

PHYTOCHEMICAL SCREENING AND ALLELOPATHIC INFLUENCE OF *EUCALYPTUS CAMALDULENSIS* ON *ZEA MAYS* AND *VIGNA* *UNGUICULATA* L (WALP)

*Lawan, S. A¹, Iortsuun, D. N², Kabiru, S¹ and Yahaya, S. U³

¹Biological Science Department, College of Arts, Science and Remedial Studies, Kano.

²Biological Science Department, Ahmadu Bello University, Zaria

³Crop Science Department, Kano University of Science and Technology, Wudil

*Correspondence author: lawansa@yahoo.com

ABSTRACT

Phytochemical screening and Allelopathic influence of aqueous leaf extracts of Eucalyptus camaldulensis on number of leaf per plant, plant height, fresh and dry plant weight, root nodule number and chlorophyll content were investigated on Zea mays and Vigna unguiculata. The results revealed that, Eucalyptus camaldulensis extracts contain tannins, flavonoids, saponins, resins, alkaloids, cardiac glycosides and acids which are all important allelopathic sources. The inhibitory effect on these crops were directly proportional to the increasing concentration of the plant extracts ($p \leq 0.05$). There were also significant reduction in number of leaves per plant, plant height, fresh and dry plant weight, root nodule number and chlorophyll content of the plants at higher extract concentrations of 15%. This study revealed that Eucalyptus camaldulensis would have inhibitory effects when grown along side with the test crops.

KEYWORDS: *Phytochemical screening, Allelopathic influence, Eucalyptus camaldulensis, Zea mays, Vigna unguiculata.*

INTRODUCTION

Plants live in association and in groups, depending upon the ecological requirements; they have generally the same structural and morphological adaptations. Whenever two or more plants occupy the same niche in nature they compete with each other for various life support requirements (Caton *et al.*, 1999). *Eucalyptus* belongs to the family Myraceae, mostly found in tropical regions and is a native to Australia. *Eucalyptus* grows under wide range climatic and edaphic conditions in their natural habitat (Dawar *et al.*, 2007). There are hundreds of secondary metabolites in the plant kingdom and many are phytotoxic (Einhellig, 2002). Reports by several authors have indicated that secondary metabolites are phytochemicals produced as by-products of primary metabolism in plants (Einhellig, 2002). Allelopathic effects of these compounds are often observed to occur early in the life cycle causing inhibition and modification of plant growth and development. A report by Singh *et al.*, (1992) showed that, aqueous extract of air dried leaf litter of *Eucalyptus citrodora* inhibited seed germination, plant height and yield in Wheat, Mustard and Gram. More so, Jayakumar *et al.* (1990) reported the inhibitory effect in root nodules number (in groundnut), number of leaves and leaf area of groundnut and maize treated with *Eucalyptus globules* leaf extract at various concentrations. They all concluded that the inhibition effects were from extracts of higher concentrations. Allelochemicals belong to different categories of secondary compounds such as phenols, benzoic and cinamic acids derivatives, flavonoids, tannins, coumarines, terpenoids, alkaloids and

polyacetylenes (Duke *et al.* 2000). These chemical compounds with allelopathic activity are regulated by environmental factors such as water potential of the environment, temperature, soil moisture, light intensity, nutrients, soil micro-organisms and perhaps others. They are distributed in varying concentrations in different organs of plants including leaves, stem and roots (Inderjit, 1996; Chon *et al.*, 2002). These are released into the environment through leaching, decomposition of residues, root exudation and volatilization. Allelochemicals such as coumarines and many alkaloids can inhibit cell division, cell wall formation and water uptake. Apart from these, flavonoids, tannins, quinines and many phenolic compounds, inhibit germination, photosynthesis, respiration and protein synthesis (Einhellig, 2002). Allelopathic interaction may among other effects inhibit germination and growth. In addition to these, it plays a key role in influencing the distribution and abundance of vegetation in nature, yield of various crops species and weeds interference (del Moral and Cates, 1991). This study was undertaken to determine the phytochemical screening and allelopathic influence of the aqueous leaf extract of *Eucalyptus camaldulensis* on *Zea mays* and *Vigna unguiculata*.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Leaves of *Eucalyptus camaldulensis* were collected and identified from Ahmadu Bello University, Samaru Zaria (Lat.11°11'N; Long. 7°39'E) located in the Guinea savanna eco-region of Nigeria. This sample was air dried at room temperature to a constant weight for two weeks.

The leaves were ground into fine powder in a mortar and pestle. *Zea mays*, var. Quality protein maize (QPM) and *Vigna unguiculata* var. (IT93K - 451 - 1) seeds were obtained from Institute of Agricultural Research (IAR) Samaru, Zaria and used as planting materials.

Extraction Process

The maceration method of extraction was employed. About 30g portions of ground powdered leaves was placed in round bottom flask containing 200cm³ of methanol and mixed. The round bottom flask was placed on a flask shaker and agitated for four hours and left to stand overnight. The residue was stirred and filtered using Whatman No. 1 filter paper and glass funnel into a beaker. The process was repeated using petroleum ether as the extracting solvent with the same quantity of powdered leaves and solvent. Evans and Trease (1999) and Cannel (2000).

Phytochemical Screening of the Extracts

The phytochemical screening of the extracts were carried out using standard procedures as described by Cannel (2000).

Tannins

A few drops of FeCl₃ (5%w/v) solution were added to extract in a test tube and shake. A dirty green or dark blue-black colouration if observed gave a positive result or confirmed the presence of tannins (Cannel, 2000).

Flavonoids

The extract (2cm³) was put into a test tube into which magnesium chips and few drops of hydrochloric acid (HCl) were added. A cloudy colouration with production of fumes was taken as a preliminary evidence for the presence of flavonoids (Cannel, 2000).

Alkaloids

A quantity (5cm³) of 10% of hydrochloric acid was added to 2cm³ of the extract in a test tube and was then left overnight and later divided into two parts for the following tests:

- a. A few drops of Dragndoff's reagent were added to one part of the extract in a test tube.
- b. A few drops of Meyer's reagent were added to the other part of the extract in a test tube. In both cases, the formation of precipitation indicated the presence of alkaloids.

Saponins

Distilled water (5cm³) was added to 2cm³ of the extract and shaken vigorously. Formation of foam following the shaking indicated the presence of saponins (Cannel, 2000).

Cardiac Glycosides

The extract was dissolved in 1cm³ of chloroform and few drops of conc. sulphuric acid (H₂SO₄) were added. A reddish brown colour at the

interface indicated the presence of cardiac glycosides (Cannel, 2000).

Resins

The extract (3cm³) was taken in a test tube, and then 3cm³ of copper acetate solution was added. The mixture was shaken vigorously and allowed to settle. A green colouration indicated the presence of resins (Cannel, 2000).

Acids

The extract (3cm³) was diluted with 2cm³ of distilled water. The solution was made alkaline by addition of 2-drops of ammonia with 2cm³ of conc. hydrochloric acid. Formation of bubbles indicated the presence of acids.

The same phytochemical test procedures were conducted for both the methanolic and petroleum ether extracts (Cannel, 2000).

Preparation of aqueous leaf extract treatment concentrations

The leaf powder (10g) was soaked in 1000cm³ of water for 24h, filtered and used as stock solution. The extract was prepared from the stock solution to get concentrations of 5, 10 and 15% using the procedure as described by Singh *et al.* (2003).

Treatments and Experimental Design

A total of five seeds each of cowpea and maize were planted in black plastic bags (15x30cm) and later thinned to two plants per bag. They were watered with about 30cm³ of the extract at three days interval and water as control. There were fifteen bags for each treatment with three replications per treatment. These were arranged in a complete randomized design. Data was collected on the number of leaves, plant height, fresh plant weight, dry plant weight, leaf chlorophyll content and root nodules number.

Statistical analysis

The data were analyzed using F- test using GLM procedure. Where significant difference exists, means of the data were separated using Duncan Multiple Range Test DMRT (Duncan, 1955).

RESULTS AND DISCUSSION

The results of the phytochemical screening of leaf extracts of *Eucalyptus camaldulensis* revealed that all the allelochemicals tested were found present except flavonoids, cardiac glycosides and acids, which were absent when methanol extract was used (Table 1).

Resins and saponins were found at higher concentrations using methanol extracts while tannins with petroleum ether extract. Alkaloid was the only phytochemical found at higher concentration when both extracts were used. Einhellig (1995) reported that presence in isolation or combination of two or more of these allelochemicals identified are all important allelopathic sources.

In other reports by Chon *et al.* (2002) and Fischer *et al.* (1994) allelochemicals such as coumarins, flavonoids, resins, phenols and many alkaloids were shown to inhibit cell division, changes in phytohormones and their balance, water uptake, and germination of seeds. Other effects include stomatal movement, pigment synthesis, nitrogen fixation, photosynthesis and perhaps others. Khan *et al.* (2008) reported that, *Eucalyptus camaldulensis* contained allelochemicals including tannins, resins, flavonoids and alkaloids, which are all allelopathic in nature.

The results of allelopathic potentialities of *E. camaldulensis* aqueous leaf extracts on number of leaves per plant, plant height, fresh and dry plant weight of *Zea mays* and *Vigna unguiculata* are shown in Tables 2 and 3. This revealed that the control treatment produced the highest mean values in leaf number, plant height, fresh and dry plant weight of the crop plants. In all cases, the other treatments showed significant reduction in all the growth rates. The inhibitory effect of these crops is directly proportional to the increasing concentrations of the aqueous extract. Such that as concentration increased the leaves number, height of the plants, fresh and dry plants weight gradually decreased. The decreases were observed from extract of higher concentration from 2-10 weeks after sowing (WAS).

Similar results have been reported by Muhammad *et al.* (2008) who reported the inhibitory effect of different concentrations (10, 15 and 20%) of aqueous leaf extract of *Eucalyptus camaldulensis* on germination and seedlings growth of wheat (*Triticum aestivum*). This effect was related to the presence of allelochemicals including tannins, flavonoids,

resins, and phenolic acid. The result is further supported by the findings of Padhy *et al.* (2000) who reported the reduction of seedlings growth and other growth parameters of finger millet using three concentrations (5, 10, 15 and 20%) of *Eucalyptus globules* leaf. The inhibitory effect was concentration dependent, as concentration increases all the growth parameters gradually decreased.

There was increase in root nodule number in cowpea with plant age. However, within the treatment significant decrease was observed at higher concentrations of the extract (Table 4). This is similar to the result obtained by El-Darier (2002) who reported that, aqueous leaf litter extracts of *Eucalyptus rostrata* reduced growth, root nodules number (in broad beans), nutrient uptake and metabolite accumulation of broad beans (*Vicia faba*) and *Zea mays* at higher concentrations.

With respect to chlorophyll content, there was highly significant reduction in chlorophyll content when plants were treated with extract of higher concentration compared to control. The reduction was based on increase in concentration of the plant extract and a decrease with plant age (Table 5). Similar result was reported by El-Khawas and Shehata (2005) that, chlorophyll content, soluble sugar and other biochemical parameters of maize and kidney beans were reduced due to application of *Acacia nilotica* and *Eucalyptus rostrata* aqueous leaf extracts. Similarly, the result correspond with one obtained by Rajangam and Arungam (1999), who reported the reduction of plant height, leaf area, total chlorophyll and starch content in rice treated with *Excoecaria agallacha* aqueous leaf extract.

Table 1: phytochemical properties of *Eucalyptus camaldulensis* leaf extract

Extraction solvent	Tannins	Flavonoids	Resins	Saponins	Alkaloids	Cardiac glycosides	Acids
Methanol	+	-	++	++	++	-	-
Petroleum ether	++	+	+	+	++	+	+

Key: - ++ present in high concentrations, + present, - absent

Table 2: Allelopathic influence of *Eucalyptus camaldulensis* aqueous leaf extract on leaf number/plant and plant height (cm) on *Zea mays* and *Vigna unguiculata*.

Treatment With <i>Eucalyptus</i> extract	Conc. (%)	Plant's Age (WAS)					
		No. of leaves/plant			Plant Height (cm)		
		2	6	10	2	6	10
<i>Zea mays</i>	0	4.7 ^e	9.3 ^c	10.4 ^c	35.0 ^{ef}	75.2 ^c	94.1 ^a
	5	4.3 ^e	8.9 ^c	10.4 ^c	31.3 ^{ef}	71.0 ^c	90.0 ^{ab}
	10	3.5 ^f	8.6 ^c	9.1 ^c	27.0 ^f	66.0 ^d	80.1 ^b
<i>Vigna unguiculata</i>	15	2.7 ^g	8.0 ^d	8.3 ^c	24.0 ^g	58.6 ^d	71.0 ^c
	0	5.3 ^h	28.0 ^b	38.0 ^a	27.0 ^f	30.4 ^e	44.0 ^{de}
	5	4.8 ^h	25.6 ^{bc}	35.0 ^{ab}	22.4 ^{gh}	28.0 ^f	33.3 ^e
	10	4.5 ^h	25.0 ^{bc}	29.0 ^b	18.3 ^h	27.0 ^f	29.0 ^f
	15	3.8 ⁱ	23.6 ^c	25.0 ^{bc}	17.0 ^h	24.3 ^g	25.2 ^g
	SE±	0.03	0.40	0.57	1.24	1.38	1.48

Means with same letter(s) within each column are not significantly different ($p \leq 0.05$).

Table 3: Allelopathic influence of *Eucalyptus camaldulensis* aqueous leaf extract on fresh and dry plant weight (g).

Treatment With <i>Eucalyptus</i> Extract	Conc (%)	Plant's Age (WAS)					
		Fresh plant weight (g)			Dry Plant weight (g)		
		2	6	10	2	6	10
<i>Zea mays</i>	0	5.8 ^h	59.0 ^c	128. ^a	1.0 ^f	3.2 ^d	16.5 ^a
	5	5.5 ^h	50.0 ^d	107 ^{ab}	0.8 ^f	2.5 ^{de}	14.0 ^{ab}
	10	4.8 ^{gh}	35.0 ^d	89.0 ^b	0.5 ^g	2.0 ^e	12.5 ^b
	15	4.6 ^{gh}	26.0 ^e	65.0 ^{bc}	0.3 ^h	1.9 ^e	8.7 ^c
<i>Vigna unguiculata</i>	0	5.3 ^h	16.0 ^f	32.3 ^d	0.6 ^g	4.5 ^d	9.4 ^c
	5	4.0 ^{gh}	13.0 ^g	26.2 ^e	0.5 ^g	3.1 ^d	7.1 ^{cd}
	10	3.5 ^{gh}	6.6 ^h	22.7 ^f	0.3 ^h	2.3 ^{de}	3.3 ^d
	15	2.6 ⁱ	5.0 ^{gh}	14.1 ^g	0.2 ^h	1.1 ^f	2.4 ^{de}
	SE±	0.05	0.34	0.43	0.02	0.31	0.41

Means with same letter(s) within each column are not significantly different ($p \leq 0.05$)

Table 4: Allelopathic influence of *Eucalyptus camaldulensis* aqueous leaf extract on root nodule number in cowpea.

Extract Conc. (%)	Plant's Age (WAS)		
	2	6	10
0	16.0 ^a	19.3 ^a	30.3 ^a
5	15.0 ^b	17.7 ^b	29.3 ^b
10	13.7 ^c	16.3 ^c	27.0 ^c
15	11.7 ^d	15.0 ^d	25.3 ^d
SE±	0.42	0.45	0.50

Means with same letter(s) within each column are not significantly different ($p \leq 0.05$)

Table 5: Allelopathic influence of *Eucalyptus camaldulensis* aqueous Leaf extract on chlorophyll content (mg/l) on *Zea mays* and *Vigna unguiculata*.

Treatments With <i>Eucalyptus</i> extract	Conc(%)	Plant's Age (WAS)	
		5	10
<i>Zea mays</i>	0	9.4 ^b	9.4 ^b
	5	8.5 ^c	7.5 ^c
	10	7.5 ^d	6.6 ^d
	15	5.6 ^e	5.2 ^e
<i>Vigna unguiculata</i>	0	9.8 ^a	9.8 ^a
	5	9.5 ^b	7.1 ^c
	10	5.6 ^e	4.5 ^f
	15	3.7 ^f	2.8 ^g
	SE±	0.21	0.23

Means with same letter(s) within each column are not significantly different ($p \leq 0.05$).

CONCLUSION

The study indicated that, allelopathic influence is determined by the interaction of several different allelochemicals and not by an individual compound. The presence of these allelochemicals in the extract of *Eucalyptus camaldulensis* leaves could be the reason for the

reduction of plant height, root nodule number and chlorophyll content of *Zea mays* and *Vigna unguiculata*. The reduction effect was concentration dependent such that as the concentration increased the extent of inhibition also increased.

REFERENCES

Cannel, R. J. P. (2000). *Methods in Biotechnology. Natural product isolation*. Humana press. New Jersey. Pp 14-36

Caton, B.P. Mortimer, A. M. Hill, T.C. Gibson, J.E. and Fisher, A.J. (1999). Weed morphology. Effects on competitiveness for light in direct-seeded rice. Proc. 17th Assian Pacific, Weed Science., Soc. Conf., Bangkok, 1.A, 116-120

- Chon, S. U. Choi, S. K. Jung, S. D. Jang, H. G. Pyo, B. S. and Kims, M. (2002). Effects of Alfalfa leaf extract and phenolics allelochemicals on early seedling growth of barnyard grass. *Crop Protection*. 5:107-138
- Dawar, S. M. Summaira, Y. Tariq, M. and Zaki, M. J. (2007). Use of *Eucalyptus* species in the control of root infecting fungi on mungbean and chicken- pea. *Pakistan Journal of Botany*.39(3): 975-979
- Duke, S. O. Dayan, E. F. Romangi, J. G. and Rimando, A. M. (2000). Natural products as source of herbicides. Current status and future trend. *Weed Research*. 10: 99-111.
- Del - Moral, R. and Cates, R.G. (1991). Allelopathic potentials of the dominant vegetation of western Washington. *Ecology*. 52 : 1030 - 1037.
- Duncan, D.B. (1955). Multiple Range and Multiple 'F' test. *Biometrics* 11: 1- 42
- Einhellig, F.A. (1995). Mechanism of action of allelochemicals in Allelopathy, Organism, Processes and Application. ACS. Symposium series 582. American Chemical Society. Washinton DC. Pp. 87 - 89.
- Einhellig, F.A. (2002). The physiology of allelochemicals action.clues and views in allelopathy. In: Molecules of Ecosystem. (Reigosa, M J and Petrole, N. Eds.). Science publishers, Einfield, New Hampshire. Pp1-9.
- El-Darier, S.M. (2002). Allelopathic effects of *Eucalyptus rostrata* on growth, nutrient uptake and metabolic accumulation of *Visia faba* (broad beans) and *Zea mays*. *Pakistan Journal of Biological Sciences*. 5(1): 6-11
- El-Khawas, S. A. and Shehata, M. M. (2005). The allelopathic potentialities of *Acacia nilotica* and *Eucalyptus rostrata* on Monocot (*Zea mays*) and Dicot (*Phaseolus vulgaris*) plants. *Biotechnology Journal* . 4(1): 23-34
- Evans, W.C. and Trease, G.E. (1999). *Pharmacognocny*. WB Saunders Company Limited, Nottingham U. K. Pp. 432.
- Fischer, N. H. Williams, G. B. Weidenhamer, J. D. and Richardson, D. R. (1994). Research of Allelopathy in Florida scrub.The role of Terpenoids. *Journal of Chemical Ecology* 20:1355-1350.
- Inderjit, I (1996). Plant phenolics in allelopathy. *Botanical Review*. 62:186-202
- Jayakumar, M. Eyini, M. and Pannirselvam, S. (1990). Allelopathic effect of *Eucalyptus globules* on groundnut and corn. *Comparative Physiology and Ecology*. 15: 109-113.
- Khan. M.A. Hussain, I. and Ejaz, A. K. (2008). Supressing effects of *Eucalyptus camaldulensis* on germination and seedlings growth of six weeds. *Pakistan Journal of Weed Reserch*. 14:201-207
- Muhammad, A. K. Iqtidar, H. and Ejaz, A. K. (2008). Allelopathic effects *Eucalyptus camaldulensis* on germination and seedlings growth of weat (*Triticum aestivum*). *Pakistan Journal of Weed Science*. 14(1-2): 9-18
- Padhy, B.B. Patanaik, P. K. and Tripathy, A.K. (2000). Allelopathic potential of *Eucalyptus* leaf litter leachates on germination and seedlings growth of finger millet. *Allelopathic Journal*. 7: 69-78
- Rajangam, M. and Arungam, K. (1999). Allelopathic effects of *Excoecaria agallocha L.* on germination, chlorophyll content and seedling growth of rice. *Journal of Environmental Ecology*. 9: 63 - 66
- Singh, A. Singh, H. S. and Mishra, S.S.(1992). Wheat response to allelopathy. Effects of some *Eucalyptus citiriodora* and their residues. *India Journal of Agronomy*. 43(2): 256-259
- Singh, H.P. Batish, D.R. Pandr, J.K. and kohli, R.K. (2003) Assessment of allelopathic properties of *Parthemium hysterophorous* Residue *Agricultural Ecosystem and Environment*. 95:537 - 541.