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The Allelopathic Potentialities of *Acacia nilotica* and *Eucalyptus rostrata* on Monocot (*Zea mays* L.) and Dicot (*Phaseolus vulgaris* L.) Plants

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Abstract: Plants may favorably or adversely affect other plants through allelochemicals, which may be released directly or indirectly from live or dead plants (including microorganisms). The objective of this study was to examine the allelopathic effects of leaf leachates of *Acacia nilotica* and *Eucalyptus rostrata* on morphological, biochemical and molecular criteria of *Zea mays* L. (maize) and *Phaseolus vulgaris* L. (kidney bean). Concerning the seedling, seedling emergence was reduced with treatment with *Acacia* and *Eucalyptus* leaf leachates and growth parameters were generally reduced in both maize and kidney-bean. With respect to the internal metabolites, a gradual increase in the ABA concentration, decrease in the chlorophyll content and consequently the soluble sugars content is proportional to the increase in the concentration of the leaf leachates. Total DNA and RNA contents are highly significant increased in all treated samples of maize with respect to the control. On the other hand, kidney-bean samples showed mostly a significant reduction. Maize samples treated with *Acacia* show a highly significant increase in the level of soluble proteins while samples treated with *Eucalyptus* show a highly significant decrease. However, there is a highly significant decrease in soluble protein contents in Kidney-bean samples treated with both *Acacia* and *Eucalyptus*. With respect to the protein pattern, the allelopathic effect of both *Acacia* and *Eucalyptus* induced the formation of stress proteins. High and mid molecular weight proteins 244, 187, 143, 51.8, 41, 40.6 and 29.7 kDa were newly synthesized in response to the allelopathic effect of *Acacia*. On the other hand, mid and low molecular weight polypeptides 121.8, 116, 62, 52.9, 46.8, 45 and 40.6 kDa were recorded in response to the allelopathic effect of *Eucalyptus*. In maize treated with both *Acacia* and *Eucalyptus*, the 55.4 kDa protein was disappeared, while the proteins 40.5, 30.5, 29.7 and 26.5 kDa were newly synthesized. Moreover, in maize seedlings treated with *Eucalyptus*, the proteins 50.8, 41, 30.5, 26.8 and 26.5 kDa were recorded. The number of *de novo* synthesized proteins (chaperones) in maize samples treated with both *Acacia* and *Eucalyptus* (12-induced proteins; 121.8, 60.9, 55, 52.9, 50.8, 46.8, 41, 50.6, 30.5, 29.7, 26.8 and 26.5 kDa) were more than that induced in kidney-bean (10-stress induced proteins; 244, 187, 143, 116, 93.7, 51.8, 45, 41, 40.6 and 27.4 kDa). The genomic DNA of each treatment was subjected to four restriction enzymes (BamHI, EcoRI, HinfI and SmaI). The resulting cleavage fragments had apparent low molecular sizes and appear as smears of digests whatever the concentrations of templates of DNA or restriction enzymes and the time of electrophoretic run. The highest degree cleavage was observed EcoRI. All studied samples showed different DNA fingerprints. All samples had on average 20-30% of their bands in common. The variability was higher in the bands in the region of the gel above 2.7 kb than in the bands below it. On the average, 50±5 bands were seen above 500 bp, ranging from 700 to 2400 bp. A clear information data was obtained using HinfI (5-base cutter) as compared to other enzymes (6-base cutter) which may indicates the presence of stimulator which facilitate the recognition of the nucleotide sequence which is specific for each enzyme and the cutting site inside it. This indicates that the percentage of shared bands in DNA fingerprints of maize and kidney-bean might be used as a measure of genetic difference and demonstrate that the restriction site mutation assay can detect mutations. Present results indicate that, *Eucalyptus* is more effective in affecting morphological, biochemical and molecular criteria and that the monocot plant (maize) is more tolerant than the dicot one (kidney-bean). We can suggest that the allelopathic chemicals of both *Acacia* and *Eucalyptus* may have the potential as either biological herbicides or templates for new herbicide classes (i.e. biological control).

Key words: Allelopathy, growth parameters, internal metabolites, protein patterns, DNA profiles

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INTRODUCTION

The interaction of plants through chemical signals (allelopathy) has many possible agricultural applications^[1]. Decline in crop yields in cropping and agro-forestry system in recent years has been attributed to allelopathic effects. Allelopathy associated problems have been observed both in monocultures and multiples cropping system, continuous monoculture causes the accumulation of phytotoxins and harmful microbes in soil, which give rise to phytotoxicity and soil thickness. Crop rotation are practiced to eliminate the effect of monoculture, but the succeeding crop may be influenced by the phytotoxins released by the preceding crop. A large number of weeds and trees possess allelopathic properties which have growth inhibiting effect on crops. Allelopathy also plays an important role in suppressing the growth of weed plants^[2-5]. Struggle for space and nutrients for propagation, continuity and universality is the most powerful law of nature. In this trend, some plants have allelopathic potential by releasing allelochemicals to their surrounding that have either deleterious or beneficial effects on other plants. These compounds inhibited plant growth by affecting many physiological processes among them, the effect on ion uptake and hydraulic conductivity (i.e. water uptake) are particularly important since the root is the first organ to come into contact with the allelochemicals in the rhizosphere^[6]. The degree of inhibition depends on their concentration. Some plant genotypes are likely to escape the allelopathic chemical(s) by being "hypersensitive". In this regards the root tip may actually be strongly affected by allelochemical(s) and have its growth rate nearly stopped^[7].

Chemicals with allelopathic activity are present in many plants and in various organs, including leaves and fruits^[8,9] and have potential as either herbicides or templates for new herbicide classes^[10,11]. Many allelopathic compounds produced by plants are regulated by environmental factors, such as water potential of the environment, temperature, light intensity, soil moisture, nutrients, soil microorganisms and perhaps others. The compounds are released to the environment by means of volatilization, leaching, decomposition of residues and root exudation. They are; firstly, the terpenoids cineole, are released to the environment by volatilization, which is noticeable under drought conditions; secondly, the water born phenolic and alkaloids are washed out by rainfall through leaching; thirdly, phytotoxic aglycones, such as phenolics and others are produced during the decomposition of plant residues in soil; fourthly, many secondary metabolites such as scopoletin, may be released to the surrounding soil through root exudation^[8,12].

Paper chromatography separation of the water soluble extract of *Washington filifera* fruits revealed the presence of two inhibiting zones of germination and growth, the fast moving beta-inhibitor was further separated by TLC into four components, one of which appears to be abscisic acid (ABA) and the rest was phenolic substances. Gibberellic Acid (GA) was found to neutralize the germination and growth inhibiting ability of the inhibitor beta-complex. Besides these, a slow moving inhibitory zone of germination and growth was due to the presence of phenolic substance^[13]. Chromatographic separation showed that the majority of the compound presents in leaf extracts of *Eucalyptus globulus* Labill were phenolic^[14]. The inhibitory effect of *Acacia* spp. on seed germination and seedlings was related to the presence of allelochemical including tannins, wax, flavonoides and phenolic acids. Moreover, the toxicity was caused due to synergistic effect rather than single one^[15]. *Eucalyptus* spp. and *Acacia* spp. have phytotoxic effects on tree crops of legumes^[16]. However the *Eucalyptus* species are considered the most notorious of allelopathic trees causing understory^[17]. In addition, Reigosa *et al.*^[2] confirmed that *Eucalyptus globus* has the most allelopathic effect and the most large number of allelochemicals (allelopathin). Moreover, the large area of the ground surface beneath *Eucalyptus* remains completely bare or with very limited vegetation due the increase of cation exchange capacity and the decrease in both pH and base saturation^[18]. Phytotoxic substances may act in many biological processes, such as to suppressing the mineral uptake by plants, inhibiting cell elongation and cell division, as well as retarding the photosynthesis, respiration and enzymatic activities, resulting in the retardation of plant growth. They may also interfere with the action of growth promoting substance, e.g., gibberellins or auxins^[19]. It also included deterioration of membrane integrity, loss of dehydrogenase activity with the concurrent drastic reduction or total failure of water absorption by the roots and the reduction of the chlorophyll contents of leaves were responsible for the inhibition^[20].

Recently, some progress has been made in the study of molecular processes involved in morphological and physiological adaptation of plants exposed to allelopathic chemicals of other plants. Development of new molecular procedures for the detection of genetic alteration has been developed as a genotypic mutation detection system capable of identifying mutations occurring in restriction enzyme sites of genomic DNA. The present research was carried out to study the allelopathic effects of leaf leachates of *Acacia nilotica* and *Eucalyptus rostrata* on morphological, biochemical and molecular criteria of *Zea mays* L. and *Phaseolus vulgaris* L. and prove that

these extracts can be used in biological control as natural herbicides to reduce the risk of manufactured herbicides.

MATERIALS AND METHODS

Plant materials: Grains of maize (*Zea mays* L. Fardy 124) and seeds of kidney-bean (*Phaseolus vulgaris* L. Pronko) were obtained from the Agricultural Research Center, Giza, Egypt. The grains and seeds were surface sterilized with 1% sodium hypochlorite for 20 min, then rinsed with distilled water several times. Fresh samples of leaves of *Acacia nilotica* and *Eucalyptus rostrata* were collected at random at the vegetative stage in early 2000 from some fields of Kalyobia Governorate. The leaves were dried in an oven at 50°C for 48 h and then crushed using a vegetative grinder. The crushed leaves were allowed to decay for 24 h in distilled water. Four different concentrations (1, 3, 5 and 10%) were prepared. Maize grains and kidney-bean seeds were soaked in the previous extracts and distilled water as control for one hour. The bioassay experiment was done in plastic pots. The percentage of germination were recorded after 5 days. Samples of 10 days were collected for morphological, biochemical and molecular analysis. All parameters were statistically analyzed according to SAS Program^[21].

Estimation of chlorophyll: Chlorophyll a and b contents were determined according to Todd and Basler^[22].

Estimation of sugars: Soluble sugars were extracted following the method adopted by Homme *et al.*^[23] and determined with the anthrone reagent^[24].

ABA (abscisic acid): ABA was extracted, separated, identified and quantified by HPLC using the method described by Shindy and Smith^[25].

Protein analysis: Soluble proteins were determined according to the method described by Bradford^[26]. The identification and characterization of different protein fractions were obtained using one-dimensional SDS-PAGE. Polyacrylamide slab gel (12.5%) was prepared according to Laemmli^[27]. To analyze the banding pattern, molecular weights and band percentage, the destined gels was analyzed Gel Doc 2000 in the presence of protein molecular weight marker using Gel Proanalyzer Version 3 Media Cybernetics Tice Imaging Experts Software.

RNA and DNA analysis: Total nucleic acids (RNA and DNA) were extracted according to Guinn^[28] and estimated using U.V. spectrophotometer (Spectrophotometer (601) as adopted by Ogur and Rosen^[29]. Genomic DNA was

extracted from 60 mg of frozen tissues (young leaves of samples grinded in liquid nitrogen) using GenomicPrep™ Cells and Tissue DNA Isolation Kit (Amersham Pharmacia Biotech Inc.) according to the instruction manual. The concentration of DNA in the samples was measured by UV-absorption spectroscopy at a wavelength of 260 nm at which DNA gives a maximum absorption of OD₂₆₀ corresponding to a concentration of approximately 50 µg mL⁻¹ of double-stranded DNA^[30]. DNA was subjected to restriction using four restriction enzymes (BamHI, EcoRI, HinfI and SmaI at 5U per Reaction) according to manufacturer's instructions. The reaction mixture was incubated for 1 h at 37°C except SmaI (25°C). The restriction fragments were size-fractionated using 2% agarose gel electrophoresis. The agarose gels were stained with ethidium bromide and photographed under UV light. The results were documented by Image Analyzer Gel Doc 2000 (Bio Rad).

RESULTS AND DISCUSSION

The allelopathic potential of *Acacia nilotica* and *Eucalyptus rostrata* on the germination of maize and kidney-bean is shown in Table 1. It is obvious that the leaf leachates both of *Acacia* and *Eucalyptus* inhibited the germination of maize and kidney-bean. It is also apparent that *Eucalyptus* perform the more inhibitory effect than *Acacia*. These results are in agreement to those obtained by Duhan and Lakshinayana^[31], who found that the growth of *Cyamopsis tetragonoloba* and *Pennisetum* growing at distance of 1-2 and 7.5 m from tree of *Acacia nilotica*, was inhibited. The percentage of germination, plumule and radicle length of rice and cowpea, were decreased with increasing concentration of *Acacia auriculiformis* leaf leachates^[32]. *Acacia auriculiformis* significantly inhibited germination and growth of rice and radicle growth of cowpeas. Responses indices revealed that the inhibition of radicle and plumule was more pronounced than that of germination. In addition, Leaf extracts of *Acacia auriculiformis* and *Acacia nilotica* were highly toxic for growing wheat^[33]. Moreover, germination and growth of aster (*Callistephus chinensis*) and chrysanthemum (*Chrysanthemum coronarium*) decreased with increased leaf soaking period in *Acacia auriculiformis*^[34]. Bora *et al.*^[35] found that, the inhibitory effect of leaf extracts of *Acacia auriculiformis* on germination of some agricultural crops was proportional to the concentration of the extract. The elongation of radicle and plumule was reduced in all treatments, with effects much more pronounced on plumule than radicle elongation. In addition, extracts of *Acacia auriflormis* inhibited rice germination, plumule and

Table 1: Effect of the allelopathic potential of *Acacia nilotica* and *Eucalyptus rostrata* on percentage of germination, shoot length and root length of *Zea mays* L. and *Phaseolus vulgaris* L. 10 days seedlings. Each value is a mean of three replicates

Sample	Percentage of germination	Shoot length (cm)	Root length (cm)
1	100.00	9.90	9.90
2	86.00*	3.50*	2.60**
3	78.00*	0.75**	1.70**
4	75.00*	0.50**	1.00**
5	58.00**	-	0.50**
6	100.00	2.50	1.50
7	36.00**	1.50	1.00
8	36.00**	1.50	0.50*
9	35.00**	1.50	0.50*
10	24.00**	-	0.50*
11	93.00*	9.00*	6.00**
12	91.00*	4.50*	2.10*
13	61.00**	3.40	0.60
14	40.00**	0.80	0.60
15	31.00**	1.80*	1.30**
16	20.00**	1.50*	2.20**
17	13.00**	1.00	0.70*
18	6.00**	1.00	0.50**
LSD at 5%	2.56	1.21	0.98
LSD at 1%	2.95	1.64	1.16

** Highly significant differences * Significant differences

Sample 1: Seedlings of *Zea mays* L. as control samples.

Sample 2: Seedlings of *Zea mays* L. treated with 1% leaf extract of *Acacia nilotica*.

Sample 3: Seedlings of *Zea mays* L. treated with 3% leaf extract of *Acacia nilotica*.

Sample 4: Seedlings of *Zea mays* L. treated with 5% leaf extract of *Acacia nilotica*.

Sample 5: Seedlings of *Zea mays* L. treated with 10% leaf extract of *Acacia nilotica*.

Sample 6: Seedlings of *Phaseolus vulgaris* L. as control samples.

Sample 7: Seedlings of *Phaseolus vulgaris* L. treated with 1% leaf extract of *Acacia nilotica*.

Sample 8: Seedlings of *Phaseolus vulgaris* L. treated with 3% leaf extract of *Acacia nilotica*.

Sample 9: Seedlings of *Phaseolus vulgaris* L. treated with 5% leaf extract of *Acacia nilotica*.

Sample 10: Seedlings of *Phaseolus vulgaris* L. treated with 10% leaf extract of *Acacia nilotica*.

Sample 11: Seedlings of *Zea mays* L. treated with 1% leaf extract of *Eucalyptus rostrata*.

Sample 12: Seedlings of *Zea mays* L. treated with 3% leaf extract of *Eucalyptus rostrata*.

Sample 13: Seedlings of *Zea mays* L. treated with 5% leaf extract of *Eucalyptus rostrata*.

Sample 14: Seedlings of *Zea mays* L. treated with 10% leaf extract of *Eucalyptus rostrata*.

Sample 15: Seedlings of *Phaseolus vulgaris* L. treated with 1% leaf extract of *Eucalyptus rostrata*.

Sample 16: Seedlings of *Phaseolus vulgaris* L. treated with 3% leaf extract of *Eucalyptus rostrata*.

Sample 17: Seedlings of *Phaseolus vulgaris* L. treated with 5% leaf extract of *Eucalyptus rostrata*.

Sample 18: Seedlings of *Phaseolus vulgaris* L. treated with 10% leaf extract of *Eucalyptus rostrata*.

radicle elongation. The inhibitory effect of *Acacia* spp. on seed germination and seedlings was related to the presence of allelochemical including tannins, wax, flavonoides and phenolic acids. Furthermore, the toxicity might caused due to synergistic effect rather than

single one^[15]. Phenolic acids have been shown to be toxic to germination and plant growth processes^[36,37]. The interruption of one plant process by the phenolic compounds released from the decomposition of *Eucalyptus* leaves usually affects other processes^[38]. Rajangam and Arungam^[39] found that, the use of 7-aqueous extracts of *Excoecaria agallocha* leaves inhibited seed germination of and plumule and radicle elongation of rice. Jose and Gillespie^[40] reported that juglone released from black walnut exhibited inhibitory effects on all measured variables including leaf photosynthesis, transpiration stomatal conductance, leaf and root respiration in corn and soybean. The inhibitory effect of *Eucalyptus* leaf extracts on germination and growth of rabi crops was attributed to the essential oil content^[41].

Under field condition, the *Eucalyptus* trees reduced the wheat crop germination^[3]. The forestry plantation residue of *Eucalyptus* (leaf and branch) suppressed seed germination and early seedling growth of the dicotyledonous species^[42]. Jacob *et al.*^[43] in a pot experiment, using *Zea mays* Cv. Kasan grains sown on soil treated with extracts of *Eucalyptus camaldulensis* or tap water found that percentage of germination of maize was reduced and germination was delayed compared with the control. In addition, *Eucalyptus grandis* inhibited germination of lettuce seeds. Duryea *et al.*^[44] and El-Darier^[45] found that the germination of *Vicia faba* L. and *Zea mays* L. was greatly decreased at 5% level of *Eucalyptus rostrata*. Moreover, All concentrations of *Eucalyptus globules* leachates studied (5, 10, 15 and 20%) considerably inhibited seed germination of finger millet^[46]. Furthermore, *Eucalyptus tereticornis* leaf extract of 4 concentration gradients (1.0, 2.5, 5.0 and 10%), the extract at 10% concentration resulted in the lowest germination percentage of garlic. Recently, Djanaguiraman *et al.*^[47] found that with the increase in concentration of leaf extracts of *Eucalyptus globules*, there was concomitant decrease in germination of greengram, blackgram and cowpea also, under field condition, the *Eucalyptus* trees reduced the wheat crop germination^[3]. Sing *et al.*^[48] found that the aqueous leaf leachates of *Eucalyptus citriodora* inhibited the germination and seedling growth of *Vigna radiata*, *V. mungo* and *Arachis hypogaea*. The leaf leachate proved more inhibitory on plumule elongation than on radicle elongation the magnitude of inhibition on plumule length followed the order *A. hypogaea* (100%) > *V. mungo* (50%) > *V. radiata* (42) over the control leaf leachates. Alexander^[18] revealed that the large area of the ground surface beneath *Eucalyptus* remains completely bare or with very limited vegetation. The result was explained due

Table 2: Effect of the allelopathic potential of *Acacia nilotica* and *Eucalyptus rostrata* on total chlorophylls, total soluble sugars and abscisic acid of *Zea mays* L. and *Phaseolus vulgaris* L. 10 days seedlings. Each value is a mean of three replicates

Sample	Total chlorophylls (mg/100 g F. wt.)	Total soluble sugars (mg/100 g F. wt.)	Abscisic acid (µg/100 g)
1	14.50	280.00	-
2	8.70**	165.00**	86.00**
3	6.40**	115.00**	245.00**
4	10.00	200.00	-
5	6.00**	118.00**	100.00**
6	4.00**	78.00**	325.00**
7	5.80**	110.00**	115.00**
8	4.30**	82.00**	330.00**
9	3.80**	75.00**	130.00**
10	2.10**	40.00**	360.00**
LSD at 5%	0.65	2.20	9.10
LSD at 1%	0.92	2.80	11.30

** Highly significant differences

* Significant differences

Table 3: Effect of the allelopathic potential of *Acacia nilotica* and *Eucalyptus rostrata* on total soluble protein, RNA and DNA of *Zea mays* L. and *Phaseolus vulgaris* L. 10 days seedlings. Each value is a mean of three replicates

Sample	Soluble protein (ug/g F. wt.)	DNA (mg/100 g F. wt.)	RNA (mg/100 g F. wt.)
1	2.02	98.00	310.00
2	2.86**	105.00**	358.00**
3	2.91**	111.00**	378.00**
4	2.89**	109.00**	365.00**
5	1.98**	94.00*	288.00**
6	1.96**	88.00**	268.00**
7	1.58**	78.00**	216.00*
8	1.76**	110.00**	371.00**
9	1.79**	115.00**	411.00**
10	1.55**	108.00**	358.00**
LSD at 5%	0.064	4.22	9.27
LSD at 1%	0.089	5.64	12.35

** Highly significant differences

* Significant differences

Lane 1: Seedlings of *Zea mays* L. as control samples.

Lane 2: Seedlings of *Zea mays* L. treated with 1% leaf extract of *Acacia nilotica*.

Lane 3: Seedlings of *Zea mays* L. treated with 3% leaf extract of *Acacia nilotica*.

Lane 4: Seedlings of *Phaseolus vulgaris* L. as control samples.

Lane 5: Seedlings of *Phaseolus vulgaris* L. treated with 1% leaf extract of *Acacia nilotica*.

Lane 6: Seedlings of *Phaseolus vulgaris* L. treated with 3% leaf extract of *Acacia nilotica*.

Lane 7: Seedlings of *Zea mays* L. treated with 1% leaf extract of *Eucalyptus rostrata*.

Lane 8: Seedlings of *Zea mays* L. treated with 3% leaf extract of *Eucalyptus rostrata*.

Lane 9: Seedlings of *Phaseolus vulgaris* L. treated with 1% leaf extract of *Eucalyptus rostrata*.

Lane 10: Seedlings of *Phaseolus vulgaris* L. treated with 3% leaf extract of *Eucalyptus rostrata*.

the increase of cation exchange capacity and the decrease in both pH and base saturation. Phenolic acids have been shown to be toxic to germination and plant growth processes and activities of many enzymes due to their interference with vital growth processes and activities of many enzymes^[36] and phytohormones^[37].

Concerning the seedling, seedling emergence was reduced with treatment with *Acacia* and *Eucalyptus* leaf

leachates and growth parameters were generally reduced in both maize and kidney-bean. Blaise *et al.*^[49] and Khan *et al.*^[50] obtained similar results in maize and wheat. The germination rate of seeds of Indian mustard (*Brassica juncea* Var. *napiformis* and wild cabbage (*Brassica oleracea* Var. *Caulorapa*) was inhibited by 69 and 72%, respectively, with treatment with *Eucalyptus*^[51]. Also, seedling lengths of greengram and pigeonpea were decreased compared to the control when treated with *Eucalyptus tereticornis* leaf extract^[52]. There was concomitant decrease in the shoot and root lengths of greengram, blackgram and cowpea^[47]. Leaf extracts of *Eucalyptus* were shown to inhibit the rooting rate, root number and length of root of mung bean cuttings by 85.71, 58.96 and 93.52%, respectively and inhibit the rooting rate of pea cutting by 100%^[51].

The morphological reduction exerted due to the allelopathic potential of both *Acacia* and *Eucalyptus* is accompanied by a reduction in the biochemical parameters of the treated plants. Table 2 showed that the total chlorophyll content and consequently the soluble sugar contents of maize and kidney-bean was reduced due to the application of both *Acacia* and *Eucalyptus* leaf leachates. Similar to this result, there was a reduction of the chlorophyll contents of leaves of *Eichornia crassipes* in response to *Parthenium hysterophorus* leaf leachates^[20]. Rajangam and arumgam^[39] found that the total chlorophyll and starch contents of rice were reduced by 61 and 55%. The inhibitory effect was due to the presence of phenolic acids such as p-hydroxybenzoic acid, syringic, vanillic, protocatechuic, caffeic and chlorogenic in *Excoecia agallocha*, which are considered to be allelochemicals. Furthermore, application of the aqueous leaf leachates of *Eucalyptus citriodora* also influenced the metabolism of seeds and seedlings, i.e. concentration of sugars, amino acids and organic acids in the seedlings of the tested crops. It has been found that the allelochemical present in the leaf leachate of *Eucalyptus citriodora* had potential to inhibit the physiological processes, i.e. germination, growth and metabolism of seeds and seedlings of *V. radiata*, *V. mungo*, *A. hypogaea*^[48]. *Eucalyptus globulus* leachates inhibited the growth of *Costus speciosus*. It also reduce diosgenin, chlorophyll and nitrogen contents. The greatest inhibition was by 10% compared to 5 and 2.5%. Leachates of mature leaves were the most inhibitory, followed by those of senescent and juvenile leaves.

The ABA concentration of maize and kidney-bean is shown in Table 2. There is a gradual increase in the ABA concentration in proportional to the increase in the concentration of the leaf leachates. The results are coincided with those obtained by the paper

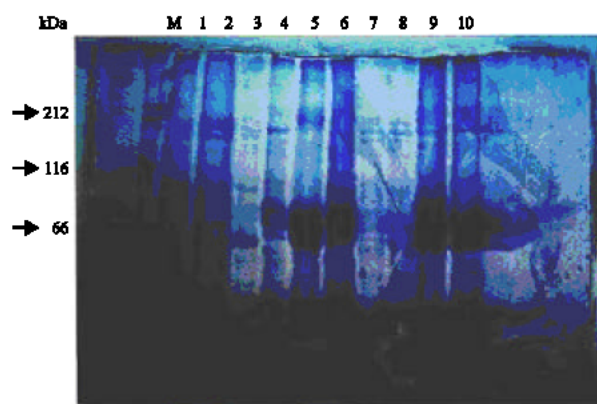


Fig. 1: Changes in protein banding patterns in seedlings of *Zea mays* L. and *Phaseolus vulgaris* L. treated with different concentrations of leaf extracts of *Acacia nilotica* and *Eucalyptus rostrata*. Lane M: Protein molecular weight marker. Lanes 1-10: seedlings of *Zea mays* L. and *Phaseolus vulgaris* L. treated with different concentrations of leaf extracts of *Acacia nilotica* and *Eucalyptus rostrata*. Lanes 1, 2, 3, 7 and 8: seedlings of *Zea mays* L. treated with different concentrations of leaf extracts of *Acacia* and *Eucalyptus* (lane 1: control sample, lanes 2, 3, 7 and 8: samples treated with 1 and 3%, respectively). Lanes 4, 5, 6, 9 and 10: seedlings of *Phaseolus vulgaris* L. treated with different concentrations of leaf extracts of *Acacia* and *Eucalyptus* (lane 4: control sample, lanes 5, 6, 9 and 10: samples treated with 1 and 3%, respectively)

chromatography separation of the extract of *Washington filifera* fruits which revealed the presence of two inhibiting zones of germination and growth. The fast moving beta- inhibitor was further separated by TLC into four components, one of which appears to be abscisic acid (ABA) and the rest was phenolic substances^[13]. Also, Noguchi *et al.*^[33] identified ABA-Ge as an allelopathic substance from *Citrus junos*.

With respect to the total soluble protein and the nucleic acids contents, Table 3 showed that, there was a highly significant increase in the level of DNA and RNA in all treated samples of maize, while Kidney-bean treated samples showed a significant reduction. Maize samples treated with *Acacia* show a highly significant increase in the level of soluble proteins while samples treated with *Eucalyptus* show a highly significant decrease. However, there is a highly significant decrease in soluble protein contents in Kidney-bean samples treated with *Acacia* or *Eucalyptus*. These results were confirmed partially by Duhan *et al.*^[31], which revealed a drastic increase in the

level of nucleic acids and decrease in the level of soluble proteins in legume crops in response to *Acacia nilotica* extracts. He attributed this effect to the enzymes of synthetic pathways. In accordance with these results, Baziramakenga *et al.*^[54] reported that many phenolic acids reduced the incorporation of phosphorus into DNA and RNA in soybean. Present results are in controversy with that obtained by Padhy *et al.*^[46], who reported that chlorophyll synthesis in leaves as well as protein, carbohydrate and nucleic acid (DNA and RNA) contents in both shoots and roots of seedling were also decreased with increases in leachate concentration. Baziramakenga *et al.*^[54] concluded that phenolic acids reduced the incorporation of certain amino acid into proteins and thus reduce the rate of protein synthesis. Phenolic acids have been shown to be toxic to activities of many enzymes^[36,37]. Moreover, *Vicia faba* L. and *Zea mays* L. at 5% of *Eucalyptus* tended to accumulate amino acids, which may be considered an adaptive mechanism to increase stress tolerance, also there was the possibility of phenolic compound phytotoxicity for the two studied species^[45]. Increase of pre- soaking time of seeds in *Eucalyptus* leachates well as increase of leachates concentration decreased the respiration rate and catalase and alpha-amylase activities. Pandey *et al.*^[20] concluded that the inhibitors that leached out of the plants work through changes in macromolecules (protein, lipid and nucleic acids), resulting in root dysfunction and other inhibitory activities both in the roots and shoots. The degree of inhibition depend on their concentration. Recently, Singh *et al.*^[48] concluded that leachates of *Eucalyptus globulus* reduce nitrogen contents of *Vigna radiate*, *Vigna mungo*, *Arachis hypogaea*. The greatest inhibition was by 10% compared to 5 and 2.5%. Leachates of mature leaves were the most inhibitory, followed by those of senescent and juvenile leaves.

In general, abiotic or biotic stress induces stress proteins^[55,56]. The allelopathic effect of both *Acacia* and *Eucalyptus* also induced the formation of stress proteins (Fig. 1 and Table 4). High and mid molecular weight proteins 244, 187, 143, 51.8, 41, 40.6 and 29.7 kDa were newly synthesized in response to the allelopathic effect of *Acacia*. On the other hand, mid and low molecular weight polypeptides 121.8, 116, 62, 52.9, 46.8, 45 and 40.6 kDa were recorded in response to the allelopathic effect of *Eucalyptus*. In maize treated with both *Acacia* and *Eucalyptus*, the 55.4 kDa protein was disappeared, while the proteins 40.5, 30.5, 29.7 and 26.5 kDa were newly synthesized. Moreover, in maize seedlings treated with *Eucalyptus*, the proteins 50.8, 41, 30.5, 26.8 and 26.5 kDa were recorded. The number of *de novo* synthesized proteins (chaperones) in maize samples treated with both

Table 4: Molecular weight (M.wt.), band percentage and mobility rate (R_m) of the different types of protein bands of seedlings of *Zea mays* L. and *Phaseolus vulgaris* L. treated with different concentrations of leaf extracts of *Acacia nilotica* and *Eucalyptus rostrata*

Band % / Lanes 1-10											R_m	M wt. (kDa)
Band No.	1	2	3	4	5	6	7	8	9	10		
1	-	-	-	-	3.52	2.60	-	-	-	-	0.10	244
2	-	-	-	-	4.20	5.13	-	-	-	-	0.12	187
3	-	-	-	-	5.24	6.11	-	-	3.69	1.55	0.15	143
4	-	-	-	-	-	-	1.65	2.33	-	-	0.16	121.8
5	-	-	-	-	-	-	-	-	4.11	3.89	0.18	116
6	-	-	-	3.54	-	-	-	-	3.66	5.11	0.21	101.5
7	5.12	4.96	1.98	-	-	-	-	-	-	-	0.23	97
8	4.44	3.63	2.36	-	1.68	4.12	2.89	4.68	-	-	0.24	93.7
9	-	-	-	-	-	-	1.33	-	2.50	-	0.26	62
10	-	-	-	2.35	2.87	1.58	3.50	4.10	-	-	0.30	60.9
11	3.60	-	2.85	-	-	-	-	-	-	-	0.33	58
12	4.23	-	-	-	-	-	-	-	-	-	0.35	55.4
13	-	-	-	2.30	-	-	1.52	-	-	-	0.37	55
14	-	-	-	-	-	-	2.96	1.95	-	-	0.40	52.9
15	-	-	-	-	5.26	1.40	-	-	2.64	3.60	0.43	51.8
16	-	-	-	4.25	-	-	4.10	6.20	-	-	0.46	50.8
17	-	-	-	-	-	-	3.62	5.20	-	-	0.48	46.8
18	-	-	-	4.50	-	-	-	-	-	-	0.53	45.9
19	-	-	-	-	-	-	-	-	2.58	4.23	0.58	45
20	-	-	-	3.66	-	-	-	-	-	-	0.62	42.7
21	-	-	-	-	8.20	-	2.90	5.12	-	-	0.64	41
22	-	2.30	1.25	-	-	-	-	-	8.21	5.21	0.69	40.6
23	-	-	-	5.24	3.60	-	-	-	-	-	0.71	32
24	6.20	5.21	-	3.64	8.50	4.25	5.13	4.11	1.25	4.68	0.73	31.6
25	-	-	5.16	4.11	2.30	4.32	3.85	2.55	-	-	0.75	30.5
26	-	-	4.12	-	-	-	-	-	-	-	0.79	29.7
27	6.12	3.50	-	-	-	-	-	-	9.12	7.18	0.80	27.4
28	-	-	-	5.21	3.14	2.15	7.12	3.65	4.13	3.14	0.82	26.8
29	-	6.12	-	1.26	4.80	5.81	9.62	3.55	1.25	2.25	0.84	26.5
30	-	-	-	2.36	6.11	4.50	-	-	6.25	5.26	0.86	25.1
Total	6	6	6	12	13	11	13	11	12	11		

Table 5: The degree of cleavage characteristics of seedlings of *Zea mays* L. and *Phaseolus vulgaris* L. treated with different concentrations of leaf extracts of *Acacia nilotica* and *Eucalyptus rostrata*, using four restriction enzymes

Sample and degree of cleavage / Lanes 1-10										
Enzyme	1	2	3	4	5	6	7	8	9	10
BamHI	++	++++	+++	+	+	++	++	++++	+++	++++
EcoRI	++	++++	++++	+	+	+	+++	++++	+++	++++
HinfI	++	++++	++++	+	+	+	+++	++++	++	+++
SmaI	++	++++	+++	++	++	++	+++	++++	+++	+++

Lane 1: Seedlings of *Zea mays* L. as control samples.Lane 2: Seedlings of *Zea mays* L. treated with 1% leaf extract of *Acacia nilotica*.Lane 3: Seedlings of *Zea mays* L. treated with 3% leaf extract of *Acacia nilotica*.Lane 4: Seedlings of *Phaseolus vulgaris* L. as control samples.Lane 5: Seedlings of *Phaseolus vulgaris* L. treated with 1% leaf extract of *Acacia nilotica*.Lane 6: Seedlings of *Phaseolus vulgaris* L. treated with 3% leaf extract of *Acacia nilotica*.Lane 7: Seedlings of *Zea mays* L. treated with 1% leaf extract of *Eucalyptus rostrata*.Lane 8: Seedlings of *Zea mays* L. treated with 3% leaf extract of *Eucalyptus rostrata*.Lane 9: Seedlings of *Phaseolus vulgaris* L. treated with 1% leaf extract of *Eucalyptus rostrata*.Lane 10: Seedlings of *Phaseolus vulgaris* L. treated with 3% leaf extract of *Eucalyptus rostrata*.

+ weak cleavage, ++ moderate cleavage, +++ strong cleavage, ++++ very strong cleavage

Acacia and *Eucalyptus* (12-stress induced proteins: 121.8, 60.9, 55, 52.9, 50.8, 46.8, 41, 50.6, 30.5, 29.7, 26.8 and 26.5 kDa) were more than that induced in kidney-bean (10-stress induced proteins, 244, 187, 143, 116, 93.7, 51.8, 45, 41, 40.6 and 27.4 kDa). The over-expression of chaperones correlates positively with the allelochemicals concentration. These proteins are responsible for folding, assembly, translocation and degradation in a broad array

of normal cellular processes such as improvement of plant growth, physiological and molecular characteristics^[57]. This may be one of the mechanisms of high tolerance in maize (monocot) as compared to kidney-bean (dicot).

The restriction site mutation assay is a genotypic mutation system capable of detecting DNA mutations induced in restriction enzyme sites in target DNA regions. When the total DNA was digested with restriction

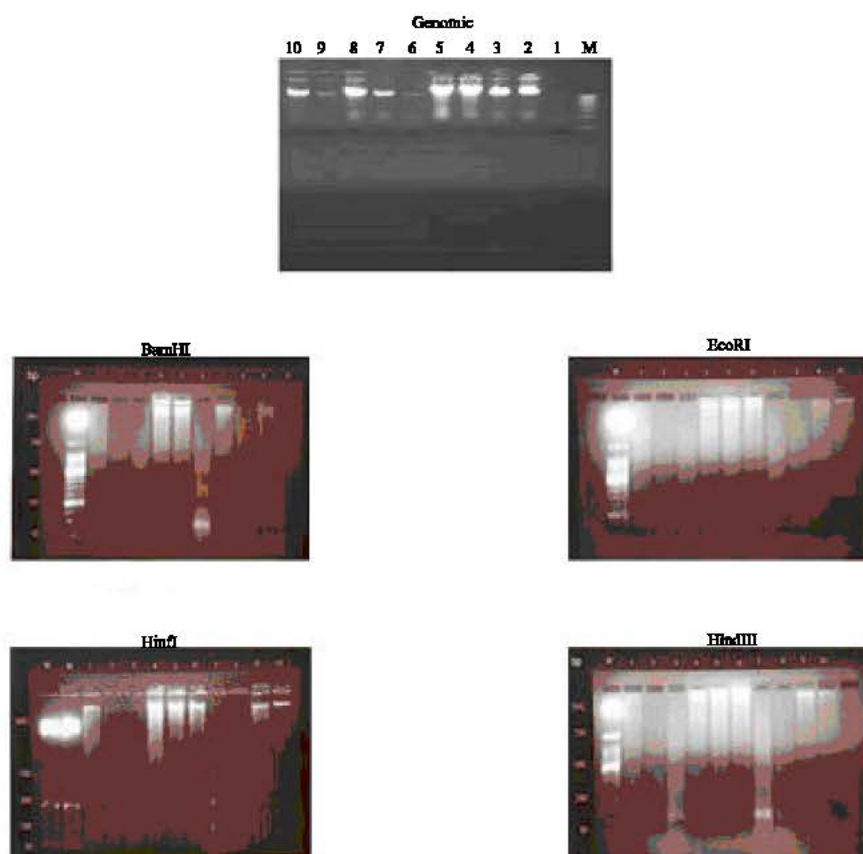


Fig. 2: Genomic DNA and restriction enzyme patterns in seedlings of *Zea mays* L. and *Phaseolus vulgaris* L. treated with different concentrations of leaf extracts of *Acacia nilotica* and *Eucalyptus rostrata* using four enzymes (*Bam*HI, *Eco*RI, *Hin*fI and *Sma*I). Lane M: DNA molecular size marker. Lanes 1-10: seedlings of *Zea mays* L. *Phaseolus vulgaris* L. treated with different concentrations of leaf extracts of *Acacia nilotica* and *Eucalyptus rostrata*. Lanes 1, 2, 3, 7 and 8: seedlings of *Zea mays* L. treated with different concentrations of leaf extracts of *Acacia* and *Eucalyptus* (lane 1: control sample, lanes 2, 3, 7 and 8: samples treated with 1 and 3%, respectively). Lanes 4, 5, 6, 9 and 10: seedlings of *Phaseolus vulgaris* L. treated with different concentrations of leaf extracts of *Acacia* and *Eucalyptus* (lane 4: control sample, lanes 5, 6, 9 and 10: samples treated with 1 and 3%, respectively)

enzymes and electrophoresed, there emerged a complex and variable multi-band pattern, characteristic of DNA fingerprints in general. The potential of the restriction endonuclease analysis to identify the genomic differentiations of DNA patterns for maize and kidney-bean in response to allelochemicals are shown in Fig. 2. The genomic DNA of each treatment was subjected to different restriction enzymes. These enzymes are *Bam*HI, *Eco*RI, *Hin*fI and *Sma*I. The resulting cleavage fragments had apparent low molecular sizes and appear as smears of digests whatever the concentrations of templates of DNA or restriction enzymes and the time of electrophoretic run. The resulting smears represent DNA fragments of low molar ratios which were not enough for any of these fragments to appear as discrete bands^[38]. The highest

degree cleavage was observed *Eco*RI. All studied samples showed different DNA fingerprints. All samples had on average 20-30% of their bands in common. The variability was higher in the bands in the region of the gel above 2.7 kb than in the bands below it. On the average, 50 ± 5 bands were seen above 500 bp, ranging from 700 to 2400 bp. This phenomenon appears to reflect a higher abundance and a lower intrinsic variability of low molecular weight bands. A clear information data was obtained using *Hin*fI (5-base cutter enzyme) as compared to other enzymes (Table 5) which may indicate the presence of stimulators or inhibitors which facilitate the recognition of the nucleotide sequence or induce new ones, which are specific for each enzyme and the cutting site inside it. This result is in agreement with the

hypothesis of Seed *et al.*^[59], who proposed that restriction enzymes recognize 5-base sequences produce a more random collection of fragments than enzymes that recognize 6-base sequences. From the above results, it can be concluded that the tested extracts may interact with the basic targets such as DNA related processes, protein biosynthesis and membrane stability. These results are in harmony with those of Wink *et al.*^[60], who deduced that, 19 natural products were found in *Acacia* and *Eucalyptus* with allelopathic properties. Most compounds affect more than one molecular target: 8 compounds interact with DNA and affect the recognition sites of restriction enzymes, 10 inhibit DNA polymerase I, reverse transcriptase and protein biosynthesis and lead to membrane leakage. Our results suggest that the percentage of shared bands in DNA fingerprints of maize and kidney-bean might be used as a measure of genetic difference and demonstrate that the restriction site mutation assay can detect mutations.

A large number of studies has confirmed that both *Eucalyptus* and *Acacia* leachates contained phenolic compounds so there is no need to analysis of leachates, hence the light was overviewed the none studied points; the biochemical and molecular aspects of allelopathy. However, chromatographic separation showed that the majority of the compound present in leaf extracts of *Eucalyptus globulus* Labill were phenolic^[14]. The reduction in the germination of *Vicia faba* L. and *Zea mays* L. at 5% level of *Eucalyptus rostrata* water leaf extract showed the possibility of Phenolic compound phytotoxicity for the two studied species^[45]. Phenolic acids reduced the incorporation of certain amino acid into proteins^[57]. Paper and gas chromatography indicated that the allelopathic phenolic compounds in *Eucalyptus* spp. (8-year-old) were caffeic, coumaric, gallic, gentisic, catechol, hydroxybenzoic syringic and vanillic acid. The inhibitory effect of leaf leachates of *Eucalyptus* hybrid was concentration dependent and thought to be caused by glycosides and polyphenols conjugation, which are present in relatively high concentration in the leachates^[61]. The fresh mulches of *Eucalyptus grandis* contained hydroxylated aromatic compounds that could have caused the allelopathic effect^[44].

The inhibitory compounds isolated from the ether fraction of aqueous extracts of *Acacia* were identified as ferulic, vanillic, caffeic, gallic, m-hydroxybenzoic and m-hydroxyphenylacetic acids. Unidentified flavonoides were also found. Chromatographic bioassay of compounds isolated from both ethyl acetate and water fractions of methanolic extracts of *Acacia* leaves also showed significant phytotoxicity but none was found in the chloroform and hexane fractions, suggesting that the

phytotoxic compounds present in *Acacia* plants are water soluble^[62]. Some plants have allelopathic potential by releasing allelochemicals to their surrounding that have either deleterious or beneficial effects on other plants. These compounds inhibited plant growth by affecting many physiological process among them, the effect on ion uptake and hydraulic conductivity (i.e. water uptake) are particularly important since the root is the first organ to come into contact with the allelochemicals in the rhizosphere^[6]. The degree of inhibition depends on their concentration.

Several modes of action-attributed to allelopathic compounds-include effecting on cell elongation and ultrastructure of roots by inhibition of cell division and accumulation of lipid globules in the cytoplasm of root cells^[8]. Another mode of action is the allelopathic effect on membrane permeability which causes an alteration in water and ion permeability of the cytoplasmic membrane or in the case of Aescin, a triterpeneglycoside, induced leakage of ribonucleotide material, nucleosides and pentose from the species *Ophiobolus graminis*. Allelochemicals can damage cell membranes through interaction with a constituent of the membrane or as a result of an impairment of some metabolic function necessary to the maintenance of membrane function^[8]. Recent studies suggest that environmental stress can increase the oxygen –induced damage to cells due to increased generation of active species (AOS). AOS brings about peroxidation of membrane lipids, which leads to membrane damage^[63]. Yet another mode of action which can occur is the interference in protein synthesis and changes in lipid and organic acid metabolism. Cinnamic acid was found to interfere in the mechanism of protein synthesis while ferulic acid diverted acetate into lipid synthesis instead of Kreb's cycle and other pathways leading to amino acid and protein synthesis. Other modes of action include disruption of mineral transport, inhibition of enzymatic activity, inhibition of germination, inhibition of seedling growth, mutations and disruption of nutrient uptake are all considered viable means of allelopathic interference^[8]. The greater inhibition of transpiration is attributed to both the damage root function in absorbing water and nutrient, and stomatal closure. It is suggested that allelopathic agents might inhibit photosynthesis by inducing peroxidation^[40]. Several studies report on the mode of action of allelochemicals, phytotoxic substances may act in many biological processes, such as to suppress the mineral uptake by plants, inhibit cell elongation and cell division, as well as retard the photosynthesis, respiration and enzymatic activities, resulting in the retardation of plant growth. They may also interfere with the action of growth

promoting substance, e.g., gibberellin or auxin^[19].

Present results indicate that *Eucalyptus* is more effective in affecting physiological, biochemical and molecular criteria of maize and kidney-bean and that the monocot plants (maize) is more tolerant than the dicot one (kidney-bean). These results were confirmed by Reigosa *et al.*^[2] who found that *Eucalyptus* has the most allelopathic effect and the most the large number of allelochemical (allelopathin) than *Acacia* spp on different crop and woody plants. We can suggest that the allelopathic chemicals of both *Acacia* and *Eucalyptus* may have the potential as either herbicides or templates for new herbicide classes. This hypothesis is supported by Seigler^[10] and Duke *et al.*^[11].

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