot dry weight of seedlings were also measured using standard methods. Data were analyzed by analysis of variance. Statistically significant ($P<0.05$) means were separated using Duncan's Multiple Range Test. Leaf extract had the highest concentrations of phenolic, flavonoid and saponin compounds compared, while the stem extract had the least metabolite concentrations. Leaf extracts exerted highest inhibition on okra germination at 2 and 7 DAS in the first trial and at 7 DAS in the second trial compared to the bark, stem, root and fruit extracts of *Olax subscorpioidea*. The seedling growth and yield parameters in treatment extracts were better than control. In conclusion, *Olax subscorpioidea* has allelopathic potential with effects on germination and seedling growth of okra. It can be used as an alternative bio-herbicide. Its production and agricultural potentials should be promoted.

Keywords: Allelopathy, *Olax subscorpioidea,* bio-herbicide, bio-fertilizer, okra production

INTRODUCTION

Allelopathy is defined as the beneficial or harmful effect of one plant or microorganism on another through the release of allelochemicals or allelochemics (An *et al*., 1996; Han *et al*., 2008). It may result in the stimulation or inhibition of growth of the same or different plant at a lower concentration (Rice, 1984). As a form or inhibitory mechanism, allelopathy can be expressed in the form of inhibition or retardation of germination rate, reduction of root or radicle and shoot or coleoptile extension, Lack of root hairs, swelling or necrosis of root tips, curling of the root axis, increase in number of seminal roots, discolouration, reduced dry weight

accumulation and lowered reproductive capacity are also some of the symptoms of allelopathy (Ayeni *et al*., 1997). The useful plants of West Tropical Africa have been classified into seven major groups. The major groups and the number of their subgroups are food (5), drinks (5), medicine (45), phytochemistry (25), agro-horticulture (13), products (19), and social (3). These uses indicate that there is a plant for every human need (Ogirigiri, 1996). More than 35,000 plant species are being used in various human cultures around the world for medicinal purposes (Lewington, 1993).

In crop production, allelopathy is explored for weed control (Kohli *et al*., 1998). For instance, it has been reported that *Chromolaena odorata* which contains a large amount of allelochemicals in its leaves, inhibited the growth of many plants in nurseries and plantations (Eze and Gill, 1992). Likewise aqueous root and shoot extracts of *Tithonia diversifolia* has been demonstrated to inhibit the germination and growth of *Amaranthus cruentus* (Otusanya *et al*., 2007).

The first chemicals used for weed control were the inorganic copper salts in the early 1900s and hydrochloroslphate VI acid sometime after that, the synthesis of 2,4-D in 1941 by Pokorny and the discovery of its plant growth regulating and herbicidal properties by Hamner and Tukey in 1944 was the first account of a synthesized organic chemical used to control weeds (Stephenson *et al*. 2001).Weed control is a necessary culture in crop production for optimum yields. The metabolites (Flavonoids, Saponins and Phenolics) are important in plant for normal growth development and defense against infections and injury. The secretion of catechin from the roots of noxious weed is one (*Centaurea stoebe*) of the described examples of negative plant-plant interactions medicated through phytotoxins . The metabolites have been shown to inhibit the root and shoot growth of alfalfa (Ohara and Ohira, 2003). In many parts of the world, use of synthetic herbicides in weed control is waning at the expense of more sustainable and environmentally friendly use of bio-herbicides, which are mainly produced from plants with allelopathic metabolites (Ayeni *et al*., 1997). Many plants contain metabolites which have allelopathic effects on other plants like sorghum, rye, and *Leucaena leucocephala* (Kohli *et al*., 1998) The phytochemical qualitative results reveals the presence of tannins, glycosides and saponins in the aqueous extract while the ethanoic extract shows the presence of tannins, alkaloids, glycosides, steroids and flavonoids.This study was premised on the observation of indigenous people of Oke Ogun area in Oyo state that *Olax subscorpioidea* Oliv exerts a territorial influence on plant communities in its vicinity. It belongs to the family Olacaceae. It can either be a shrub or tree, growing up to 10 m or more in height. The plant is widely distributed in Nigeria, Zaire and Senegal parts of Africa. It is already being used for various purposes. The stem of the plant has medicinal properties to cure ulcer,mental illness and fever (Jimoh and Kolapo, 2008). It has been reportedly used in the control of mycotoxin producing fungi (Jimoh and Kolapo, 2008). Thus, it could contain phytochemicals which could serve as a bio-herbicide. Its allelopathic potentials has however, not been documented. *Olax subscorpioidea* appears to be suppressing the growth of plants around it which necessitate a detailed assessment of its allelopathic potentials.

Studies on allelopathy are vast in developing world, but allelopathic potentials of a few indigenous species remained unstudied.

This study assayed the bio-herbicidal potentials of *Olax subscorpioidea* in Okra (*Abelmoschus esculenthus* [L.] Moench) production in southwest Nigeria.. Okra is widely cultivated in Nigeria and many parts of tropical Africa; however, a fresh fruit yield loss of 16.4% to 92.2% could occur from weed infestation (Awodoyin and Olubode, 2009). Therefore, the objective of the study was to determine the allelopathic potentials of aqueous extracts of shoot and root extracts of *Olax subscorpioidea* on germination and growth of okra.

MATERIALS AND METHODS

Collection and Pre-Processing of Plant Materials

Olax subscorpioidae was collected from Oke-Awon village, in Oyo town. The plant was separated into leaf, seed, bark, stem and root. The roots, stems, barks, leaves and fruits were cut into small pieces with a steel kitchen knife before air drying at 80^0C to constant weight for two weeks in the Ecology Research Laboratory of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan, Ibadan. The air dried plant materials were then macerated into small pieces and soaked in distilled water.

Preparation of Aqueous Extracts

Air-dried leaf, fruit, root, stem and bark of *Olax subscorpioidea* plant were soaked separately in distilled water for 72 hours in 15-litre transparent plastics containers at concentration of 200 g of plant part in 1000 ml of distilled water to extract water soluble alellochemicals following the methods of Bhowmik and Doll (1982) and Reigosa *et al*. (1999). The mixture was sieved and stored at 25-28 ^{0}C temperature. All extracts were prepared in three replicates.

Experiment I: Laboratory Bioassays In Petri Dishes

The seed germination test was conducted with 10 seeds of okra evenly placed on Whatman No.1 filter paper in petri dishes (9cm). Two (2) ml of aqueous solutions of the different extracts (leaf, bark, fruit, stem and root) were added to the maize seeds in the petri dishes at using syringe. Distilled water was applied on the control seeds. Five replicates of each treatment were used. Germinated seed (defined as the emergence of radicle) were counted a day after the commencement of the experiment and continuously for seven days after sowing (7 DAS). At 7 DAS, the length of plumules and radicles were measured (using meter rule). Five germinating seeds were randomly picked in each petri dish for the measurement.

Parameters Measured

Germination was observed on daily basis for 7 days. The numbers of seed that germinated for 2, 4, 7 days after sowing (DAS) was counted in each of the treatments containing 10 seeds per petri dish, with five replicates per treatment. All data were transformed using square root transformation. Also at 7 DAS, the lengths of plumules and radicles were measured using meter rule. Five germinating seeds were randomly picked in each petri dish for the latter measurements.

Experiment II: Plant Growth Bioassay (Pot Trial)

The plant growth bioassay was conducted using extracts from the plant parts of *Olax subscorpioidea* at Roof-top garden of the Department of CPEB, University of Ibadan from May to July, 2015. Plastic pots with top-diameter 26.5 cm and base-diameter of 17.5 cm were filled with 8 kg of soil collected from the Crop Garden of the Department. Each pot was sown with 2

seeds at a depth of 1 cm. The seedlings were thinned to 1 plant per pot 3 weeks after sowing (WAS). The experiment followed completely randomized Design with 3 replications. The extract (100 ml) was added to treated pot once in a week while water was used as control. The pots were watered twice every week. Data on the crop plant height and number of leaves were taken at weekly interval from 3 to 8 WAS.

Measurement of Growth and yield Parameters

The plant height was measured as a distance between the bases of the shoot at soil level to the tip of the terminal bud of the plant using a meter rule. The number of leaves was obtained by counting the number of leaves on each plant by simple visual counting; while dry weight of plant parts was obtained after oven drying to constant weight.

The number of leaves was counted at 3, 4, 5, 6, 7 and 8 weeks after sowing and the dry weights of the plant shoot and root were determined. The biomass determination was conducted by removing each plant from a pot with the ball of earth attached to it. It was lowered into a bucket of water to loosen the soil for easy removal without damaging the root as much as possible (Awodoyin and Ogunyemi, 2005). Each plant harvested was separated into shoot and root in different labeled envelope and oven dried to constant weight at 80ºC in a Gallenkamp oven. The dried samples were later weighed on Metler balance (Model P1210) to obtain its dry weight.

Phytochemical Analysis

The extracts of the leaves, stem, root, bark and fruits of *Olax subscorpioidea* were evaluated for quantitative presence of selected metabolites; flavanoid, saponin and phenol following Sofowora (2008) protocol. The phytochemical analyses were conducted at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Data Analysis

All data were analysed using analysis of variance with DSAATAT software (version 1.101) and the means that were significant were separated using Duncan's Multiple Range Test (DMRT) at 5% level of probability.

RESULTS AND DISCUSSION

Effects of Aqueous Extract of *Olax subscorpiodea* Plant Parts on germination, Radicle and Plumule Length

The germination in okra increased as the day after sowing (DAS) progressed. The germination inhibition varied based on the treatments and duration .The mean cumulative germination ranged from (1.93±0.24) for 2 DAS to (3.08±0.06) for 7 DAS. There was no significant difference between control and other treatments except leaf extracts which is significantly lower than control and other treatments from (1.09a±0.19) at 2 DAS in the first trial to (1.96±0.14) at 7 DAS and from (1.09±0.19) at 2 DAS to (2.02±0.07) at 7 DAS in the second trial (Table 1), This agrees with the finding of Ayeni *et al*. (1997) that the allelopathic effects may be inhibition of germination rate, or reduced root or radicle and shoot or coleoptile extension. A similar trend was reported by Aisha *et al*. (2010) and Monica *et al*. (2011), where the aqueous extracts of *Ascarum europaeum* L. inhibited the germination and growth of *Lycopersicum esculentum*. Eze and Gill (1992), reported that *Chromolaena odorata* contains a large amount of allelochemicals especially in the leaves, which inhibit the growth of many plants in nurseries and plantations. Otusanya *et al*.,2007 also reported that aqueous extract and shoot extract of *T. diversifolia* was inhibitory to the germination and growth of *Amaranthus cruentus*. Similarly, Bhatt et al. (1994) have

reported that the bark, leaf and leaf extract of Quercus glauca and Q.leucotricophora significantly reduced germination, plumule and radicle length of wheat (Triticum sp.) andmustard seeds. Although, the principle of allelopathy in mustard seeds was suggested to be allelochemicals or toxins which are released from the weed by the action of micro-organisms during decomposition, which may interfere with the plant growth processes (McCalla and Haskins, 1964; Pandya, 1975). Bark extracts showed the highest degree of inhibition on radicle length having (0.44±0.14) compare to control which was (1.57±0.70) and (4.43±0.40) for the first and second trial respectively. There was no significant difference between control and other treatments. But the highest degree of plumule inhibition was recorded in leaf extract which was (0.18±0.34) and (0.12±0.28) for the first and second trial respectively. There was significant difference between the leaf extracts and the control (Table 2). The study agreed with the report of Abu-Romman (2010) that allelochemicals released into the surrounding might have inhibited or retarded the growth of root or radicle and shoot or coleoptile of plants.

Phytochemical Analyses

Leaf has the highest concentration of phenol, flavonoid and saponin which are 6.37±0.48, 1.83±0.22 and 3.89±0.38 respectively. This was followed by concentrations recorded in the bark and stem extracts respectively. Fruit which tends to have stimulatory properties had the lowest amounts of the secondary metabolites (Table 3).

Biomass accumulation

The root and shoot dry weights were significantly higher in all the treatments than control exceptIn the bark (1.34±0.42 g/plant) and root (3.16±1.80 g/plant) extracts respectively at 8 WAS . However, they were not significantly different from one another at P<0.05 (Table 6).

Growth parameters

The trend observed in biomass accumulation was repeated in growth parameters. The number of leaves **(**3.33±1.08) and plant height (19.50±4.31) of the okra plants treated with the bark extracts were lower than control at 8 WAS, but they were not significantly different(Table 4 and 5).

Table 1: Mean Germination percentage of okra seeds treated with the water extract of different parts of *Olax subscorpioidea* at 2, 4 and 7 days after sowing in Ibadan, Nigeria in 2015

	TRIAL 1			TRIAL 2		
TREATMENT	2DAS	4DAS	7DAS	2DAS	4DAS	7DAS
CONTROL	2.09bc±0.19	2.70bc±0.10	2.74bc±0.09	2.70bd±0.15	2.84b±0.12	2.88b±0.09
ROOT	1.93b±0.24	2.98c±0.10	3.02c±0.07	2.41cd±0.17	2.73b±0.15	2.77b±0.14
STEM	2.28bc±0.20	3.05c±0.04	3.08c±0.06	2.15c±0.30	2.69ab±0.15	2.73b±0.13
LEAF	1.09a±0.19	1.89a±0.21	1.96a±0.14	1.09a±0.19	2.23a±0.07	2.02a±0.07
BARK	2.07bc±0.10	2.55bc±0.07	2.59bc±0.04	1.62b±0.16	2.42ab±0.14	2.46ab±0.12
FRUIT	.42bc±0.10	2.94c±0.11	2.94c±0.11	2.02bc±0.26	2.39ab±0.24	2.48ab±0.18

Mean Percentage values with the same letters on the same column are not significantly different using Duncan's Multiple Range Test (DMRT) at P=0.05; (DAS=Days after sowing) .Control=Distilled water only.

Table 2: Effects of Aqueous extract of *Olax subscorpiodea* plant parts on radicle and plumule length (cm) at 7 days after sowing in Ibadan, Nigeria 2015

	OKRA			
	RADICLE LENGTH		PLUMULE LENGTH	
TREATMENT	TRIAL 1	TRIAL2	TRIAL 1	TRIAL 2
CONTROL	1.57ab±0.70	4.43b±0.40	2.00cd±0.86	4.30b±0.68
ROOT	0.84ab±0.26	1.19a±0.29	1.44bc±0.27	1.69a±0.59
STEM	2.07b±0.55	1.50a±0.48	3.10d±0.69	1.61a±0.50
LEAF	0.47a±0.11	0.47a±011	0.18a±0.34	0.12a±0.28
BARK	0.44a±0.14	0.32a±0.11	0.51ab±0.36	0.18a±0.23
FRUIT	1.63ab±0.47	0.33a±0.43	1.97cd±0.55	0.56a±0.60

Mean Percentage values with the same letters on the same column are not significantly different using Duncan's Multiple Range Test (DMRT) at P=0.05; .Control=Distilled water only.

Table 3: Phytochemical components of different parts of *Olax subscorpioidea* in (mg/g)

TREATMENTS	PHENOLIC	FLAVONOID	SAPONIN
ROOT	4.60±0.21	0.83±0.02	1.94±0.08
STEM	2.63±0.05	0.51±0.01	1.28±0.03
LEAF	6.37±0.48	1.83±0.22	3.89±0.38
BARK	4.24±0.22	1.26±0.19	2.43±0.20
FRUIT	2.97±0.27	0.66±0.02	1.66±0.04

Table 4: Effect of Aqueous extract of *Olax subscorpioidea* plant parts on number of leaves of okra in Ibadan, Nigeria in 2015

TREATMENTS	3 WAS	4 WAS	5 WAS	6 WAS	7 WAS	8 WAS
CONTROL	6.00a±0.00	6.00a±0.71	5.67a±0.41	5.33a±0.41	3.67a±0.82	4.67a±0.82
ROOT	5.67a±0.41	6.33a±0.41	6.33a±0.41	6.33a±0.41	4.00a±0.00	4.33a±1.08
STEM	5.00a±0.71	6.00a±0.71	6.67a±0.41	6.33a±0.41	4.67a±1.48	5.33a±0.82
LEAF	6.00a±0.00	7.00a±1.23	6.33a±0.41	6.00a±0.71	4.33a±0.41	5.00a±0.71
BARK	6.00a±0.00	6.00a±1.22	5.33a±1.48	5.33a±1.08	3.67a±1.08	3.33a±1.08
FRUIT	6.00a±0.00	5.67a±0.41	6.67a±0.41	6.33a±0.41	4.67a±1.48	4.33a±1.64

Mean values with the same letters on the same column are not significantly different using Duncan's Multiple Range Test (DMRT) at P=0.05; (WAS=Weeks after sowing) Control=Distilled water only

Table 5: Effect of Aqueous extract of *Olax subscorpioidea* plant parts on Plant Height (cm) of okra in Ibadan, Nigeria 2015

TREATMENTS	3 WAS	4 WAS	5 WAS	6 WAS	7 WAS	8 WAS
CONTROL	8.10ab±0.26	11.90a±1.35	15.77a±2.23	18.43ab±1.64	22.93a±1.19	24.17a±0.21
ROOT	7.60ab±0.59	11.47a±2.25	15.83a±2.55	20.80ab±2.74	25.43a±3.85	23.33a±4.82
STEM	6.57b±1.57	11.03a±2.32	14.63a±2.10	19.23ab±2.43	23.67a±2.60	26.43a±3.06
LEAF	7.57ab±0.30	11.87a±0.99	15.83a±2.14	20.17ab±2.75	20.17ab±2.75	24.67a±4.63
BARK	8.47a±0.11	10.60a±0.95	13.50a±2.45	15.53b±3.18	20.23a±2.15	19.50a±4.31
FRUIT	9.10a±0.91	11.93a±2.04	17.07a±2.48	21.33a±3.35	22.17a±1.25	24.57a±1.27

Mean values with the same letters on the same column are not significantly different using Duncan's Multiple Range Test (DMRT) at P=0.05; (WAS=Weeks after sowing) .Control=Distilled water only

Table 6: Effect of Aqueous extract of *Olax subscorpiodea* plant parts on shoot and root dry weight (g/plant) of okra in Ibadan,Nigeria

TREATMENTS	OKRA	
	Root dry weight	Shoot dry weight
CONTROL	0.92a±0.23	3.35a±0.57
ROOT	0.80a±0.51	3.16a±1.80
STEM	1.50a±0.20	4.37a±0.38
LEAF	1.22a±0.50	4.56a±1.90
BARK	1.34a±0.42	3.20a±0.81
FRUIT	1.16a±0.11	4.25a±0.62

Mean values with the same letters on the same column are not significantly different using Duncan's Multiple Range Test (DMRT) at P=0.05; (WAS=Weeks after sowing)
Control = Distilled water only

CONCLUSION

In the tropics, tree stands on the farm or the cropping plots are a common occurrence for variety of reasons. Many bio-pesticides have been developed mostly in area of insecticides and fungicides but there no any documentation on bio-herbicides .This experiment confirmed the potential benefit of *Olax subscorpioidea* as a bio-herbicide. The study showed its inhibitory characteristics on both the germination and growth components of okra The leaves of the plant which contained the highest concentration of all the secondary metabolites could be said to account for the inhibitory allelopathic activities in the wild; which the farmers observed. Therefore, having confirmed the basis of the bio-herbicidal potential of *Olax subscorpioidea*, caution should be exercised in its crude application since its inhibitory effects, as expressed in okra could be broad spectrum. We recommend further screening and development of extracts/allelochemicals in *Olax subscorpioidea* into a non-selective bio-herbicide. Farmers should therefore ensure that their field is cleared of the *Olax subscorpioidea* plant on their farmland most especially its leaf extract.

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