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Allelopathic Effects of*Eucalyptus Saligna* on Germination Growth and Development of *Vigna Unguiculata* L.Walp

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Abstract: *Allelopathic effects of Eucalyptus are widely reported and considered the major factor limiting the establishment of native species in eucalyptus forest. Allelopathy is used here to refer to the inhibitory and stimulatory effects of one plant species upon another by the compounds arising from the products of their metabolism. Improved germplasm of Eucalyptus developed through traditional breeding, tissue culture technique and other biotechnology researches are becoming an attractive alternative to other hardwood tree species for wood, pulp and paper, and bioenergy applications in many countries. In Eucalyptus plantations established for research and commercial end use, allelopathy has been raised as a concern for possible negative effects on crops grown in local areas, especially in agroforestry systems. In this study Eucalyptus saligna is used. E. saligna is thought to possess phenolic acids released from the leaves, bark and roots that have deleterious effects on other plant species. Cowpea (Vigna unguiculata L.Walp), an annual legume which originated in Africa is widely grown for grain crop, animal fodder and as a vegetable. The main objective of the study was to determine the allelopathic effects of Eucalyptus saligna on the germination, growth and development of Vigna unguiculata L. Walp. The study was conducted at Maseno University, Kenya, to investigate specifically the allelopathic effects of E. saligna on the percentage germination of seeds, rate of germination, growth of different parts of the plant species and synthesis of chlorophyll of seedlings of cowpea (Vigna unguiculata). Cowpeas seeds were subjected to four different concentrations of the fresh shoot aqueous extracts which comprised of 0% (distilled water), 25%, 50%, 75%, and 100%. Ten seeds of cowpeas were germinated in sterilized petri dishes lined with a layer of filter paper Whatman no. 1, moistened with 10ml of each of the 0%, 25%, 50%, 75% and 100% extract aqueous concentration,respectively. Germination percentage were determined at the end of the study. Individual cowpeas seedlings were planted in 4.5 litres plastic pots containing soil and irrigated with different amount of eucalyptus fresh shoot litter extracts (25g, 50g, 75g, 100g) and 500ml of distilled water. The treatments were replicated five times and the pots laid out in a completely randomized design in the glasshouse. Data on shoot height and number of leaves per plant was recorded on weekly basis. Leaf area, shoot and root dry weights and leaf total chlorophyll, chlorophyll a and b concentration were determined at the end of the experiment. Data collected was subjected to analysis of variance (ANOVA) using SAS statistical package. Treatment means were compared using the least significant difference (LSD) at ($P \leq 0.05$). The results revealed that the shoot litter of E. saligna has both inhibitory and stimulatory effects on Vigna unguiculataat differentstages of plant growth and development. Seed germination decreased with increasing concentration of shoot extracts from 25%, 50%, and 75% to 100% even though there were no significance differences among treatments. Shoot heights, leaf number, leaf area, shoot and root dry weights, total chlorophyll increased significantly with increasing fresh shootextract aqueous concentration. This research data can be used to inform agriculturist, farmers, policy makers and advisers on the consequences of E. saligna in the agroforestry programmes. Further, the results can be used at an advisory level to inform efficient and effective use in the agroforestry systems to improve crop bioproductivity for increasing population in the world.*

Keywords*: Allelopathy, Development, Eucalyptus saligna,Germination, Growth and Vigna unguiculata*

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I. Introduction

Allelopathy is a biological phenomenon by which an organism produces one or more biochemical substances that influences the germination, growth, survival, and reproduction of other organisms. This biochemical substances can have promotory effects (positive allelopathy) or detrimental (negative allelopathy) effects on the targeted organism and the community (Poore and Fries, 1985)[1].The biochemical substances are released by plant leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems (Kruse *et al.,* 2000)[2]. Studies have shown the allelopathic effects of different parts of the plant species affect different plant organsin different ways in different plant species.The effects can vary in the intensity, duration and developmental stage during plant development. Here, allelopathic effect refer to the inhibitory and stimulatory effects of one plant species upon another by the compounds arising from the products of their metabolism. For example, *Leucaena leucocephala*, the miracle tree promoted for revegetation, soil and water conservation and animal improvements in India, possess a toxic, non-protein amino acid in leaves and foliage that inhibits the growth of other trees but notits own seedlings [1]. Also, studies show that *Leucaena* species reduce the yield of wheat but increase the yield of rice. Leachates of the chaste tree or boxelder can retard the growth of pangola grass but stimulate growth of bluestem, another pasture grass. Foliar and leaf litter leachates of *Eucalyptus* species, for example, are more toxic than bark leachates to some food crops.*Eucalyptus* species are said to reduce both the diversity and abundance of plantation of Forbes and graminoids, the productivity of adjoining crops through release of allelochemicals[1]. Debris from the pruning and litter fall reduces agricultural yields, whereas the leaves are unpalatable and possible allelopathic to pasture growth. Allelochemical concentrations in the producer plant may also vary over time and in the plant tissue produced.

The basic model applied in allelopathic research for agricultural crops has been to screen both crop plants and natural vegetation for their capacity to suppress weeds. In the laboratory, plant extract and leachates are usually screened for their effects on seed germination and seedling phases. This is followed with the isolation and identification of the active allelochemical compounds from greenhouse tests and field soil to confirm the observed data of laboratory experiments. The interaction of plants through chemical signals ‘allelopathy’ has many agricultural and ecological application (Nelson, 1996)[3]. Furthermore, allelochemistry may provide baseline indicator structures or templates for developing new synthetic herbicides. Incorporation of allelopathic traits from wild or cultivated plants into crop plants through traditional breeding or genetic engineering methods could also enhance the biosynthesis and release of allelochemicals.

Several studies (Alexander, 1989[4]; Kohli *et al.*, 1988[5]; Moore, 1997[6]; Paulino *et al.*, 1987[7]) reveal that large area of the ground surface beneath the *Eucalyptus spp* remain completely bare and ground vegetation is very limited in extend, tend to be concentrated in wetter hollows. Evidences were obtained about the progressive degradation of the soil under *Eucalyptus* through the increase of the cation exchange capacity(CEC) and decrease in both pH and base saturation [4].The allelochemicals released include phenols, terpenoids and alkaloids and there derivative. They may inhibit shoot and root growth, nutrient uptake or may attack the naturally occurring symbiotic relationship thereby destroying the plant usable source of nutrient. Detrimental effects of allelochemicals from *Eucalyptus* species on plant germination and growth have been reported (Nandal, 1994[8]; Bogatek *et al.,* 2006[9]).

Allelopathic effects of *E.saligna* have always been considered as one of the main factors for poor performance of associated companion crops (Del Moral and Mullar, 1970) [10] besides other limiting factors such as moisture, nutrient and light stress. Commonly cited effects of allelopathy include reduced seed germination and seedling growth. Like commonly cited effects of allelopathy include reduced seed germination and seedling growth. Like synthetic herbicides, there is no common mode of action or physiological target site for all allelochemicals. However, known sites of action for some allelochemicals include cell division, pollen germination, nutrient uptake, photosynthesis, and specific enzyme function.

Allelopathic inhibition is complex and can involve the interaction of different classes of chemicals like phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates, and amino acids, with mixtures of different compounds sometimes having a greater allelopathic effect than individual compounds alone. Furthermore, physiological and environmental stresses, pests and diseases, solar radiation, herbicides, and less than optimal nutrient, moisture, and temperature levels can also affect or modify allelopathic activities. Different plant parts, including flowers, leaves, leaf litter and leaf mulch, stems, bark, roots, soil and soil leachates and their derived compounds, can have allelopathy activity that varies over a growing season. Allelopathic chemicals can also persist in soil, affecting both neighboring plants as well as those foliar and leaf litter leachates of *Eucalyptus* species, for example, are more toxic than bark leachates to some food crops [10]

The present species of study, the *E. saligna* and *Vigna unguiculata* L.Walp have characteristic chemical compounds of significances. The leaf essential oil of *E.saligna* is dominated by a-pinene (24.40%) and 1, 8-cineole (24.26%). Due to this property the essential oils are promising insecticides and repellents to be used against insect pests of stored food grains. *E.saligna* contain phenolic acids released from the leaves, bark and roots that have deleterious effects on other plant species (Sasikumar *et al*., 2002) [11]. These compounds might be responsible for *Eucalyptus saligna*inhibiting growth and development of many agricultural crops leading to low yield production by modifying the environment of plant and other plants growing in their vicinity.

Vigna unguiculata L.Walp has a relatively low cost and high quality source of protein and is between 19.5% and 27.3% w/w;the amino acid score and protein digestibility for the whole bean was determined at 0.44 and 86.7%, respectively (Frota, Lopes, SilvaandArêas, 2017) [12]. For the isolate, these values were 0.60 and 96.7%, respectively[12]. Correcting each score by the digestibility resulted in an estimated nutritive value of 38% and 58% for the whole bean and the isolate, respectively. Therefore, by having higher digestibility and increasing the bioavailability of essential amino acids, cowpea protein isolate is of interest for inclusion in food products, especially cereal-based products, which contain lysine as a limiting amino acid and are rich in methionine. Its nutritional value is generally reduced by inhibitors, lectins, tannins, and polyphenols. The carbohydrates contents of cowpea seeds have been published much less than those of proteins, which is more common for plant seeds.

Though the allelopathic effects of *Eucalyptus* species have greatly been investigated (Alves *et al*, 1999[13]; Willis, 2010[14]; Ferreira and Aquila, 2000[15], Ferreira *et al*, 2007[16]; Yamagushi *et al*, 2011[17]), no further information and research has been made on the effects of *E.saligna* on the germination, growth and development of cowpea (*Vigna unguiculata* L. Walp). Thus, thisresearch gap on the *Eucalyptus* information on the crops, especially on the *Vigna unguiculata* L. Walp, is scanty. More information is therefore required to clearly understand the allelopathic effects of *E.saligna.* Hence, the present study is meant to determine allelopathic effects of *E.saligna* which is a type of common woody tree widely grown in various of Kenya and how it affect common crops. This information would be important in the selection of better tree variety to practice agroforestry by most farmers.

The general objective was to determine the allelopathic effects of concentrations of an aqueous extract of *Eucalyptus saligna*shoot on germination, growth and development of *Vigna unguiculata* L. Walp. The specific objectives were as follows:

i) To determine the allelopathic potential of *E.saligna* on percentage germination of *Vigna unguiculata* L.Walp.
ii) To determine the allelopathic effect of *Eucalyptus saligna* on rate of growth of *Vigna unguiculata* L.Walp.
iii) To determine the allelopathic effect of *E.saligna* on change in fresh weight and dry weight of *Vigna unguiculata* L.Walp.
iv) To determine the allelopathic effects of *E.saligna* on the concentration of chlorophyll of *Vignaunguiculata* L.Walp

II. Material And Method

2.1 Description of the area of study

The study was carried out at Maseno University Botany Laboratory and in the glasshouse in the University Botanic Garden, Maseno University, Kenya.

2.2 Collection and screening of plant germplasm

Collection of *Eucalyptus saligna* was collected from Maseno University Amphitheater. *Vigna unguiculata* L.Walp was obtained from Kenya Forestry Research Institute,Muguga, Nairobi, Kenya. The seeds were thoroughly washed with distilled water and surface sterilized in 90% ethanol for 2 minutes, and was followed by soaking for 15 minutes in a solution of 5% sodium hypochlorite, then rinsed four times with distilled water, a method of EL-Darier (2002) [18].

2.3 Preparation of Fresh Shoot aqueous Extract of *E.saligna*

2.3.1 Fresh shoot extract in distilled water

One hundred and eight (108) grams of fresh shoot of *E.saligna* was harvested at vegetative stage and cut into small pieces of about 4cm length. The small pieces was finely grounded with pestle and mortar, and then soaked in 0.5litres of distilled water in larger beaker for 24 hours at room temperature. The collected extracts was filtered through cheese cloth to remove debris and finally filtered using Whatman No. 1 filter papers to have 100% concentration. Aqueous extracts of 25%, 50%, 75% concentration was made by diluting the original extract with distilled waterexcept in 100% extract solution. The four extract levels besides the control (distilled water) was chosen to undergo the first experiment.

2.3.2 Fresh shoot extract in ethanol and water

One hundred and eight (108) grams of fresh shoots of *E.saligna* was harvested at vegetative stage and cut into small pieces of about 4cm length. The small pieces were finely grounded with pestle and mortar, and then soaked in 0.5litres containing 5% ethanol and 95% water in larger beaker for 24hrs.The collected extracts was filtered through cheese cloth to remove debris and finally filtered using Whatman No. 1 filter papers to have 100% concentration. Aqueous extracts of 25%, 50%, 75% and 100% concentration was made by diluting the original extract with distilled water.

2.3.3 Using ethanol

One hundred and eight (108) grams of fresh shoots of *E.saligna* was harvested at vegetative stage and cut into small pieces of about 4cm length. The small pieces were finely grounded with pestle and mortar, and then soaked in 0.5liters containing 100% ethanol in larger beaker for 24hrs.The collected extracts was filtered through cheese cloth to remove debris and finally filtered using Whatman No. 1 filter papers to have 100% concentration.

2.4 Germination Experiment
Germination tests was carried out in a Botany Laboratory in department of Botany of Maseno University at room temperature. The treated seeds were placed in dried petri dishes lined with two layers of Whatman No. 1 filter papers and moistened with 10ml of the respective aqueous extracts (treatment) and distilled water (control). The treatments were replicated three times, the arrangement was randomized, 10ml of each level of *Eucalyptus saligna* extract was added daily to the replicate and data on seeds germinating each day was recorded and germination percentage calculated at the end of the experiment.

2.5 Growth Experiment
Growth tests was carried out in a glasshouse in the University Botanic Garden, Maseno University, Kenya. Four and half (4.5) litter plastic pots with were filled with 2kg of humus soils collected from Botanic garden which was solarized (sun sterilized) for at least two days to prevent fungal growth. The pots were perforated at the bottom to avoid water logging. 10 seeds of *Vigna unguiculata* L.Walp were sown in each of the pots containing soil with different amount of *Eucalyptus* fresh shoot-litter (25g, 50g, 75g, and 100g).Control experiment was set using 100% distilled water treatment. The pots were laid in complete randomized design. Corresponding 350ml of *E. saligna* shoot aqueous extract of 25%, 50%, 75% and 100% concentration was used to water labelled pots 25%, 50%, 75% and 100% concentration every morning to the end of four weeks, respectively. After two weeks, the seedlings in each pot were thinned down to 5 plants per pot. The treatments were replicated four times. Data was collected at the end of experimental period.

2.6 Measurement of Parameters
2.6.1 Germination percentage
The number of seeds germinating every day after treatment was counted and the total used to calculate the final germination percentage in each treatment.
2.6.2 Determination of shoot height
Shoot height was measured from the soil level to the upper point of the terminal bud of the seedling using a meter rule, every week up to the end of the experiment.
2.6.3 Determination of leaf number
Seedlings number of fully expanded mature leaves per plant was counted and recorded on each plant every week up to the end of the experiment.
2.6.4 Determination of leaf area
Leaf length and leaf width used to calculate leaf area were measured using a meter rule. Leaf area was then calculated using the formula of Otusanya et al (2007) [19], as shown below:
A= 0.5(L X W) where, A= leaf area, L= Length of leaf and W= Maximum width.
2.6.4 Determination of root and shoot fresh and dry weights
At the end of the experiment, the plants was carefully uprooted from the soil, cleared off the attached soil, separated into root and shoot and then measured separately using an electronic weighing balance. Fresh plants (roots and shoots) were packaged separately in envelopes and dried to constant weight at 80°C in an oven. Root and shoot dry weights was determined on an electronic weighing balance, and then mean weights calculated.
2.6.5 Determination of leaf chlorophyll concentration
Determination of chlorophyll concentration the formula of Arnon (1949) [20] was used. The fourth fully expanded leaf from shoot apex was sampled from all the treatments. 0.5g of these leaves were grounded in 20ml of 80% (v/v) acetone using mortar and pestle. The resulting substrate was read using UV- Visible Spectrophotometer and calculation done using formula;
$= (20.2(\mathrm{D}645) * 8.2(\mathrm{D}663) * \mathrm{V}/(1000 * \mathrm{W})$:
where: D- is the optical density reading of the chlorophyll extract at the specific indicated wavelength; V- is the volume of acetone chlorophyll extract; andW- is the fresh weight in grams of the tissue extract.
Data obtained from the study was subjected to analysis of variance (ANOVA) in SAS statistical package. Treatment means was separated and compared using Least Significance Difference (LSD at 0.05).

III. Results

3.1 Effect of fresh aqueous extracts of *E.saligna* on germination rate of cowpeas
The study examined the allelopathic effects of *Eucalyptus saligna*shoot aqueous extracts on germination and growth of *Vigna unguiculata*L. Walp. Table 1, shows the effects of *Eucalyptus saligna*shoot aqueous extract on germination and seedling growth of *Vigna unguiculata*L. Walp. The germination percentage of cowpea seeds

decreased with increasing concentration of shoot aqueous extracts of *Eucalyptus saligna* (Table 1). However, there were no significant differences ($p<0.05$) in germination percentages among the treatments except in ethanol extract. As shown in Table 1, among various aqueous extracts, higher germination percentage and germination rate were observed in aqueous extract of water only and water and ethanol, while lower germination percentage wasrecorded in ethanol extract.

Table 1. Effects of fresh shoot aqueous extracts (water, ethanol and water, Ethanol) of *E.saligna* on germination of cowpeas**.**

Concentration (%)	Water Extract	Water and Ethanol extract	Ethanol Extract
0	71.33a	67.33a	59.33a
25	69.33a	64.67a	55.33ab
50	68.00a	64.00a	50.67ab
75	67.33a	58.67a	48.00ab
100	67.33a	42.67a	36.00a
LSD	19.76	33.70	20.47

Means followed by the same letters within each column are not significantly different according to LSD ($p < 0.05$). (0) control, 25, 50, 75 and 100 denote the concentration of shoot aqueous extract of *E.saligna.*

3.2 Effects of *E.saligna* fresh shoot litter on shoot heights and leaf number of *Vigna unguiculata*L. Walp seedlings.

Shoot heights increased significantly ($P\leq0.05$) with increasing shoot litter of *E.saligna* in all the treatments (Table 2). Number of leaves increased significantly ($P\leq0.05$) with increasing concentration of the shoot litter (Table 2). There was increase in number of leaves at the highest concentration of shoot litter (100g).

Table2. Effects of fresh shoot litter of *E.saligna* on shoot heights and number of leaves of cowpeas.

Concentration g	Leaf number	Shoot length
0	9.09b	4.62b
25	11.89a	6.13a
50	10.49ab	5.38ab
75	11.33a	4.63b
100	11.70a	5.56a
LSD	1.859	0.763

Means followed by the same letters within each column are not significantly different according to LSD ($p \leq 0.05$). (0) control, 25, 50, 75 and 100 denote the concentration of extract of *E. saligna.*

3.3 Effects of shoot litter on dry weight and fresh weight of shoot and root, chlorophyll concentration of *Vigna unguiculata*L. Walp

Leaf area significantly ($P\leq0.05$) increased with increasing fresh shoot litter *E.saligna* with 100g showing the highest growth effect (Table 3). There was significant ($P\leq0.05$) difference in dry weight of shoots and roots among treatments (Table 3). The highest fresh and dry weight measurements were obtained from roots of plants supplied with 100g of fresh shoot litters of *E.saligna* treatment. Total leaf chlorophyll concentration increased by treatment with fresh shoot litter of *E.saligna* (Table 3).There was significant ($P\leq0.05$) differences between seedlings treated with fresh shoot litter and control seedlings.

Table 3: Effects of fresh shoot litter of *E.saligna* on growth and chlorophyll concentration

Concentration g	F.W Shoot	D.W Shoot	F.W Root	D.W Root	Root length	Leaf Area	Chlorophyll concentration
0	1.45a	0.18a	0.26a	0.01b	8.10a	11.12bc	7.82a
25	2.13ab	0.25ab	0.53a	0.04b	11.22a	18.73ab	7.84a
50	1.18b	0.18b	0.53a	0.01b	13.12a	8.89c	8.29a
75	1.60b	0.18b	0.55a	0.02b	18.65a	17.28ab	7.35a
100	2.78a	0.32a	0.55a	0.10a	18.65a	23.93a	8.33a
LSD	1.011	2.131	0.307	0.035	1.076	7.963	1.637

Means followed by the same letters within each column are not significantly different according to LSD ($P\leq 0.05$). (0) control, 25, 50, 75 and 100 denote the concentration of extract of *E.saligna.*

IV. Discussion

Allelochemicals and released by certain plants (through leaching, volatilization, exudation or decomposing of remains) are one of the important components of the stresses that may influence seed germination, plant growth and nutrient uptake of the associated species (Bhowmik and Doll, 1984) [21]. Plants may favorably or adversely affect other plants through allelochemicals, which may be released directly or indirectly from live, produced by dead plants or organic residues. Therefore this study examined the inhibitory and stimulatory nature of interference of aqueous leaf extract of *Eucalyptus saligna* on *Vigna unguiculata*L. Walp. The results of this study indicated that the allelopathic effects of *Eucalyptus saligna* on *Vigna unguiculata*L. Walpare both inhibitory and stimulatory. The fresh shoot aqueous extract of *Eucalyptu*s *saligna*inhibited the germination of *Vigna unguiculata*L. Walpseeds. Inhibition of seed germination and seedling growth of some herbaceous plants by leaf extracts of eucalyptushave been reported (Siddiqui *et al.*, 2009) [22].

Several workers have reported on the allelopathic potential of common weeds on germination, seedling growth and yield of several crop species (Inderjit and Dakshini, 1998[23]; Singh *et al.*, 2003[24]; Kong *et al.*, 2007[25]; Ilory*et al.*, 2011[26]; Otusanya, 2014[27]). Chivinge (1985) [28] and Hagin (1989) [29] found that soil extracts or weed residues and decompose of organism possess allelopathic potential to increase the growth and yield of some crops. Similarly, allelopathic water extract applications at lower concentrations have been observed to stimulate germination and growth of different crops (Anwar *etal.*, 2003[30]; Cheema *et al.*, 2012[31]). Chon *et al.*, (2003a [32], b [33]) reported that aqueous extracts of some plants from Asteraceae family at low concentration increased the root length of alfalfa up to 13-33%. El-EL-Rokiek *et al* (2006) [34] showed that application of the rice straw aqueous extract at lower and moderate concentration significantly stimulated the shoot elongation and shoot biomass of soybean plants at both vegetative and flowering stages of growth. Rice hull extract had stimulatory effect on stem length of *Silybum marianum* and also enhanced the dry weight of *Echinochloa crusgalli* (Seyyednijad *et al.*, 2010) [35].

Abdalla (2013) [36]reported that fertilization of rocket plants (*Eruca vesicaria*) with 2% leaf and 3% twig aqueous extracts of *Moringa oleifera* potentially increased the plant height, fresh and dry herb weight, photosynthetic rates, stomatal conductance, chlorophyll a and b, carotenoids, total sugars, total protein, phenols, ascorbic acid, nitrogen, phosphorus, potassium, calcium, magnessium, iron as well as growth promoting hormones (auxins, gibberellins and cytokinins)*Tithonia diversifolia* (Hemsl) A. Gray and *Chromolaena odorata* (L.) King and Robinson, both in the family Asteraceae are highly invasive environmental weeds due to their aggressive growth rate and heavy seed production (Muoghalu and Chuba, 2005[37]; Zachariades *et al.*, 2009[38]). These weeds are most prominent in the rain forest region of Nigeria, infesting severely the natural habitats and plantation crops. The release of the allelochemicals in these weeds into the environment during the rainy season has been reported to influence both physiological and biochemical characteristics of crops. For example, Akobundu (1987) [39] observed that the growth of other plants was hampered in the areas where *C.odorata* grows. Tijani and Fawusi (1989) [40] reported on the allelopathic activities of crude methanol extract of *C. odorata* on seed germination and seedling growth of tomato. Eze and Gill (1992) [41] stated that *C. odorata* contains a large amount of allelochemicals especially in the leaves, which inhibit the growth of many plants in nurseries and in plantations. Rafiqul-Hoque *et al*, (2003) [42] found that different concentrations of *C. odorata* leaf aqueous extract significantly inhibited the germination, root and shoot elongation and development of lateral roots of *Cicer arietinum, Brassica juncea, Cucumissativus, Phaseolus mungo, Raphanus sativus* and *Vigna unguiculata*L. Walp. These investigators [39] [40] [41] [42] emphasized that the inhibitory effect was proportional to the concentration of the extracts, that is, higher concentration had the stronger inhibition whereas in some cases, the lower concentration showed stimulatory effect. Adetayo *et al.* (2005) [43] observed 14% and 8% reduction in seed germination of cowpea and soybean treated with the water extract of *C. odorata* leaves and stems, respectively.

Sarmin (2014)[44] studied the interaction between *Moringa oleifera* and *Triticum aestivum* using five different concentrations (0% i.e. fresh water, 25%, 50%, 75% and 100%) for each of Moringa leaf, bark and root extracts to examine the effects on germination and growth of *Triticumaestivum*. Results showed that germination percent and plumule length reduced compared to control. Further, the bark extract concentration of 25% produced the longest hypocotyls (58mm) and flag leaf (17.28cm); and 50% produced longest (60.75cm) plant whereas highest soil plant analytical development (SPAD) value (60) was recorded in 50% concentration of leaf extract applied plant. The tiller number of *T. aestivum* was positively affected by 50%, 100% leaf and 50% bark extracts. Soil moisture percent was increased with extract application. Longest (11.08cm) and shortest (9.5 cm) spike was recorded on 75% bark and 25% root extract application [44]. All concentrations of leaf and root extract showed negative result for the root and shoot weight of the *T. aestivum* species. Maximum and minimum *Triticum aestivum* grain weight was recorded at 50% *Moringa* bark and 50% *Moringa* leaf extract, respectively.

Leather (1983) [45] conducted out laboratory, greenhouse, and field studies to determine the allelopathic potential of *Helianthus annuus* L. cultivars to suppress weed germination and growth. Germination of wild mustard [*Brassica kaber* (DC.) L.C. Wheeler var. *pinnatifida* (Stokes) L.C. Wheeler] seeds at 25 C in undiluted aqueous extracts of sunflower leaf tissue was inhibited 75%, but was stimulated by up to 150% at 10- and 100-fold dilutions. Stem-tissue extracts at all concentrations stimulated wild mustard seed germination [45]. Further, he indicates that germination response of other weed species varied with the sunflower cultivar and concentration of tissue extract. Furthermore, in sand culture, leachates of dried sunflower leaf and stem tissue inhibited broadleaf-weed seedling growth, but had little or no effect on the growth of grass weeds. Finally, sunflower root exudates inhibited seedling growth, but were less effective than leaf and stem tissue leachates. Germination of weed seeds was unaffected by root exudates.

According to Otusanya *et al.* (2008) [46], water root exudates of *T. diversifolia* significantly inhibited the germination, growth and chlorophyll accumulation of tomato. On the other hand, studies [26] shown that the germination of cowpea seeds was significantly enhanced through treatment with the fresh shoot aqueous extract of *T. diversifolia* and *Helianthus annuus.* Application of fresh shoot aqueous extract of *C. odorata* significantly enhanced the shoot height, leaf area, fresh and dry weight of *Celosia argentea* (Ilori *et al.,* 2011) [47]. The recent findings [27] showed that the root exudates of *T. diversifolia* significantly inhibited the germination, growth and chlorophyll contents of *Amaranthus dubius.*

Present investigations has shown that a single species exercise allelopathic effect in different ways and to different organs of the plant. The aqueous shoot extracts of *E.saligna* affected the germination of cowpeas seeds (Table 1). The results indicated that shoot extracts are desirable in inhibiting germination of cowpeas. Decrease in germination and growth of the cowpeas may be due to action by some allelochemicals. In most cases, the variation in the result of germination percentage is due to concentration differences. With the increase of concentration, the inhibitory effect was progressively increased. Highest inhibitory effect was exerted by T_5 treatment (aqueous extract in 100% solutions) in all cases except in all extract. Among the treatments, the highest inhibitory effect was recorded from the ethanol extract, this is followed by water and ethanol extract and lastly the water extract.

Table 1 shows the effects of the aqueous water and ethanol treatments on germination. The germination percentage cowpeas seeds reduced with increasing concentration of shoot extracts of *Eucalyptus saligna*. However there were no significant differences ($p \leq 0.05$) in germination percentages among the treatments. Presence of polar solvent (ethanol) exhibited highest amount of bioactive compounds (phytochemicals). Low percentage germination might also be due to acidity resulting from the reaction taking place between water and ethanol that formed ethanoic acid.

The results of this study showed that the solvent extract (ethanol) had most allelopathic effects on germination and seedling growth of cowpea (Table 1). Inhibition increased with increasing concentration. These results agree to those obtained by (Mohamadi and Rajaie, 2009) [48] that studied the effect of leaf extract of *E. camaldulensis* on sorghum and kidney bean. The results of present study were similar to those of Malik(2004) [49], El-Khawas and Shehata (2005) [50], [17] that studied allelopathic effects of *E. globulus* leaf extract on germination and seedling growth of some vegetable and crop plants. According to the results, solvent extract reduced germination percentage and germination rate and the highest inhibition was observed in highest concentration. This is because ethanol is a solvent with high isolating mechanism compared to water and a mixture of water and ethanol. Similar results were obtained in tomato by Fikreyesus *et al* (2000) [51], in wheat by Khan *et al* (2008) [52] and in cucumber by Allolli and Narayanareddy (2000) [53] through leaf extract of various *Eucalyptus* species. Several phenolic compounds such as caffeic, coumaric, gallic, gentisic, hydroxybenzoic, syringic and vanillic acids have been identified in leaf extracts of three *Eucalyptus* hybrids (Chapius-Lardy *et al*.,2000) [54] that have allelopathic potential (Rice, 1984) [55] and can inhibit the activity of gibberellic acid (GA). GA is known to regulate enzymatic activity and conversion of reserved materials to transferable materials during seed germination and embryo growth (Das *et al.*, 2011) [56]. Therefore, it is possible that these processes were inhibited by *Eucalyptus* leaf extracts.

From the present study of growth, that is, shoot height, leaf number, leaf area of cowpea seedlings treated with *Eucalyptus* fresh shoot litter were observed to be significantly stimulated to grow compared to the control plants (Tables2 and 3). The leaves of the treated plants were healthier and more green compared to the control plants. Possibly the plants irrigated with leaf litter of *E.saligna* accumulated more materials for development which was reflected in the shoot height, leaf number, leaf area and shoot and root dry weights increments as opposed to control seedlings, suggesting that cowpeas under study could adapt to the environment of *E. saligna* allelopathic influence with a little harm. A similar effect was reported on germination and growth of spider plant (*Cleome gynandra)* treated with shoot aqueous extract of *Tithonia diversifolia*. The present study also confirm observation made by Rice (1995) [57] that allelochemicals of *Eucalyptus* species stimulated plant growth of some plants.Biomass production and distribution can be important mechanisms by which plant species can survive under environmental stress. In this study, *E.saligna* fresh shoot litter stimulated shoot and root dry weights. These findings are in agreement with previous results by Bano *et al*. (2012) [58], where neem leaf extracts significantly stimulated root growth of wild oat seedlings. Increase in leaf area may lead to increased surface area for light harvesting hence increased photosynthesis and increased biomass gain and shoot growth. These findings indicate a maximum carbon–fixation adaptation mechanism in this cowpeas variety in relation to allelopathy. The response of allelochemicals may be concentration dependent and inhibit the growth of some species at certain concentrations or even stimulate the growth of the same or different species at different concentrations (Azania *et al*., 2003) [59]. Allelopathy as a mechanism and future strategy for agricultural pest control and farm management and the potential use and development of some allelochemicals as natural pesticides or plant growth regulators are becoming significant in agroforestry systems and practices (Qasem and Foy, 2001[60]; Bhadoria, 2011[61]; Klein and Miller, 1980[62]; Chou, 1999[63]; Duke, 2015[64]).

Different substances have been cited to cause allelopathic effects in various plant species by affecting morphological, anatomical, physiological, cytological and biochemical processes. The substances include cyanide-releasing complexes, ammonia releasing substances, organic acids, unsaturated lactones especially coumarin, parasorbic acid and protoanemonin aldehydes, essential oils, alkaloids and phenolic compounds (Deka and Rao, 2015) [65]. Further, Khanh *et al.*, (2007) [66] found that inhibitory substances involved in allelopathy are mainly terpenoids and phenolic substances. Bertholdesson (2005) [67] showed that volatile oil of *Artemisia afra* have several biological activities, notably antibacterial, antifungal and anti-oxidative properties. Monoterpene vapors may cause anatomical and physiological changes in plant seedlings and exposure to volatile terpenes can lead to accumulation of lipid globules in the cytoplasm, reduction in organelles including mitochondria and disruption of membranes surrounding mitochondria and nuclei (Monari *et al.*, 2005) [68]. The root tip cells subjected to the alkaloids gramine and hordenine caused damages to the cell walls, disorganization of organelles, increase cell vacuoles and the appearance of lipid and globules, showing food reserves (Rasmussen *et al.*, 2004) [69]. Large amounts of monoterpene hydrocarbons and sesquiterpenes are found to lower the antimicrobial activity of essential oils (Alexa *et al.*, 2004) [70]. The substances occur not only in seeds but in leaves, roots and other parts of the plants and leached out or released during decay and may cause inhibition in germination of seeds or root development in the vicinity of the parent plant. Thus, the substances are root-based, vegetative-based and flower-fruit-based in origin whose effect is effected in the rhizosphere region of the affected plant species. The effect can be synergistic or singular in nature, causing inhibition or promotory mechanism during germination and subsequent growth phases. During the development stages specific plant processes such as cell division and elongation, action of inherent growth regulators, mineral uptake, photosynthesis, respiration, stomatal opening, protein synthesis, membrane permeability and specific action are affected (Rice, 1974) [71]. As a result, interference of growth and development may occur when one plant species fails to germinate, grows more slowly, shows symptoms of damage or does not survive in the presence of another plant species [65]. The allelopathic effect on the DNA i.e. the influence imparted on the genes and genetic discourses requires investigation. This will inform how the mechanism of allelopathy mode of action which can be simulated to control the weeds in agricultural practices and related bioproductivity to sustain the populace in the world.

V. Conclusions

From the present study, it can be concluded that solvent extract (ethanol) of leaves of *E.saligna* had the highest allelopathic effects on germination of *Vigna unguiculata*L. Walp compared to other extracts (water and a mixture of water and ethanol. The extracts reduced germination and growth of seedlings and this inhibitory effect increased with increasing extract concentration. On the other hand *Eucalyptus* fresh leaf litter stimulated shoot and root dry weights. The results indicate that fresh shoot aqueous extracts of *E.saligna* have both inhibitory and stimulatory effects on *Vignaunguiculata*L. Walp. Fresh shoot aqueous extracts of *E.saligna* inhibited cowpea seed germination percentage; this was evidenced by the decrease percentage of seed germination with increase in extract concentration. The fresh leaf litter also stimulated shoot height growth, number of leaves, individual leaf area and fresh and dry weight of shoots and roots of cowpea. These results confirm both inhibitory and beneficial functions of the allelochemicals in *E.saligna* leaf litter. The study clearly demonstrated that water soluble inhibitory and promotory substances were present within the leaf extracts and leaf of *E.saligna*. The soluble substances have the potential to significantly affect germination and growth of*Vigna unguiculata*L. Walp, by leaching and decomposition of the leaf green manures. Many Compositae plants have allelopathic potentials, and the activities and types and amount of causative compounds differ depending on the plant species. The incorporation of allelopathic substances into agricultural management may reduce the use of pesticides and lessen environmental deterioration (Chon and Nelson, 2010)[72].These results confirm beneficial functions of the allelochemicals in *E.saligna*shoot litter use in agroforestry Therefore, *E.saligna* can be recommended to be used as shoot leafy biomass in farms growing *Vigna unguiculata* L. Walp. This will improve their growth and productivity. However, it needs further researches on the mechanisms'mode of action for efficient and effective applications in the agroforestry set upinthe cultivated lands as bio herbicides in future.

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