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Research Article

Allelopathic Influence of *Eucalyptus* on Common Kenyan Agricultural Crops

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Abstract

Eucalyptus trees introduced to Kenya are suspected of releasing allelochemials that negatively affect small farm food production. For the purpose of this study, the leaves of the *Eucalyptus grandis* W. Hill ex Maiden was used as the suspected mode for chemical release and inhibition of nearby crops. Ground leaves from *E. grandis* were applied at specific concertinos into the soil where tomato, corn and amaranth seeds were placed. These seeds types were selected because they are commonly grown on small farmer plots in Kenya near *Eucalyptus* woodlots and provide a food staple for the region aqueous solutions were also used to determine if allelochemicals in the leaf litter were inhibiting the ability of the same seed types to germinate. The results of the soil leaf litter experiment strongly suggest that alleochemicals are negatively influence the growth of the plants with amaranth showing the highest influence. The aqueous solution indicated an influence of the *Eucalyptus* on germination primarily with the amaranth.

Keywords: Africa; Allelopathy; *Eucalyptus*; Farming; Introduced species

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Introduction

Allelopathy is the ability of a plant to release chemicals, known as allelochemicals, which can influence growth and development in a nearby species [1]. The chemical release can come from a variety of sources on the plant and not exclusively from any single source. Plant leaves, roots, fruit, flowers, nuts or stems can all be allelopathic. The recognition that plants can influence other nearby species has been recorded for nearly 2,000 years in an agricultural context in ancient cultures such as those found in China and India [2]. This knowledge has been recorded as beneficial or harmful depending on the interactions observed. One example of a well-known allelopathic plant is the black walnut tree (*Juglans nigra L*.). All parts of the black walnut have the allelopathic chemical juglone. Juglone is a respiration inhibitor that affects plants by causing the leaves wilt leading to the eventual death of the plant. Juglone, while present in the entire tree, is concentrated in the buds, nut hulls and roots [3,4]. More recently, some varieties of rice have shown negative allelopathic effects towards certain aquatic plants. This has generated interest in transferring those genes to help increase rice yields. This area of interest goes beyond rice to other areas where there is potential for genetically altering organisms to reduce the dependence on herbicides by incorporating allelopathic traits in a selected plant [2]. Due to the large variety in allelochemicals, withover 100,000 identified to date, not all plants are affected the same way and it is important to note that allelochemicals can be beneficial or harmful to other organisms [2]. The allelochemicals can be both water soluble and degrade in the environment very quickly or they can build up in the soil layer over time as they are leached out from decaying leaves or dropped fruits. Environmental factors can also impact the allelopathic effects on other species [4]. There is some indication that *Eucalyptus* may contain allelochemicals that negatively affect nearby plants by inhibiting growth and seed germination. A study of *Eucalyptus camaldulensis* (Dehnh) in California found that there was a zone of limited growth surrounding the woodlot. Competition factors such as available sunlight, nutrient and water availability were ruled out with the finding of allelochemicals in the soil and tissues of the *Eucalyptus* that suppress growth [5].

Several species of *Eucalyptus* have been introduced throughout the world. The fast growing tree has become an important source of wood for many different industries globally [6]. Farmers in Mexico have objected to the large scale *Eucalyptus* pulp wood plantations that have arisen since the 1990's due to possible effects on their crops [7]. In China, *Eucalyptus* has become one of the most widely propagated introduced trees. It is also suspected of inhibiting crops near plantations [8]. This is a concern that is shared in many more countries where the *Eucalyptus* tree has been introduced; including Kenya. Several studies have used samples of collected soils from various *Eucalyptus* species wood lots for greenhouse experiments. These studies assessed the possible effect of the tree on the germination and growth in other plants [7]. Other studies have used leaf litter applied to the soil as a way to test the allelopathic effects of *Eucalyptus* on the growth and development of other plants. Predominately, the results have shown a negative effect on germination and growth, although the influence varied by tested species as well as by species of

Eucalyptus used [9-13]. Variation in allelopathic influence is highlighted in the study by which examined the effect of leaf litter on three common Chinese crops - the cabbage, radish, and cucumber [13]. They found that at lower leaf litter concentrations, the cucumber actually experienced an increase in germination rates with two of the three *Eucalyptus* species. Conversely, the cabbage and radish were negatively affected by the leaf litter, and the impact was more pronounced with an increase in concentration. These studies were the basis for developing the concentrations and protocols for the greenhouse experiment carried out in this study. The selections of seeds used were based on commonly grown crops in Kenya, where the *Eucalyptus* leaves were collected. They included corn, tomato and amaranth. The latter is an indigenous plant widely consumed in Kenya while corn and tomato are introduced crop species [14].

One way for *Eucalyptus* to influence nearby crops is from the leaching of allelochemicals from leaves; these allelochemicals are then transported with runoff water to nearby farms. The practice of "trenching" was observed in *Eucalyputs* woodlots on tree plantionation in Kenya (Figure 1A). This was presumably done to prevent the allelochemicals from influencing nearby crops. This study examined a potential mode for allelopathic influence on crops-leaf litter. This was done by adding different concentrations of leaf litter to soil and by examining germination percentages of various seeds placed in aqueous solutions. It is suspected that the higher the concentration of leaf litter the greater effect on the growth and development of the plants, with shorter and less dense plants measured at higher concentrations. It is also suspected that the indigenous plants would exhibit a greater impact from the leaf litter in both the growth and development experiment and the germination experiment.

Materials and Methods

Leaf litter greenhouse experiments

Leaves from *Eucalyptus grandis* W. Hill ex Maiden was collected near the Kenya Forest Research Institute, which is located approximately 10 km north of Nairobi, Kenya. Small stems were gathered from newly harvested trees. These stems were allowed to dry outside until the leaves were free of moisture. The leaves were then removed from the stem and packaged for shipment to the University of Arkansas.

The greenhouse component of the study examined the influence of chemicals in *Eucalyptus* leaves on the growth and development of the seeds of three types of plants-tomato (*Solanum lycopersicum L.*), corn (*Zea mays L.*) and amaranth (*Amaranthus L.*). The leaves, shipped from Kenya, were ground up through a series of grinders to reach a consistency able to pass through a 1 mm filter. Commercially available top soil was purchased from a local seed coop and air dried in the greenhouse. Once the soil was completely dry, it was sifted through a filter to remove large particles in order to create an even consistency.

Planting pots and trays were obtained from a local greenhouse. The planting pots were black, plastic, six pack pots with a growing space of approximately five square centimeters. The three groups were (1) a control, (2) 1% *Eucalyptus* to soil mixture and (3) a 10% *Eucalyptus* to soil mixture. A total of one hundred and twenty seeds were used for each type of plant. Each pot contained four seeds. The soil was mixed in one hundred gram batches for each of the experimental groups. For the 1% group, one gram of ground *Eucalyptus* was added to 99 grams of soil. This was used to fill the pots and when the pots were half full then they were watered thoroughly. More mixed soil was added to fill each pot and then it was watered again. The seeds were then placed, four per pot, an equal distance apart. A small amount of mixed soil was then added to the top. The pots were placed in the tray and watered thoroughly. This same procedure was repeated for the 10% *Eucalyptus* to soil mixture. This resulted in three trays, consisting of one control, one 1% mixture and one 10% mixture. The trays consisted of ten filled pots for each seed type, with four seeds in each pot. The pots were maintained in a greenhouse located on the campus of the University of Arkansas. The greenhouse was set at 89 degrees for a daytime high temperature and 52 degrees for the night time low temperature. The greenhouse was maintained at 38% relative humidity. The pots were watered every Monday, Wednesday and Friday, with seedling counts taken every Monday and recorded.

The corn was harvested 48 days after planting and the amaranth and tomato were harvested 68 days after planting. Before the plants were removed from the pots, a measurement of height was recorded for each plant (Figure 1B). The plants were then harvested, with the excess soil removed from the roots. The plants were allowed to dry for 24 hours in the greenhouse then they were placed in paper bags and moved to a drying facility for 48 hours. The dry weight was recorded for (1) the whole plant and (2) just the above ground portion of the plant (Figure 1C). The corn and tomato plants were measured individually, but the amaranth plants were measured in groups due to the small amount of plant material available.

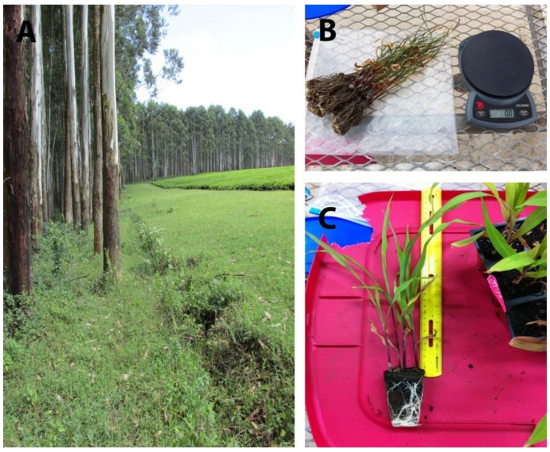

Figure 1 (A, B and C): A: Example of trenching technique between tea plants and *Eucalyptus* trees. B: Weighing the dried plants from the greenhouse experiment to determine if treatment of *Eucalyptus* impacted biomass. C: Fresh plants being measured for analysis.

Petri seed germination experiment

Leaves from two species of *Eucalyptus* (*E. paniculata* and *E. grandis*) were collected and dried. The dried leaves were shipped to the University of Arkansas from Kenya. Aqueous solutions were prepared from these dried leaves for both species.

The solutions were prepared for a 10g/l solution and a 20g/l solution. The solutions were placed on a shaker table for 24 hours at 250 rev/min. The soaked leaf litter was strained through a filter and the remaining solutions were then placed in spray bottles to be used for application.

Disposable Petri dishes were used for the germination chambers. The bottom of each Petri dish contained one 90 mm piece of filter paper. This paper was sprayed with the particular solution concentration. Ten seeds were placed on top of the paper then another piece of 90 mm filter paper was placed over the seeds. The top filter paper was sprayed again with the solution concentration to get an even moist environment. The lid of the Petri dish was placed on it, and the dish was placed in a dark cabinet. Each seed/concentration combination had 10 Petri dishes containing 10 seeds each. The Petri dishes were sprayed three times a week to maintain an even moisture environment. On the fourteenth day, the seeds were examined for germination and recorded. This was done with the use of a stereomicroscope. A seed was considered to have germinated if there was a noticeable interruption in the seed coat. The results were recorded and reported in table 1.

Eucalyptus paniculata				*Eucalyptus grandis*			
Seed Type	**Control**	**10g/l**	**20g/l**	**Seed Type**	**Control**	**10g/l**	**20g/l**
Corn	100	97	95	Corn	100	96	95
Amaranth	90	36	28	Amaranth	90	5	0
Tomato	100	92	89	Tomato	100	86	86

Table 1: Summary of the germination results for both of the *Eucalpytus* species used as well as the different concentrations used.

Results

Leaf litter greenhouse experiments

The results of the plant height greenhouse experiment using corn, tomato and amaranth seeds are recorded. The corn germination rates for the control were 77.5 %, and 82.5% in the 1% *Eucalyptus* mixture and 67.5% in the 10% *Eucalyptus* mixture. These results are slightly lower than the germination study results. The mean corn plant height in the control was 20.18 cm, 18.38 cm in the 1% mixture and 14.94 cm in the 10% mixture (Figure 2). The dry weight results where 19.83 g in the control 23.03 g in the 1% mixture and 12.55 g in the 10% mixture (Figure 2). The tomato germination rates were not different from the control or the different mixture concentrations with 15, 15, and 16, respectively, but the overall germination rate was at or under 40%. The oven dry weights were 0.46 g in the control, 0.33 g in the 1% *Eucalyptus* mixture and 0.15 g in the 10% *Eucalyptus* mixture (Figure 3). The mean height for the control group was 4.46 cm, with the 1% *Eucalyptus* mixture measuring 3.86 cm and the 10% *Eucalyptus* mixture measuring 2.97 cm (Figure 3).The amaranth seeds germinated in the control and 1% mixture with 16 and 13 seeds, respectively, but no seeds germinated in the 10% *Eucalyptus* mixture. The mean height for the control group was 2.84 cm, with the 1% *Eucalyptus* mixture measuring 2.03 cm (Figure 4). The oven dry weight of the control group was 0.05 g, with the 1% *Eucalyptus* mixture weight at 0.04 g (Figure 4). The small amount of vegetative matter made it difficult to determine a difference between the above ground dry weight and the whole plant, so the above ground measurement was not taken.

Petri seed germination experiment

The results of the seed germination numbers are presented in table 1. With the 10 g/l solution of *E. paniculata*, 97 corn seeds germinated, and with the 20 g/l solution 95 seeds germinated. For the *E. paniculata* 10g/l solution 92 tomato seeds germinated and with the 20 g/l solution 89 seeds germinated. The amaranth seeds had the fewest germinated seeds with the *E. paniculata* solution. Under the 10 g/l solution 36 seeds germinated while 28 seeds germinated with the 20 g/l solution.

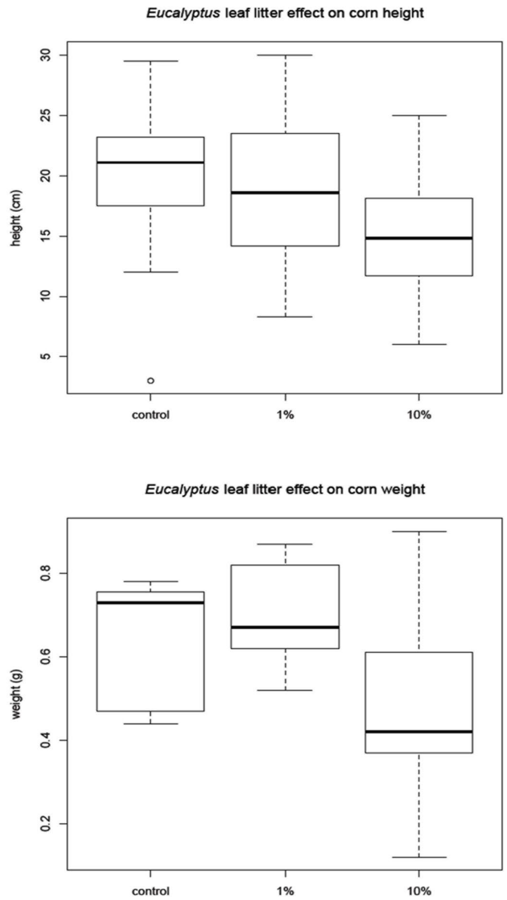

Figure 2: Results of the height and dry weight of corn plants after harvest. The graph plots median height, range and central 50% of the sample heights and weights. The height and weight measurements were statistically different between the control and 10% leaf litter mixture.

The results of seed germination under the *E. grandis* solution are given in table 1. Ninety-six corn seeds germinated in the 10 g/l solution and 95 seeds germinate in the 20 g/l solution. The tomato seeds had 86 germinate in the 10 g/l *E. grandis* solution and the same number, 86, germinated in the 20 g/l solution. The amaranth seeds had 5 germinate in the 10 g/l *E. grandis* solution. In the 20 g/l *E. grandis* solution, none of the amaranth seeds germinated.

Discussion

An ANOVA statistical test was carried out for each of the different plant treatment results, with a Tukey post hoc test performed to determine where there was a significant difference, if that was the case (Table 2). For the corn experiment, both the height and weight showed significant difference with a 99% confidence interval for the data sets. The same results were reported for the tomato and amaranth data sets. Using the Tukey post hoc test it was possible to determine which data sets in each category showed statistical differences from others. The corn data showed statistical difference between the 10% *Eucalyptus* solution in both the height and weight measurements. The 1% *Eucalyptus* solution did not have a statistical difference between the height and weight of the corn plants when compared to the control. For the corn, the influence was more evident in the higher solution concentration. The Tukey post hoc test also revealed that weight measurements for the both the tomato and amaranth did not have a significant difference between 1% *Eucalyptus* concentration and the control. It is important to note that the sample size of plants that germinated in these groups was small. The amaranth plants were so small that they could not be accurately weighted individually and had to be weighed in groups to get a reading on the scale. A larger sample size might reveal a significant difference if the experiment was repeated. The overall results of the greenhouse experiment demonstrate that *Eucalyptus* does have an effect on the growth and development of common crop seeds in Kenya. This supports the hypothesis that *Eucalyptus* tree are allelopathic and can influence the growth and development of nearby plants. This is similar to the results of greenhouse experiments using soil from *Eucalyptus* woodlots on agricultural crops conducted different concentrations by Espinosa-Garcia et al., which found that the effect was the least on corn and greatest on the other vegetables tested [7].

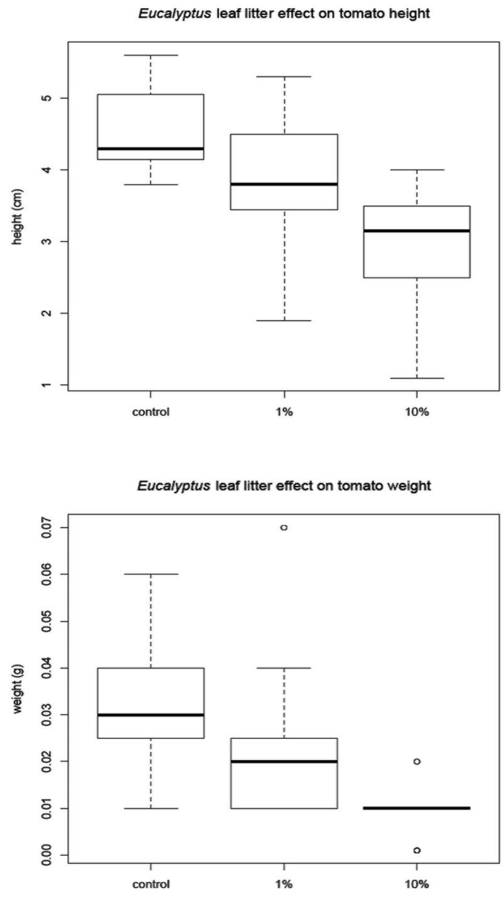

Figure 3: Results of the height and dry weight of tomato plants after harvest. The graph plots the median height, range and central 50% of the sample heights and weights. The height and weight measurements indicated a statically significant difference between the control and the 10% *Eucalyptus* leaf litter mixture.

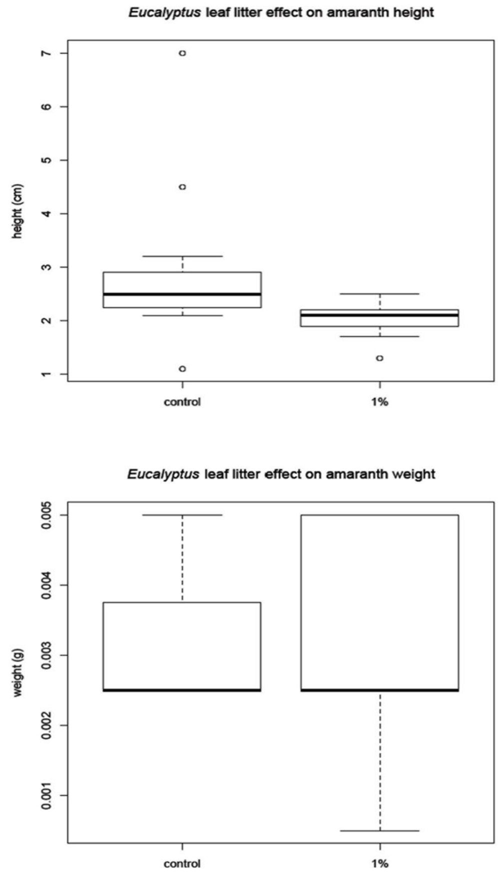

Figure 4: Results of the height and weight of the amaranth plants after harvest except for the 10% *Eucalyptus* leaf litter concentration, in which amaranth seeds did not germinate. The graph illustrates the median height, range and central 50% of the sample heights and weights. The 10% *Eucalyptus* leaf litter group did not germinate which indicates a significant difference.

The experiment to determine the effect of the aqueous *Eucalyptus* solution on the germination of seeds yielded some significant results. The corn showed very little effect from either the *E. paniculata* or the *E. grandis* solutions. The tomato displayed a slightly greater effect from both of the solutions but with little difference from the increased concentration. The amaranth seeds had the greatest effect from both of the *Eucalyptus* solutions. Approximately one third of the seeds germinated in the *E. paniculata* solution but only five seeds germinated in the 10 g/l *E. grandis* solution. In the 20 g/l *E. grandis* solution, none of the seeds germinated (Figures 5 and 6).

The amaranth was the only seed that displayed a statistically significant difference in the germination percentages from the control group. This was verified using a chi-square analysis. The results were significant for both *Eucalyptus* species as well as in both concentrations. The corn, tomato and *Eucalyptus* are all introduced species to Kenya, while the amaranth is a native species. This may provide some insight into the why the amaranth is more susceptible to the secondary metabolites that are found in the *Eucalyptus* leaves. The recent interaction between the two species has not been a long enough time for defense mechanisms to evolve to combat the allelopathic effects of the *Eucalyptus* tree. The evidence from this study also indicated that allelochemicals found in *Eucalyptus* could contribute to lower crop yields in rural farming areas and have a significant impact on food security for subsistence farmers.

	Control		1%		10%	
	Height (cm)	Weight (g)	Height (cm)	Weight (g)	Height (cm)	Weight (g)
Corn mean	20.18	0.64	23.5	0.70	14.94	0.46
Tomato mean	4.56	0.03	3.86	0.02	2.96	0.01
Amaranth mean	2.84	0.003	2.03	0.003	0	0

ANOVA	DF	Sum Sq	Mean Sq	F value	Pf(>F)
Corn height	2	405.3	202.65	7.78	0.000774
Corn weight	2	0.8512	0.4256	18.61	1.82e-07
Tomato height	2	19.75	9.876	18.92	1.28e-06
Tomato weight	2	0.003520	0.0017601	11.14	0.000126
Amaranth height	1	4.741	4.741	4.789	0.0375
Amaranth weight	1	2.000e-08	1.660e-08	0.01	0.92

Tukey	T1-ControlPval.	T2-ControlPval.	T2-T1Pval.
Corn height	0.34	0.0005	0.29
Tomato height	0.03	00000007	0.004
Amaranth height	0.04	NA	NA
Corn weight	0.28	0.00009	0.0000002
Tomato weight	0.15	0.00008	0.02
Amaranth weight	0.92	NA	NA

Table 2: Summary of statistical data for the greenhouse experiment using corn, tomato and amaranth at different mixtures of ground *Eucalyptus grandis* leaf litter. The numbers represent the height in centimeters and the weight in grams of each plant grown. Anova and Tukey post hoc results are provided for all data sets.

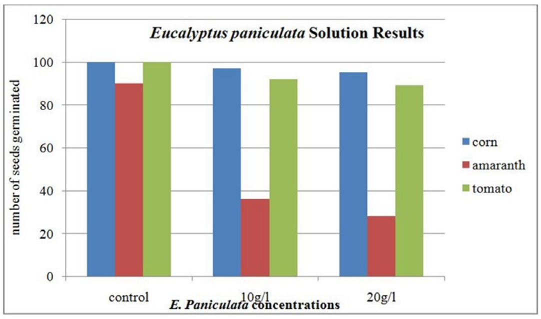

Figure 5: Seed germination results for the *Eucalyptus paniculata* solution on corn, amaranth and tomato seeds.

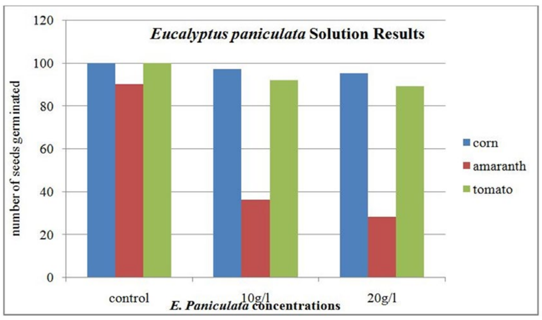

Figure 6 : Seed germination results for the *Eucalyptus grandis* solution on corn, amaranth and tomato seeds.

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