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EFFECTS OF ALLELOCHEMICALS OF SOME *EUCALYPTUS* SPECIES ON GERMINATION AND RADICLE GROWTH OF *ARACHIS HYPOGEA*

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ABSTRACT
A laboratory experiment was conducted to assess the effects of allelochemicals of Eucalyptus camaldulensis, Eucalyptus citriodora and Eucalyptus globules on germination and root elongation using leguminous crop ground nut (Arachis hypogea) as bioassay material. The experiments were conducted in sterilized petridishes. The effect of different concentration of aqueous extracts was compared with distilled water (control). The result revealed that different concentrations of E. globulus and E. camaldulensis extracts caused highly significant (p=0.05) inhibitory effect on germination and root elongation. The bioassays indicated that the inhibitory effect was proportional to the concentrations of the extracts so that higher concentration has a stronger inhibitory effect. The study also revealed that inhibitory effect was much pronounced in root development rather than seed germination.
Key words: Allelochemicals, Eucalyptus species, Root length, Germination, Arachis hypogea

INTRODUCTION

Plants that live in association, in groups, depending upon the ecological requirement; usually have 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 (Chon *et al*., 2005). The term "allelopathy" signifies the interactions between plants which might lead to either stimulation or inhibition of growth. Different groups of plants like; algae, lichens, crops and annuals and perennial weeds have wide known allelopathic interactions (Ahmad *et al.,* 2004; Uddin *et al.,* 2007). Several phytotoxic substances causing germination and/or growth inhibition have been isolated from plant tissues and soils. These substances are collectively known as allelochemicals. They are secondary plant products or waste products of main metabolic pathways of plant (Whittaker and Feeny 1977; Ashrafi *et al.,* 2007). Allelochemicals belong to different categories of secondary compounds such as phenols, benzoic and cinamic acids derivates, flavonoids, tannins, coumarines, terpenoids, alkaloids and polyaccetylenes (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). The effects of secondary substances released by these mechanisms can be long-lasting or quite transitory and can ultimately influence practices like fertility, seeding and crop rotations (Kaletha *et al.*1996; Kumar, 2006).

Melkania (1986) reported that allelopathic effects are selective and vary with different trees since these plants will vary in the amount of indigenous secondary metabolites and would release different amount of the phytotoxins. Generally leaves are the most potent source of allelochemicals, (Whittaker and Feeny 1977, Melkania, 1986). However, toxic metabolites are also distributed in all other plant parts in various concentrations. The effects of these compounds are often observed to occur early in the life cycle causing inhibition and modification of plant growth and development (Ahmed *et al*., 2007). A report by Singh *et al.* (1992) showed that, aqueous extract of air dried leaf litter of *Eucalyptus citriodora* 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. Higher plants (tree crops) release some phytotoxins into soil which adversely affect the germination and yield of crops (Kaletha *et al*., 1996; Kumar *et al*., 2006). Such type of tree crop interactions called phytochemical ecology/ecological biochemistry.

Eucalyptus species belongs to the family Myraceae, mostly found in tropical regions and is a native to Australia. *Eucalyptus* species grows under wide range of climatic and edaphic conditions in their natural habitat (Dawar *et al*., 2007).

Muhammad *et al,* (2008) reported that *Eucalyptus* species release volatile compounds such as benzoic, cinnamic and phenolic acids which inhibit growth of crops and weeds growing near to it. The leaves of *Eucalyptus* species are main releasing source of toxic compounds (Khan *et al.,* 2008). The present study was aimed at investigating the effect of allelochemicals present in some *Eucalyptus* species aqueous leaf extract on germination and radicle length of *Arachis hypogea*.

MATERIALS AND METHODS

The experiment was conducted to determine the effect of allelochemicals present in some *Eucalyptus* species aqueous leaf extracts on germination and radicle length of *Arachis hypogea* in the Biological Science Department laboratory, College of Arts, Science and Remedial Studies, Kano. The *Eucalyptus* species studied were *E. camaldulensis, E. globules* and *E.citriodora* which were considered as the donor plants. Fresh matured leaves of the different plant species were collected from the College campus, College of Arts, Science and Remedial Studies, Kano. The receptor agricultural crop selected was groundnut (*Arachis hypogea*) Var. EX-Dakar obtained from Kano Agricultural and Rural Development Authority (KNARDA).

Preparation of Aqueous Extracts

The dried leaves were grounded to fine powder using thistle and motar. Ten grammes (10 g) of the powder was weighed and soaked in 1000 cm^3 of distilled water for 24 hours. The solution was filtered through double layer of muslin cloth followed by No.1 Whatman filter paper. The concentration of various extracts were made, thus (15 gL^{-1} , 30 gL^{-1} and 45 gL^{-1}) from the leaf extract and stored in cornical flasks. Distilled water was used as control (0 %) as procedure described by Jafari *et al.* (2007).

Treatments

The following treatments were used in the experiment.

T_0 = Seeds of receptor plants grown in distilled water only (control)

T_1 = Seeds of receptor plants grown in each plant extract of 15gL^{-1} concentrations

T_2 = Seeds of receptor plants grown in each plant extract of 30 gL^{-1} concentrations

T_3 = Seeds of receptor plants grown in each plant extract of 45 gL^{-1} concentrations

Germination and Growth Records

The germination test was carried out in sterile petridishes of 9 cm in diameter placing a Whatman No.1 filter paper on the petridishes . The extract of each concentration was added to each petridish of respective treatment daily in such an amount just to keep the seeds moist enough to get favorable condition for germination and growth. The control was treated with distilled water only. Five seeds of *Arachis hypogea* were placed in the petridish replicated three times and arranged in complete randomized design. The petridishes were kept at a room temperature of 28-30^{0}C. The experiment extended over a period of five days to allow the last seed to germinate and the measurement of shoot and root length. The seed was considered as germinated when the radicle emerged and the germination count was recorded daily. The results were determined by counting the number of germinated seeds and measuring the length of primary root. The data were used to calculate the followings:

Ratio of germination and elongation were calculated, using the following formula as suggested by Rho and Kil (1986), Oyun (2006), Sazada *et al.* (2009).

$$\text{Relative germination Ratio (RGR)} = \frac{\text{Germination ratio of tested plant}}{\text{Germination ratio of control}} \times 100$$

$$\text{Relative elongation Ratio (RER)} = \frac{\text{Mean root length of tested plant}}{\text{Mean root length of control}} \times 100$$

Percentage inhibition on germination and radicle elongation of treatment plants to control were calculated, using the following formula as suggested by Sundra and Pote (1978), Oyun (2006), Sazada *et al.* (2009).

$I = 100 - (E_2 \times 100/E_1)$

Where, I = % inhibition, E_1 = Response of control plant, E_2 = Response of treatment plant.

Statistical Analysis

The data were analyzed by one way analysis of variance (ANOVA). Different means were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Germination

The effect of 3-donor plants on relative germination ratio of the bioassay species is shown in Table 1. The inhibition extent of germination has been determined to depend on both aqueous leaf concentration and incubation period. In most cases, variation percent varied evenly due to different concentrations. With the increase in concentration the inhibitory effect was progressively increased.

In all cases, the maximum inhibitory effect was found at T_3 treatments except *E. globules* which also occur at T_2 in 48 and 96 hours. The maximum relative germination ratio (RGR) (88.2%) was found for *E. citriodora* at T_2 treatment in 120h. Among the donor plants, E. *camaldulensis* shows less significant effect at all treatments to the receptor crop in comparison to others. It was also observed that all leaf extracts delayed the germination significantly in receptor crop compared to control treatment.

Root elongation

The root length of bioassay species was found to be greatly inhibited with the increase of the concentration of extracts. In all cases the results revealed that highest inhibitory effect was much more pronounced at T_3 concentration followed by T_2 and T_1 concentrations respectively. This is with the exception of *E. citriodora* where highest percent inhibition recorded was at 72h(-36.9%) 96h (-41.1%) and 120h with (-39.5) all at T_2 concentration (Table 2). Results in Table 3. showed that *E.camaldulensis* leaf extract recorded the highest inhibitory effect with (-83.9%) at T_3 then T_2 with (-75.8%) and T_1 with (-64.1%) treatment of same plant extract. This is followed by *E. globules* which show moderate effect (Table 4). Complete inhibition of root elongation was also shown at 24h of T_3 concentration of same *E. camaldulensis* plant extract with (-100%). Least inhibitory effect was also recorded from *E. citriodora* plant with (-29.6%) (Table2). Maximum elongation of root (7.54±1.48cm) was observed in *E. citriodora* followed by (7.44±0.45cm) in *E. camaldulensis.*

Considering the foregoing results, it seemed that there are significant phytotoxic effects of *E.citriodora , E. camaldulensis and E. globules* on germination and root elongation. These results correlated with the findings that leaf extract of *Eucalyptus camaldulensis* have allelopathic effect on seed germination and seedling growth of wheat (*Triticum aestivum* L.) (Muhammad *et al.,* 2008). Padhy *et al.* (2000) also reported the suppressing effects from *Eucalyptus* leaf leachates on germination and seedlings growth of finger millet. This observation also confirmed the findings that *Eucalyptus globules* have phytotoxic effect on germination and radicle growth of groundnut and corn and root growth was more sensitive to the increasing concentration of the aqueous extract in comparison to seed germination (Jayakumar *et al.,* 1990). The result also confirms that allelopathy is a concentration dependent phenomenon as concentration increased the extent of inhibition also increased (Hoque *et al.,* 2003; Ahmed *et al.,* 2007; Siddiqui *et al.,* 2009).

Table 1: Effect of aqueous leaf extract of *E.citriodora, E.camaldulensis and E. globules* on relative germination of *A. hypogea.*

		Duration				
		24h	48h	72h	96h	120h
Treatments	**Plants spp.**					
T_0	*E. citriodora*	70.2	75.6	77.4	80.3	85.6
T_1		55.3	72.3	75.4	78.4	80.8
T_2		43.4	70.4	73.5	75.5	88.2
T_3		30.3	67.6	70.6	73.6	75.1
T_0	*E. camaldulensis*	54.7	70.1	72.3	75.4	76.1
T_1		50.2	55.3	62.4	65.0	67.6
T_2		33.6	48.6	55.1	59.3	61.8
T_3		25.0	33.2	46.8	50.4	53.2
T_0	*E. globules*	69.8	74.8	78.0	80.3	84.6
T_1		54.7	68.9	75.4	79.0	83.2
T_2		43.4	56.1	69.1	72.2	79.0
T_3		40.8	58.1	73.3	74.8	77.4

Table 2: Root elongation (cm) of receptor crop to distil water (T_0) and different concentrations of *E.citriodora* extracts (T_1-T_3).

	Duration				
Treatments	**24h**	**48h**	**72h**	**96h**	**120h**
T_o	0.50	3.35	5.94	7.06	7.54
T_1 (PIE)	0.48(-4.0)	3.35(-32.5)	3.6(-39.4)	5.62(-20.4)	6.26(-16.5)
T_2 (PIE)	0.48(-4.0)	2.29(-31.7)	3.75(-36.9)	4.16(-41.1)	4.54(-39.5)
T_3 (PIE)	0.29(-42.0)	1.76(-47.5)	3.89(-34.5)	4.86(-31.2)	5.28(-29.6)

(PIE = Percent of inhibitory effect, -ve = inhibitory effect, +ve = stimulatory effect)

Table 3: Root elongation (cm) of receptor crop to distil water (T_0) and different concentrations of *E. camaldulensis* extracts (T_1-T_3).

	Duration				
Treatments	**24h**	**48h**	**72h**	**96h**	**120h**
T_o	1.64	3.41	6.55	7.18	7.44
T_1 (PIE)	1.44(-12.2)	1.9(-44.3)	2.35(-64.1)	2.84(-60.5)	3.40(-54.3)
T_2 (PIE)	0.48(-70.7)	0.99(-70.9)	1.39(-75.8)	1.82(-74.2)	2.26(-69.6)
T_3 (PIE)	0.00(-100)	0.86(-74.8)	1.62(-83.9)	1.9(-76.6)	1.9(-77.9)

(PIE = Percent of inhibitory effect, -ve = inhibitory effect, +ve = stimulatory effect)

Table 4: Root elongation (cm) of receptor crop to distil water (T_0) and different concentrations of *E. globules* extracts (T_1-T_3).

	Duration				
Treatments	**24h**	**48h**	**72h**	**96h**	**120h**
T_o	1.24	3.41	6.55	7.18	7.44
T_1 (PIE)	1.04(-16.1)	2.57(-24.6)	3.93(-40.0)	4.46(-37.9)	4.78(-35.8)
T_2 (PIE)	0.91(-26.6)	2.46(-27.9)	3.56(-45.7)	3.84(-46.6)	4.27(-42.6)
T_3 (PIE)	0.78(-37.1)	1.91(-44.0)	3.02(-53.9)	3.26(-54.6)	3.69(-50.4)

(PIE = Percent of inhibitory effect, -ve = inhibitory effect, +ve = stimulatory effect)

CONCLUSSION

The present studies showed that all the 3-donor plants (*E. citriodora, E. camaldulensis and E. globules*) are allelopathic in nature, but *Eucalyptus camaldulensis* exhibited the highest Allelopathic potentials. . However, long term field based studies must be carried out before incorporation of these trees in any arable system.

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