

**ALLELOPATHIC EFFECT OF COWPEA (*Vigna unguiculata*) SHOOT AND ROOT AQUEOUS EXTRACTS ON GERMINATION AND GROWTH OF MAIZE IN IBADAN, NIGERIA**

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**SUMMARY**

Plants generate various secondary metabolites referred to as allelochemicals that can be released into the environment and influence the growth and development of other plants. The germination and growth response of seedlings of *Zea mays* L. to aqueous shoot and root extracts of *Vigna unguiculata* was assayed in the laboratory and screen house. Shoot and roots of *V. unguiculata* Walp were harvested, air-dried and milled into fine powder. Milled samples (144, 72, 36 and 0 g), were dissolved in 1 L distilled water to obtain 100, 50, 25 and 0% concentration of Aqueous Shoot Extracts-ASE and Aqueous Root Extracts-ARE. Ten seeds were germinated in Petri dishes lined with filter paper, then soaked with aqueous extract solutions. The treatments (7) were replicated three times. After seven days, data was collected on percentage germination, radical and plumule length. Twenty-eight (10 kg) polyethylene bags comprise of seven treatments and four replicates was used. Two maize seeds were sown and later thinned to one seedling per bag. Thereafter, 300 mls of each treatment was administered from two to eight weeks after sowing. Growth parameters were obtained and data analyzed following standard procedure. Above 70% germination was recorded for maize in all the ASE concentration and ARE-100 gave least (53.33±1.08%). The ASE-25 had highest Dried Shoot Weight-DSW (31.78±1.19g) and Dried Root Weight-DRW (11.90±0.66g), respectively. The ARE-100 gave least DSW (20.02±0.74g) and DRW (6.52±0.87g) in maize. *Vigna unguiculata* Walp aqueous shoot extract enhanced germination, while aqueous shoot extract at 25% encouraged dry matter accumulation.

**Keywords:** Allelopathy, Allelochemicals, Plant, Extracts, Germination, *Vigna unguiculata*

MAIZE is an annual cereal plant of the family Poaceae, widely cultivated for its edible grains. The grains are harvested when immature (green) or matured (dried) and they are widely eaten in

South Western Nigerian as it's provide a rich source of carbohydrates. Weed interference has been a major constraint to its production. Biological control of weeds has been established to be environmentally safe but has not been widely used in maize cropping system. In order to achieve sustainable production, ecological interactions within the agro-ecosystem must be recognized (Xingxiang *et al.*, 2009). Allelopathic interference occurs in crop association or natural stands, alongside nutrients, water, light and space competition (Schenk, 2006).

Natural selection is a process that enables nature to choose at any point in time the structure and number of species living in a specific ecosystem (Scavo *et al.*, 2019). Allelopathy is a biological process in which the organism produces and release exudates of one or more biochemical compounds that influence the growth or not of germination phylogeny of other species. These biochemical substances can have a promontory (positive allelopathy) or harmful (negative allelopathy) effect on the target organism and its population (Uddin *et al.*, 2014). The biochemical substances are released by plant leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems (Metten *et al.*, 2009). Studies have shown that allelopathic effects of different parts of the plant species affect different plant organs in different ways in different plant species. The effect can vary in the intensity, duration and developmental stage during plant development. Here allelopathic effect refers to the inhibitory and stimulatory effects of one plant species upon another by the compounds arising from the products of their metabolism. For example, when investigating the effects of leaf extracts on two perennial weeds, fresh leaf leachates of *Eucalyptus globulus* Labill. inhibited the growth of bermuda grass (*Cynodon dactylon* L. Pers.) but promoted the growth of purple nutsedge (*Cyperus rotundus* L.) (Khalil *et al.*, 2010). Similarly, germination of cereal crops was less affected than legume crops by fresh and dry leaf extracts of *Pinus halepensis* and *Quercus coccifera* (Alrababah *et al.*, 2009).

Allelopathy inhibition is complex and can involve the interaction of different classes of chemicals such as phenolic compounds, flavonoids, alkaloids, terpenoids, coumarins, glycosides, and

glucosinolates. The chemicals called secondary metabolites are known to be released by plants to suppress emergence or growth of other plants. These substances are phytotoxic and can be suggestive of their potentials as natural herbicides, provided they suppress weeds not primary crop on the field (Uddin *et al.*, 2014). These secondary metabolites released by plants may influence resource competition, nutrient dynamics, microbial ecology, mycorrhiza, and even soil abiotic factors (Musilovia *et al.*, 2016). When plants are exposed to allelo-chemicals, their growth and development are affected through inhibition of seed germination or seedling growth decrease. The readily visible effects include inhibited or retarded germination rate (Oyerinde *et al.*, 2009), seeds darkening and swelling, reduced root or radicle and shoot or coleoptiles extension (Khalil *et al.*, 2013), swelling or necrosis of root tips, curling of root axis, discolouration, lack of root hairs, reduced dry weight accumulation, and lowered reproductive capacity (Farooq *et al.*, 2014).

Allelopathy had been studied by many researchers (Field *et al.*, 2006; Inderjit *et al.*, 2006). In Nigeria, allelopathy studies so far concentrated on allelopathic potentials of crops on weeds. This includes the work of Okon and Amadu (2003) on Siam weed and Usuah *et al.* (2013) on some weed species such as *Chromolaena odorata* and *Panicum maximum* and *Aspilia africana*. Recently, Ayeni and Kayode (2013) reported the allelopathic potentials of some crop residues on agricultural crops (*Cajanus cajan* stem, Maize inflorescence and Rice husk) on the germination and growth of *Sphenostylis sternocarpa*. The present research emphasizes the allelopathic effects of cover crop on the germination and growth of maize, an important cereal widely consumed in Nigeria. This study aimed at examining the allelopathic potentials of aqueous shoot and root extracts of *Vigna unguiculata* on the germination and growth of maize.

## MATERIALS AND METHODS

### *Study site*

The research work was carried out in the laboratory and screen house at the Department of Crop Protection and Environment Biology, University of Ibadan (Latitude 7<sup>o</sup>27'N; Longitude 3<sup>o</sup>53'E; Elevation 218 m Above Sea Level (ASL)).

### *Source of Seed for Bioassays*

Viable seeds of maize were obtained from International Institute of Tropical Agriculture, Ibadan.

### *Source and Preparation of Aqueous Shoot and Root Extract*

Plant material of *V. unguiculata* L. comprising shoots and roots were collected from cultivated field plot. Plant materials of *V. unguiculata* L. were then washed thoroughly in running water and subsequently cleaned with distilled water. The plant materials of *V. unguiculata* L. were partitioned into shoots and roots. These were later slice into chips (650 g) with the use of a sharp knife and shade dried at a room temperature of 27 °C for fourteen days. After shade drying, the specimens were grounded into fine particles, using mechanical blender (Thomas milling machine) and made to go through a strainer with a diameter of one milliliter. One hundred and forty four grams of dried milled plant parts (shoots and roots) were used to prepare the aqueous shoot and root extracts. It was then soaked in a litre of distilled water in a beaker covered with aluminum foils for a period of twenty four hours and subsequently made to pass through a muslin cloth and Whatman No. 1 filter paper. The stock distillate of 100% w/v was prepared per plant part. Other concentrations of the aqueous extracts 50%, 25% and 0% (distilled water) were achieved through sequential dilution in distilled water (w/v). The botanicals of *V. unguiculata* Walp were kept in the fridge at 20 °C before application to avoid disintegration and degradation of the allelochemicals which might be inherent in them.

**Experiment 1:** This experiment was conducted in the laboratory to determine potential allelopathic effect of *Vigna unguiculata* Walp aqueous shoot and root extracts on germination of maize seeds. Seeds of *Zea mays* L. were disinfected using five percent NaClO (bleach) for ninety seconds to avoid contamination by fungus; afterwards they were washed in running water for 5 minutes. The experiment was laid out in a Complete Randomized Design (CRD) on the laboratory bench at a room temperature of  $27 \pm 2$  °C and repeated three times. A total of twenty one Petri-dishes used for the experiment were disinfected using five percent NaClO, completely cleaned and lined with Whatman No. 1 (9 cm) filter paper. The treatments were; Distilled water (control), 100% Shoot Extract (SE100), 50% Shoot Extract (SE50), 25% Shoot Extract (SE25), 100% Root Extract (RE100), 50% Root Extract (RE50), 25% Root Extract (RE25) to give a total of seven treatments. Petri dishes were lined with moistened Whatman No. 1 filter papers and ten seeds of maize placed in each Petri dish. Two milliliter of each treatment concentration was applied to its respective replicates in the petri dishes daily using hypodermic syringe, while the control received only distilled water accordingly. Germination of test crop seeds in the Petri dishes was observed on daily basis for 7 days at laboratory room temperature of 27°C. The protrusion of radicle and plumule were taken as evidence of complete germination. Parameters observed for assessment of treatment effect were total germination percentage at 7 days after sowing (7 DAS i.e one week) and seedling sprout length {i.e. sum of plumule and radicle lengths (cm)}.

**Experiment 2:** This experiment was carried out under the screen house conditions at the Department of Crop Protection and Environmental Biology, University of Ibadan. It was conducted to determine possible allelo-pathic effect of *V. unguiculata* Walp aqueous shoot and root extracts on early growth of maize plants. Twenty eight bags (each 22 cm surface diameter and 30 cm deep) were filled with 10 kg top soil obtained from the Crop garden of the Department of Crop Protection and Environment Biology, University of Ibadan and the base were perforated to ensure good drainage of water. The 28 bags were arranged in a Completely Randomized Design. Each treatment comprised of four bags. Two disinfected seeds of maize were planted per bag. Ten

days later, the seedlings were thinned to one seedling per bag. Thereafter, 300 mls of each treatment was administered daily from two to eight weeks after sowing, during which the effect of the treatment applied were observed.

### **Measurement of Parameters**

#### **Germination percentage**

The number of seeds germinating every day after treatment was counted and the total was used to calculate the final germination percentage in each treatment.

#### **Determination of plant height (cm)**

Plant height was measured from the soil level to the upper point of the terminal bud of the seedling using a meter rule, every two weeks up to the end of the experiment.

#### **Determination of shoot and root dry weight (g)**

At the end of the experiment, the plants were carefully uprooted from the soil, with the ball of earth placed in a bucket of water to loosen the soil from the root. Thereafter, it was washed gently under a running water to keep it free of soil so that the root could be recovered fully. Plants were separated with the use of a knife into shoot and root. Fresh plants (shoots and roots) were packaged separately in envelopes and dried to constant weight at 80°C in an oven for 72 hours. Shoot and root weights were determined on a top loading Metler Balance (Metler P1210) and mean weights were calculated.

All data collected were analyzed by means of Analysis of Variance (ANOVA) and means differentiated by using the LSD at 5 percent Level of Probability.

## RESULTS

The Aqueous Shoot Extracts - ASE100 significantly impaired plumule length of *Z. mays*. A plumule length of 2.57 cm and 1.62 cm were obtained in both trials, which was significantly lower than the plumule length obtained from other concentration of the aqueous extracts (Table 1). The control treatment which received no aqueous concentration of the extract recorded the highest plumule length of 7.89 cm and 7.64 cm respectively, for both trials. As with the plumule higher concentrations of akidi impaired radicle length (Table 1). The ASE100 significantly impaired *Z. mays* radicle growth. The ASE100 had a radicle length of 0.36 cm and 0.25 cm obtained in the first and second trial which were significantly less than those of other treatments (ASE50, ASE25, ARE100, ARE50, ARE25 and Distilled Water) across the two trials (Table 1).

The application of all the concentration of the aqueous extracts had no significant effect on the plant height of maize at 2 WAS, across the two trials (Table 2). At 4 WAS, the shoot extract which ranged between (39.57 to 46.95 cm) were more allelopathic than the root extracts which ranged between (46.11 to 53.30 cm) for both trials. The dried shoot weight of maize was significantly affected by the treatments. The highest shoot weight (31.78 g) was recorded under ASE 25 which was significantly higher than all other values in the first trial and 30.89 g in the second trial which was also better than all other treatments evaluated in the trial. The least values of 20.42 g and 20.02 g, respectively for 1<sup>st</sup> and 2<sup>nd</sup> trial were recorded with ARE100 which indicated that the root extracts was more allelopathic than the shoot extract when applied at 100% (Table 3).

As with the dried shoot, the highest root weights were recorded under the ASE 25 (11.90 and 11.60 g) in both trials, while the least values of 2.78 g and 4.94 g were observed under ASE 100 and ASE 50, respectively for the first trial and 2.66 g and 5.04 g respectively for the second trial. There were no significant ( $P = 0.05$ ) difference between the dried root weight of maize treated with ARE 100 and ARE 50 (6.67 g and 6.95 g) respectively for the first trial and 6.57 g and 6.89 g,

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respectively for the second trial when compared with the control treatments (7.95 g and 7.88 g) respectively for the first and second trial (Table 3).

**Table 1:** Effect of different concentrations of aqueous extracts of dried shoot and roots of cowpea on the germination percentage of *Zea mays* seeds after seven days in a laboratory

Treatments	Germinated Seeds	Plumule Length (cm)	Radicle Length (cm)
First Trial			
100% w/v shoot extract (ASE100)	70.00 ± 0.71	2.57 ± 0.49	0.36 ± 0.07
50% w/v shoot extract (ASE50)	73.33 ± 0.41	5.99 ± 0.46	0.56 ± 0.08
25% w/v shoot extract (ASE25)	76.67 ± 0.82	6.19 ± 0.58	0.67 ± 0.09
100% w/v root extract (ARE100)	53.33 ± 1.08	4.50 ± 0.45	0.49 ± 0.05
50% w/v root extract (ARE50)	63.33 ± 0.81	5.65 ± 0.55	0.60 ± 0.06
25% w/v root extract (ARE25)	86.67 ± 0.41	6.33 ± 0.46	0.72 ± 0.06
Control (distilled water)	93.33 ± 0.82	7.89 ± 0.52	1.10 ± 0.13
LSD (0.05)	1.872	1.251	0.203
Second Trial			
100% w/v shoot extract (ASE100)	90.00 ± 0.71	1.62 ± 0.43	0.25 ± 0.05
50% w/v shoot extract (ASE50)	90.00 ± 0.71	6.26 ± 0.53	0.67 ± 0.10
25% w/v shoot extract (ASE25)	93.33 ± 0.82	6.23 ± 0.51	0.73 ± 0.09
100% w/v root extract (ARE100)	80.00 ± 0.71	4.26 ± 0.19	0.48 ± 0.06
50% w/v root extract (ARE50)	83.33 ± 0.82	6.47 ± 0.63	0.67 ± 0.12
25% w/v root extract (ARE25)	90.00 ± 0.71	6.90 ± 0.48	0.78 ± 0.11
Control (distilled water)	93.33 ± 0.41	7.64 ± 0.62	1.10 ± 0.10
LSD (0.05)	1.751	1.251	0.232

Values are mean ± standard error, n = 4

LSD (0.05) = Least significant difference at 5% level of probability

**Table 2:** Effect of different concentrations of aqueous extracts of dried shoot and roots of cowpea on plant height (cm) of Maize in pot

Treatments			2 WAS	4 WAS	6 WAS	8 WAS		
First Trial								
100% w/v shoot extract (ASE100)	23.38	± 1.01	41.63	± 1.11	70.03	± 1.07	98.43	± 0.95
50% w/v shoot extract (ASE50)	24.20	± 0.92	42.68	± 2.13	70.58	± 1.42	104.33	± 0.91
25% w/v shoot extract (ASE25)	24.40	± 0.56	46.95	± 1.48	72.10	± 1.29	106.90	± 0.95
100% w/v root extract (ARE100)	23.78	± 0.60	47.33	± 1.51	70.45	± 1.84	102.28	± 0.90
50% w/v root extract (ARE50)	24.10	± 0.57	51.58	± 1.05	71.58	± 1.10	103.48	± 0.78
25% w/v root extract (ARE25)	24.38	± 0.87	53.30	± 1.22	71.90	± 1.00	107.13	± 0.86
Control (distilled water)	24.98	± 1.02	55.43	± 1.23	78.75	± 1.17	118.20	± 0.91
LSD (0.05)		2.082		3.642		3.304		2.282
Second Trial								
100% w/v shoot extract (ASE100)	21.96	± 1.15	39.57	± 1.13	68.30	± 1.35	96.87	± 2.38
50% w/v shoot extract (ASE50)	22.01	± 0.95	41.51	± 0.80	71.32	± 1.63	102.82	± 2.14
25% w/v shoot extract (ASE25)	22.13	± 0.88	44.61	± 0.80	75.98	± 1.52	105.88	± 1.25
100% w/v root extract (ARE100)	22.00	± 1.06	46.11	± 1.34	68.41	± 1.63	101.60	± 1.06
50% w/v root extract (ARE50)	22.04	± 0.82	51.03	± 0.94	70.42	± 1.95	102.19	± 1.51
25% w/v root extract (ARE25)	22.06	± 1.25	52.59	± 0.86	70.91	± 3.38	106.19	± 0.92
Control (distilled water)	22.21	± 0.75	52.70	± 0.93	77.36	± 0.89	115.40	± 0.83
LSD (0.05)		1.921		2.521		4.865		3.939

Values are mean ± standard error, n = 4

LSD (0.05) = Least significant difference at 5% level of probability

WAS = Week after Sowing

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**Table 3:** Effect of different concentrations of aqueous extracts of dried shoot and roots of cowpea on dry weight (g) of *Z. mays* in pots

Treatment				Dried Shoot	Dried Root
				Weight (g)	Weight (g)
First Trial					
100% w/v Aqueous Shoot Extract	(ASE100)			23.46 ± 0.84	2.78± 0.28
50% w/v Aqueous Shoot Extract	(ASE50)			25.12 ± 0.75	4.94± 0.35
25% w/v Aqueous Shoot Extract	(ASE25)			31.78 ± 1.19	11.90± 0.66
100% w/v Aqueous Root Extract	(ARE100)			20.42 ± 0.96	6.67± 0.51
50% w/v Aqueous Root Extract	(ARE100)			25.96 ± 0.82	6.95± 0.65
25% w/v Aqueous Root Extract	(ARE100)			27.30 ± 0.85	11.71± 0.65
Control (distilled water)				25.75 ± 1.15	7.95± 0.61
LSD (0.05)				2.419	1.345
Second Trial					
100% w/v Aqueous Shoot Extract	(ASE100)			22.77 ± 0.87	2.66 ± 0.44
50% w/v Aqueous Shoot Extract	(ASE50)			24.94 ± 1.20	5.04 ± 0.36
25% w/v Aqueous Shoot Extract	(ASE25)			30.89 ± 1.10	11.60 ± 0.61
100% w/v Aqueous Root Extract	(ARE100)			20.02 ± 0.74	6.52 ± 0.87
50% w/v Aqueous Root Extract	(ARE100)			24.90 ± 0.94	6.86 ± 0.75
25% w/v Aqueous Root Extract	(ARE100)			26.70 ± 1.26	11.61 ± 0.74
Control (distilled water)				25.16 ± 1.16	7.88 ± 0.79
LSD (0.05)				2.680	1.713

Values are mean± standard error, n = 4

LSD (0.05) = Least significant difference at 5% level of probability

## DISCUSSION

Allelo-chemicals are released by certain plants (through leaching, volatilization, exudation or decomposing of remains) and are one of the important components of the stresses that may influence seed germination, plant growth and nutrient uptake of the associated plant species (Komal, 2011). Plants may favourably or adversely affect other plants through allelochemicals, which may be released directly or indirectly from live, dead plant or organic residues.

As observed, seed germination of *Z. mays* L. was more adversely affected by 100% w/v shoot aqueous extract (ASE100) of *Vigna unguiculata* than seedling growth. There was significant variation in the radicle and plumule length in the *Z. mays* germination, but the radicle length was more negatively affected when compared with the control treatment. The radicle length reduced with increase in the concentration of extracts which also suggested that the effect of the extracts was concentration dependent. These responses might be due to the fact that the radicle is more sensitive to allelochemicals during seed germination (Dorning and Cipollini, 2006). This might be due to rapid inhibiting effect on respiration of root tips, which might ultimately reduce its elongation (Krue *et al.*, 2000), also, most of the time allelochemicals are present in water soluble state (Soltys *et al.*, 2013), and roughly all innate allelochemicals in plants above-ground material are drained into soils by water (Turk *et al.*, 2003). Also, Shahid *et al.* (2006) reported that different plant extracts significantly reduced wheat and its weeds.

Often the allelopathic effect is not observed in the final germination percentage, but rather in the speed of germination, which can provide important indications of the allelochemical (Ferreira, 2004). Delays in seed germination of any species can have important biological implications, because this will affect the establishment of seedlings in natural conditions (Chaves *et al.*, 2001) and their chances of competing for resources with neighboring species (Xingxiang *et al.*, 2009). The nature of the inhibitory effect of allele-chemical to seed germination could be attributed to inhibit water absorption which is a precursor to physiological processes that should occur in seed

before germination is triggered (Pianka, 2000). Therefore, effects of allelochemicals on seed germination appear to be mediated through a disruption of normal cellular metabolism rather than through damage of organelles (Onelli *et al.*, 2017).

In the present study, plant heights of *Zea mays* treated with the aqueous shoot and root extract of *V. unguiculata*, the shoot extract were observed to be more allelopathic than root extracts. Hence, inhibitory effect was observed to be part specific, as the allelopathic impact was greatly a function of the plant part being used. These findings corroborated the earlier report by Khalil *et al.* (2010) who found out that the root and shoot length and seedling vigour of maize were decreased with increasing the concentration of *Gliricidia sepium*. These results agrees to those obtained by Mohamadi and Rajaie (2009) that studied the allelopathic impact of leaf leachate of *E. camaldulensis* on germination, growth, morphological and physiological parameters of sorghum (*Sorghum bicolor*) and kidney-bean (*Phaseolus vulgaris*). Leaf leachate was tried at 5, 10 and 20% concentrations and sterilized distilled water used as control. Seed germination, seedlings dry matter, shoot/root length were significantly reduced by all concentrations in both species ( $P < 0.05$ ). Decrease in chlorophyll content, soluble sugar content and consequently protein content is proportional to the increase in concentration of leaf leachate in both species. The result of present study were similar to that of Popoola *et al.* (2020) that studied allelopathic effect of some selected weeds on seed germination, plumule length, radicle length, fresh and dry weights of plumule and radicle of two varieties of cowpea. They observed that although, all the extracts reduced germination and seedling, growth, shoot extracts at 75% concentration of selected weeds significantly inhibited germinated and seedling growth of variety IT99K-573-1-1 compared with the control which produced 97% (germination %); *C. odorata*, *E. heterophylla* and *T. procumbens* shoot extracts produced 22, 20 and 50% germination, respectively. Consequently, *C. odorata*, *E. heterophylla* and *T. procumbens* shoot extracts produced 25, 18 and 28% germination respectively for variety IT07K-292-10 while the control yielded 99%.

A significant decrease of dry weight was observed in maize treatments applied with higher concentration of botanicals. The result revealed that the inhibitory effect was proportional to the concentrations of the extracts; higher concentrations had the stronger inhibitory effect. This is in agreement with findings of Ahmed *et al.* (2007) who observed that bioassays indicated that the inhibitory effect was proportional to the concentration of the extracts and higher concentration had the strongest inhibitory effect whereas the lower concentration showed stimulatory effect in some cases. Also, Jabeena and Ahmed (2009) reported that shoot extract of *Fumaria indica* decreased dry weight of maize with increasing concentration of aqueous extracts.

## CONCLUSION

The aqueous extracts of *Vigna unguiculata* Walp inhibited seed germination, plumule length, radicle growth and dry matter production of maize. Furthermore, allelopathic effects of *V. unguiculata* Walp are plant part specific and concentration dependent. Among the two aqueous extracts obtained from *V. unguiculata* Walp aqueous root extracts had more inhibitory effect on maize seed germination, while aqueous shoot extracts had greater inhibitory effect on plumule length, radicle growth, plant height and dry matter accumulation. In the case of *V. unguiculata* Walp plant parts, shoot extracts were more harmful than the root extracts.

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