Allelopathic effects of *Eucalyptus camaldulensis* and *Eucalyptus globules* leaf extracts mixture on Germination and Growth of Sorghum

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ABSTRACT

Allelopathy results when living organisms produce bioactive compounds which enter the environment and produce direct or indirect effects on the growth and development of the same or other species. Many investigations have been carried out on allelopathic effect of Eucalyptus leaf extracts on several cereals. *E. camaldulensis* and *E. globulus* contain allelochemicals that inhibit growth of agricultural crops. This study was done to investigate the allelopathic effect of a mixture of *E. camaldulensis* and *E. globules* on germination and growth of *Sorghum bicolor*. The seeds of *Sorghum bicolor* were germinated in sterilized petri dishes lined with a layer of filter paper Whatman No.1, soaked daily withtap water(control) and four different concentrations (25%, 50%, 75% and 100%) of fresh leaf extract mixture of *E. camaldulensis* and *E. globulus* in Department of Botany laboratory of Maseno University. Growth tests were carried out in the greenhouse using 4.5l plastic pots. Five plastic pots were half filled with top humus soil and different amounts of *E. camaldulensis* and *E. globulus* leaf segments mixture incorporated (0, g, 50g, 100g, 150g and 200g). The treatments were replicated five times and laid in completely randomized design. Data on seeds germinating, coleoptile and radicle length, plant shoot height, leaf length and width, leaf area and number of leaves was recorded. Data obtained was subjected to analysis of variance (ANOVA). Treatments means were compared using Least Significant Difference **(**LSD) at ($p \leq 0.05$).The fresh leaf aqueous extract mixture of *E. camaldulensis* and *E. globules* significantly reduced germination of seeds, coleoptile and radicle lengthof *S. bicolor*.The fresh leaf segments mixture of *E. globulus* and *E. camaldulensis* incorporated to soil significantly reduced shoot height, leaf area, fresh weight and dry weight and chlorophyll content of *S. bicolor*.The study indicated that fresh aqueous leaf extract mixture and leaf segments of *E. camaldulensis* and *E. globules have* inhibitory effect on *S. bicolor.*

Keywords: Allelopathy, E. camaldulensis and E. globules, leaf segments

1.0 INTRODUCTION

Allelopathy is the direct influence of a chemical released from one living plant on the development and growth of another (Bano *et al*., 2012; An et al., 1996)). Allelopathy occur when living organisms produce bioactive compounds which enter the environment and produce direct or indirect effects on the growth and development of the same or other species (Seigler, 1996). Allelopathy has been described as the inhibitory or stimulatory effect of the plant (donor) on another plant (receiver) (Taiwo and Makinde, 2005).According to Taiwo and Makinde (2005) allelochemicals may inhibit shoot and root growth, nutrient uptake (McWorthier, 1984).

Eucalyptus species grow under a wide range of climatic and edaphic conditions in their natural habitats (Dawar *et al*., 2007). This species has high potential of allelochemicals and also essential oils. Iqbal *et al*. (2003) found 16 components in essential oils of *Eucalyptus camaldulensis.* Eucalyptus is a tree planted among smallholder farmers in Kenya, owing to their fast growth and their rising demand for paper and plywood. Many farmers in Kenya combine the growing of Eucalyptus on the hedges with crops such as Sorghum on their farms. Sorghum production is low which may be attributed to the allelopathic effect of Eucalyptus in agroforestry systems, but this has not been documented. The leaf, root and stem extracts from different Eucalyptus species have inhibited germination and seedling growth of many crops. Sasikumar *et al*. (2002) reported that the allelopathic influence of Eucalyptus has been attributed to the production of several volatile terpenes. Eucalyptus grows in almost all tropical and sub-tropical areas and is cultivated in many other climates (Dawar *etal*., 2007). It is a predominating tree planted among small holder farmers in Kenya.It is preferred over other species due to the number of merits that address the need of farmers (Djanaguiraman *et al*., 2002). Eucalyptus oil is used in creams, ointments to relieve muscle and joint pain (Wendorf *et al*., 2007). According to Chen *et al*. (2006) Eucalyptus oil has a broad spectrum of medicinal use; it is prescribed for a wide variety of respiratory conditions including asthma, bronchitis, coughs and flu. It is also used as an insect repellant hence ingredient in garden sprays (McWhorther, 1984). In Kenya, Eucalyptus *(E. camaldulensis* and *E. globulus*) is preferred due to its fast growth, wider adaptability and high productivity.

There are many compounds in Eucalyptus species which are found in the dry and fresh leaves,buds,mature fruits and bark (Samuel *et al*., 2005).The essential oils in the leaves are commonly used for medicinal purpose; they include cuminaldehyde, epiglobulol,d-linalool, rutinand tannins (Holmes *et al.,* 2009). Eucalyptus species contain high levels of phenolic and

terpenoid which can be toxic. Some have antimicrobial properties and others impose allopathic effect on other plants (Del Moral *et al.*, 1978). Phenolic compounds include; coumaric, gallic, gentisic, cathechol, hydrobenzoic, syringic and vanillic acid (Pimentel *et al*., 2005). Phenolic compounds interfere with the phosphorylation pathway and interference in cell division, mineral uptake and biosynthetic processes (Sasikumar *et al*., 2002). Studies indicate that Eucalyptus leachates have harmful effect on germination and growth of wheat, barley, chick pea, mustard and many weeds (Samuel *et al*., 2005). This has been attributed to the decreased α- amylase activity in these seeds which regulate starch break down.

Romel *et al*. (2008) reported that chlorophyll content was reduced significantly in kidney-bean. The reduction in chlorophyll content was due to degradation of chlorophyll pigment or reduction in its synthesis due to the action of phenolic compounds present in leaf leachets (Sala *et al.*, 2001; Netwing, 2008). Though many investigations have been carried out on allelopathic effect of Eucalyptus leaf extracts on several cereals, there is still scanty information on combined allelopathic effect of aqueous leaf extracts *E. camaldulensis* and *E. globulus* on germination of Sorghum bicolor and also a combined allelopathic effect of fresh leaf segments of *E. camaldulensis* and *E. globulus* growth parameters of *Sorghum bicolor*.

Allelochemicals were reported to be highest in foliage of many plants; these chemicals were found to be released in the soil ecosystem through volatilization, root exudation and leaching from foliage (Holmes *et al*., 2009). Laboratory experiments have been undertaken to investigate the allelopathic activity of Eucalyptus species in the soil and it has been reported that the leachates caused significant reduction in seedling growth of black gram and rice. However, there is scanty information on allelopathic effect of *Eucalyptus globulus* and *Eucalyptus camaldulensis* on sorghum.

Eucalyptus globules and *Eucalyptus camaldulensis* are allelopathic tree species in forest plantation and the leachates contain large number of allelochemicals. Allelopathic interactions are mediated by a mixture of many allelochemicals. Leaf extracts of Eucalyptus inhibit seed germination and seedling growth of cereals and legumes. Many Eucalyptus species are grown in the agricultural fields where crops such as Sorghum are also grown. Sorghum production is low which may be due to combined allelopathic effect of fresh leaf segment mixture of *Eucalyptus globulus* and *Eucalyptus camadulensis* which are common Eucalyptus grown. This has not been documented. Studies indicate that aqueous extract of

*Eucalyptusglobulus*and*Eucalyptuscamadulensis* exhibit allelopathic effect on some food crops, however, there is scanty information on allelopathic effect of fresh leaf extract mixture and fresh leaf segment mixture of *Eucalyptus globulus* and *Eucalyptus camadulensis* on germination and growth of sorghum.

Sorghum (*Sorghum bicolor* (L.) Moench is one of most important cereal crops widely grown as food, fodder and fuel in the semi-arid tropics of Asia, Africa, America and Australia (Belium *et al*., 2010). Sorghum is adapted to a wide range of ecological conditions. It is mostly cultivated in hot, dry regions although it can still survive in cool weather as well as waterlogged habitats (Rich *et al*., 2004). Sorghum is considered the 'camel of the crops' a true testament to its hardness and ability to grow in dry, nutrient-poor soils and withstand prolonged droughts.

Sorghum bicolor is grown by farmers in arid and sub-humid regions of Africa. In Kenya it is cultivated in Northern Rift valley, Western, Eastern and some parts of Central province. It is generally suited to hot and dry agro ecologies where it is difficult to grow other food grains (Anglami, 1998).Sorghum is grown as a rain-fed crop in Kenya. Subsistence farmers rarely add fertilizer; it is intercropped with legumes such as beans, cowpeas, pigeon peas and green gram.

Sorghum is also alleged to be allelopathic due to the production of sorgholene. Studies indicate that sorgholene suppresses weeds and enables sorghum to thrive better (Awika *et al*., 2004). Sorgholene is produced by sorghum roots as root exudes, it is not present in the leaves or the above parts of sorghum plant unlike allelochemicals of Eucalyptus which is present in the whole plant.In addition, these allelochemicals are inhibitory to germination and growth of other plants (Sasikumar *et al*., 2002) but sorgholene mainly inhibits growth of weeds.

Sorghum bicolor is a native to Africa and an important crop worldwide, used as food, fodder and production of alcohol beverages and biofuels. It is a drought tolerant and important crop in arid regions where the grain is one of the staple foods for poor and rural people. Sorghum has high levels of potassium and also is a good source of energy. Recently, the high demand for sorghum by East Africa Breweries Limited has enhanced sorghum popularity among Kenyan farmers. Therefore, Sorghum not only thrives in harsh conditions but has won a place in heart and plates of local farmers which is increasingly there food security and source of income (Anglami, 1998). Sorghum is grown in close proximity to these two Eucalyptus species *(E. camaldulensis* and *E. globulus).* Eucalyptus, though, a potential industrial crop is not recommended as an intercrop in agroforestry system. There is need to provide information to farmers about combined allelopathic effect of *E. camaldulensis* and *E. globulus* fresh leaf

segment mixture on germination and growth of *Sorghum bicolor*which is sensitive to phytotoxic compounds. The broad objective of this study was to investigate the combined allelopathic effect of *Eucalyptus globulus* and *Eucalyptus camaldulensis* on germination of seeds and growth of seedling of *Sorghum bicolor*.

2.0 MATERIALS AND METHODS

2.1 Collection of Plant Material

Eucalyptus globulus and *Eucalyptus camaldulensis* fresh leaves were collected from University Botanic Garden-Maseno and sorghum seeds were collected from Kenya seed branch-Eldoret, Kenya.

2.2 Preparation of Mixture of Fresh Leaf Extracts of *Eucalyptus globulus* and *Eucalyptus camaldulensis.*

Two hundred and fifty grams of fresh leaves of *Eucalyptus globulus* and two hundred and fifty grams of fresh leaves of *Eucalyptus camaldulensis* were harvested at the vegetative stage. They were cut into small pieces of about 5cm in length, mixed thoroughly and finely ground using pestle and mortar and then soaked in 1 litre of distilled water in a large beaker for 24 hours (Djanaguiraman *et al.*, 2002). The extracts were filtered using a sieve to remove debris and then whatman no.1filter paper was used to have 100% concentration. Aqueous extracts of 25%, 50%, 75% concentration of a mixture of *E. camaldulensis* and *E. globulus* were made by diluting the original extract with distilled water.

2.3 Germination Tests

Germination tests were carried out in the Maseno University Botany Laboratory. Seventy five treated seeds of *Sorghum bicolor* were placed in dried petri dishes lined with layers of Whatman no.1 filter papers. It was soaked with the respective mixed aqueous extracts (treatment) of *E. camaldulensis* and *E. globules* and distilled water (Chiapusio *et al*., 1997).The treatments were replicated five times and laid out in completely randomized design. Data on seed germinating each day was recorded and germination count determined. The coleoptiles length and radicle length was measured daily.

2.4 Preparation of Mixture Fresh Leaf Segments of *Eucalyptus globules* and *Eucalyptus camaldulensis.*

Two hundred and fifty gramsof fresh leaves of *Eucalyptus globulus* and two hundred and fifty grams of *Eucalyptus camaldulensis* were cut into small pieces using a scissor and the segments

were mixed thoroughly. Leaf segments wereincorporated into 4.5 liter pots together with 1200g of top humus soil collected from botanic garden. A mixture of leaf segments of 50g, 100g, 150g and 200g (Seigler, 1996) of *E. camaldulensis* and *E. globulus* were mixed thoroughly with top humus soil and irrigated with 500ml of distilled water for five days prior to sowing *S. bicolor* seeds. Pots with top humus soil only without the fresh leaf segments formed the control.

2.5 Experimental Design and Treatment

Growth tests were carried out in the greenhouse. Five 4.5 litre plastic pots with dimensions 21cm in height and 19cm in diameter were filled with top soil collected from Maseno University botanic garden (Musyimi *et al*., 2012). One pot with top humus soil only formed the control and the remaining four pots contained top humus soil mixed with different concentrations of mixed fresh leaf segments (50g, 100g, 150g and 200g) of *E. camaldulensis* and *E. globulus* (Seigler, 1996). The treatments were replicated five times and laid out in a completely randomized design (Plate 1). Twenty treated seeds of *Sorghum bicolor* were sown in each pot. Watering was done every morning with 250ml tap water per pot till then end of 2 weeks. Data collection commenced after seed germination. After two weeks, the seedlings in each pot were thinned down to five plants per pot (Plate1).

Plate 1: Sorghum seedlings growing in plastic pots containing soil plus various treatments of fresh leaf extract mixture of *E. camaldulensis* and *E. globules*

3.0 MEASUREMENT OF PARAMETERS

3.1 Germination

Germination was established by counting the seeds germinating every day after treatment allotment and calculating the final germination count in each treatment for presentation.

3.2 Coleoptile and radicle length

Coleoptile and radicle length of the seedlings was measured using transparent meter rule every day to the end of experiment.

3.3 Shoot height

Shoot height was measured from soil level to the upper point of the terminal bud of the seedling using a meter rule every three days to then end of the experiment.

3.4 Number of leaves

Number of mature leaves per plant was counted and recorded every three days up the end of experiment.

3.5 Leaf length and width

Leaf length and width was measured using a meter rule every three days to the end of experiment.

3.6 Leaf area

Leaf area was determined at the end of experiment using the formula of Otusunya *et al*. (2007) as indicated below:

$LA=0.5(L*W)$

Where L=length of leaf

W=maximum width.

3.7 Root and shoot fresh weight

At the end of the experiment, the plants were carefully uprooted from the soil, cleared off debris, separated into shoot and root and measured separately using electronic weighing balance.

3.8 Root and shoot dry weights

Fresh plants (roots and shoots) were packaged separately in envelopes and dried to constant weight at 80◦c in on oven. Root and shoot dry weights were then weighed on an electronic weighing balance; and then mean weights calculated.

3.9 Leaf chlorophyll concentration

Determination of chlorophyll will follow the formula of Combs *et al* (1985). The third fully expanded leaf from shoot apex was sampled from all the treatments after one month. Leaves of about 3 grams were grounded in 10ml of 80% (V/V) acetone using mortar and pestle. They were then left overnight for 24 hours to allow maximum extraction of chlorophyll. The resulting extract were read at 645nm and 664nm using UV-visible spectrophotometer. Chlorophyll a, b and total concentration were calculated as follows:

Chlorophyll a =13.19 A664-2.57 A645 (mgg-1 fresh weight)

Chlorophyll b =22.1 A664-5.26 A664 (mgg-1 fresh weight)

Total Chlorophyll =7.93 A664+19.53 A645 (mgg-1 fresh weight)

Where A664 is the absorbance at 664nm and A645 is the absorbance at 645nm

4.0 DATA ANALYSIS

Data obtained from the study will be subjected to the analysis of variance (ANOVA) in SAS statistical package. Treatment means were separated and compared using the Least Significant Difference (LSD at 0.05) (Steel *et al*., 1992).

5.0 RESULTS

5.1 Germination

The results presented in tables 1 and 5 showed that germination of *Sorghum bicolor* decreased significantly ($P \leq 0.05$) by 100% of fresh leaf extract mixture of *E. camaldulensis* and *E. globulus*. One hundred percent leaf extract mixture had the greatest effect on growth response compared to control. The control treatment with 14.17 seed count was significantly ($P \leq 0.05$) different from 25%, 50% and 100% of fresh leaf extract mixture of *E. camaldulensis* and *E. globulus*. Treatment containing 50% and 75% fresh leaf extract of *E. camaldulensis* and *E. globulus* were not significantly ($P \geq 0.05$) different. Germination count generally decreased with increasing Eucalyptus extract. All the treatments 25%, 50%, 75% and 100% fresh leaf extract

mixture of *E. camaldulensis* and *E. globulus* had inhibitory effect on the germination of *Sorghum bicolor*.

5.2 Radicle and coleoptile length

The results in tables 1 and 5 showed that the radicle and coleoptile length of *Sorghum bicolor* at 100% of *E. camaldulensis* and *E. globules* fresh leaf extracts mixturewere significantly lower than (P≤0.05) other treatments. Radicle and coleoptile lengths of control (0%) were longer compared to treatments 25%, 50%, 75% and 100% of fresh leaf extract mixture of *E. camaldulensis* and *E. globules* and were significantly (P≤0.05) different from the treatments. There was no significant (P≥0.05) difference in radicle length among 50%, 75% and 100% concentration of fresh leaf extracts mixture of Eucalyptus. In coleoptile length, there was no significant (P≥0.05) difference between 75% and 100% of fresh leaf extracts of Eucalyptus.

TABLE 1: Allelopathic effects of mixture of leaf extracts of *E. camaldulensis* and *E. globules* on seed count, radicle length and coleoptile height of *Sorghum bicolor*

Treatments (%)	Seed counts	Radicle length (cm)	Coleoptile length (cm)
0%	14.17a	5.39a	3.15a
25%	12.54b	2.96b	2.18b
50%	11.46c	1.09c	1.59c
75%	10.60c	0.50d	1.51c
100%	7.31d	0.27d	1.31c
LSD	0.93	0.38	0.34

Means with the same letter within column are not significantly different.

5.3 Shoot height

There was a significant (P≤0.05) lower shoot height among treatments 50g, 100g, 150g and 200g of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*as compared to control (0g) as shown in table 2 and 6. There was no significant (P≥0.05) difference among treatment with 100g, 150g and 200g of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*. There was a significant (P≤0.05) difference between treatment with 50g and 100gof fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*as well as those with 150g and200g of

fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*. The control recorded the highest (10.58cm) shoot height compared to other treatments (50g, 100g, 150g and 200g) of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*. The minimum shoot height (3.91cm) was observed at200g of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*.

5.4 Leaf length

The results presented in tables 2 and 6 indicates that the leaf length of *Sorghum bicolor* decreased significantly (P≤0.05) between 150g and 200g of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*. The highest leaf length (31.00cm) was recorded in control while the lowest length (13.86cm) was recorded at 200g concentration of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*. Leaf length decreased with increasing concentration. There was marked significant (P≤0.05) difference among all the treatment except in treatment containing 150g and 200g of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*.

5.5 Leaf width

The results presented tables 2 and 6 showed that there was significant (P≤0.05) difference between control and treatment with 200gof fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*. The control had the highest leaf with (1.16 cm) as compared to 200g of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*which had 0.47cm.There was slight significant difference (P≤0.05) in leaf width in treatments containing 50g, 100g and150g of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus.*

5.6 Leaf number

The number of leaves decreased with increase in the concentration of the treatment as follows 0g >50g>100g>150g>200gof fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*. The results presented in tables 3 and 7 indicate that the leaf number was significantly (P≤0.05) reduced over control. The least (3.8571) leaf number was recorded in treatment with 200g of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*and the highest (7.0286)leaf number in the control.

5.7 Leaf Area

Significant (P≤0.05) reduction in leaf area was observed on Sorghum grown on treatment with 200g of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus* as indicated in table 3 and 5. Largest leaf area was determined from the control treatment (19. 41cm^2) and the least area (3.631cm^2) was recorded from treatment with 200g of fresh leaf segments mixture of *E.*

camaldulensis and *E. globulus*. There was no significant (P≥0.05) difference in leaf area between 150g and 200g treatments of Eucalyptus leaf segments mixture.

TABLE 2: Allelopathic effect of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus* on **shoot height, leaf length and leaf width**

Treatments (g)	Shoot height (cm)	Leaf length (cm)	Leaf width (cm)
0	**10.58a**	**31.01a**	**1.16a**
50	**7.61b**	**28.06b**	**0.84b**
100	**4.74c**	**20.26c**	**0.73bc**
150	**3.97c**	**16.00d**	**0.65c**
200	**3.91c**	**13.86d**	**0.47d**
LSD	**1.55**	**2.91**	**0.16**

Means with the same letter down the column are not significantly different.

5.8 Total chlorophyll content

The results presented in tables 3 and 6 indicate that the total chlorophyll content was significantly (P≤0.05) different among treatments containing 0g, 50g and 100g of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus* as compared to the treatment containing 150g and 200gof fresh leaf segments mixture of *E. camaldulensis* and *E. globulus.* There was no significant (P≥0.05) decrease in content of chlorophyll between 0g and 50g of Eucalyptus leaf segments mixture treatments as well as between 50g and 100g of Eucalyptus leaf segments mixture treatments. There was significant (P≤0.05) difference in total chlorophyll content between treatments with 100g and 150gof fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*. The control treatment had the highest value (31.061 mg) of chlorophyll, and the least chlorophyll content (17.795mg) was recorded in treatment with 200g of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*.

5.9 Shoot and root fresh weight

There was a significant (P≤0.05) reduction in fresh weight as shown in Table 4 and 7 indicate that control plants had the highest value (3.97g) of fresh weight and the least (1.15g) which was observed in treatment with highest amounts (200g) of fresh leaf segments mixture of *E.*

camaldulensis and *E. globulus.* There was significant (P≤0.05) difference in root and shoot fresh weight between control and the remaining treatments of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus.* There wasno significant(P≥0.05) difference in root fresh weight among the treatments (50g, 100g, 150g and 200g) of fresh leaf segments mixture of *E. camaldulensis* and *E. globules* but there was significant (P≤0.05) difference in shoot fresh weight between treatment 150g and 200g of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus.*

TABLE 3: Allelopathic effectof fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*on leaf number, leaf area and total chlorophyll content of Sorghum.

Treatments (g)	Leaf number	Leaf area (cm2)	Total chlorophyll (mg)
0	7.03a	19.41a	31.06a
50	5.51b	12.92b	26.61a
100	4.89bc	8.05c	26.42a
150	4.69c	6.03cd	20.65b
200	3.86d	3.63d	17.80b
LSD	0.77	3.40	5.69

Means with the same letter down the column are not significantly different.

5.10 Shoot and root dry weight

Three weeks old seedlings were uprooted and oven dried. The data on dry weight was taken after 24 hours. The analyzed data presented in tables 4 and 7 indicate that different amounts of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus* significantly (P≤0.05) reduced sorghum root and shoot dry weight.

There was significant (P≤0.05) difference between control and other treatments of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus.* Highest root dry weight (0.62g) and shoot dry weight (1.88g) was recorded in the control. Root dry weight of treatments 50g, 100g, 150g and 200g of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus* were not significantly (P≥0.05) different but shoot dry weights of treatments 50g, 100g and 150g of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*. Eucalyptus leaf segments were significantly (P≤0.05) different from treatment containing 200g of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*.

TABLE 4: Allelopathic effect of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus* on root and shoot fresh weight and root and shoot dry weight

Treatments (g)	Root fresh weight (g)	Shoot fresh weight(g)	Root dry weight(g)	Shoot dry weight (g)
0	**3.97a**	**12.73a**	**0.62a**	**1.88a**
50	**2.07b**	**9.52b**	**0.30b**	**1.06b**
100	**1.77b**	**7.81b**	**0.30b**	**1.04b**
150	**1.49b**	**7.42b**	**0.22b**	**1.00b**
200	**1.15b**	**3.05c**	**0.20b**	**0.34c**
LSD	**1.09**	**2.27**	**0.19**	**0.35**

Means with the same letter down the column are not significantly different.

Table 5: Analysis of variance of germination, coleoptiles and radicle of Sorghum seedlings

	Source	DF	Mean of Squares	Sum of Square	F Value	P>.0001
Germinating seeds	Mode	4	915.46	228.53	58.39	<.0001
	Error	170	666.29	3.92		
	Corrected error	174	1581.75			
Coleoptile length	Mode	4	77.42	19.36	36.72	<.0001
	Error	170	89.61	0.53		
	Corrected error	174	167.03			
Radicle Length	Mode	4	646.11	161.53	246.68	<.0001
	Error	170	111.32	0.65		
	Corrected error	174	757.43			

TABLE 6: Analysis of variance of shoot height, leaf number, leaf width, leaf length and leaf area of Sorghum seedlings

	Source	DF	Mean of Squares	Sum of Square	F Value	P>.0001
Shoot height	Mode	4	1173.75	293.44	27.35	<.0001
	Error	170	1824.18	10.73		
	Corrected error	174	2997.93			
No. of Leaves	Mode	4	196.31	49.08	18.66	<.0001
	Error	170	447.08	2.63		
	Corrected error	174	643.39			
Leaf Length	Mode	4	7807.37	1951.84	51.29	<.0001
	Error	170	6469.04	38.05		
	Corrected error	174	14276.41			
Leaf width	Mode	4	9.17	2.29	19.09	<.0001
	Error	170	20.42	0.12		
	Corrected error	174	29.59			
Leaf Area	Mode	4	5500.63	1375.16	26.51	<.0001
	Error	170	8820.05	51.88		
	Corrected error	174	14320.68			

TABLE 7: Analysis of variance of chlorophyll content, shoot fresh weight, root and shoot dry weights of Sorghum seedlings

	Source	DF	Mean of Squares	Sum of Square	F Value	P>.0001
Chlorophyll content	Mode	4	554.85	138.71	7.76	0.0008
	Error	20	371.80	18.59		
	Corrected error	24	926.65			
Shoot fresh weight	Mode	4	247.51	61.88	20.95	<.0001
	Error	20	59.00	2.95		

	Corrected error	24	306.51			
Root fresh weight	Mode	4	24.39	6.09	8.96	0.0003
	Error	20	13.60	0.68		
	Corrected error	24	37.99			
Shoot dry Weight	Mode	4	5.97	1.49	21.58	<.0001
	Error	20	1.38	0.07		
	Corrected error	24	7.35			
Root dry weight	Mode	4	0.57	0.14	6.90	0.0012
	Error	20	0.42	0.02		
	Corrected error	24	0.99			

6.0 DISCUSSION

Fresh leaf extract mixture of *E. camaldulensis* and *E. globules* inhibited seed germination and growth of *Sorghum bicolor*. Seed germination is considered to be the most critical stage especially under stress conditions. During germination, biochemical changes takes place, which provide the framework for subsequent growth and development. The inhibition of seed germination of crop plants may be due to the disturbance in the activities of peroxidase, alpha-amylase and acid phosphates (Bano *et al,* 2012). These results agree with those reported by McWorther (1984).He reported inhibition of seed germination and seedling growth of some herbaceous plants such as chick pea, maize,pea and by aqueous leaf extracts of *E. camaldulensis* and *E. globules*. The highest concentration of fresh leaf extract mixture of *E. camaldulensis* and *E. globules* inhibited significantly the germination of Sorghum. There was direct relationship between the extract concentration and normal seedlings. It is very clear from these results that Sorghum sown in the soil is containing fresh leaf extract mixture of *E. camaldulensis* and *E. globules* may be adversely affected and its germination (%) will be reduced up to about 49%.

It was observed that the fresh leaf extract mixture of *E. camaldulensis* and *E. globulus* delayed as well as hindered the emergence of radicle and coleoptile significantly in the receptor plant compared to control. The results also revealed that radicle elongation was much more inhibited than coleoptile elongation. Dawar *et al.* (2007) reported that undiluted leaf extract of *E.*

camaldulensis and *E. globules* impeded the radical elongation of cucumber, onions, tomatoes and sorghum.The interaction among various concentration of Eucalyptus extract and Sorghum depicted that the extract at 25% had the lowest inhibitory effect. This might be attributed by the strong sensitivity of radicle development to increasing concentration of aqueous extract as compared to control.

The results presented in table 3 indicate that control treatment had the highest shoot height (10.58cm). The minimum shoot height (3.91cm) was observed in treatment containing 200g of fresh leaf segment mixture of *E. camaldulensis* and *E. globulus*. The inhibition of shoot height may be due to the presence of higher amount of volatile chemicals like α-pinene, β-pinene, and cineole (Del Moral and Muller, 1978). This shows that Eucalyptus possess different allelochemicals which might have inhibited cell division. These phenolic compounds might have interfered with phosphorylation pathway or inhibiting the activation of magnesium ions and ATPase activity or might be decreased synthesis of total carbohydrates, proteins and nucleic acids, interference of mineral uptake and biosynthetic processes (Sasikumar *et al.,* 2002). Romel *et al.* (2008) reported that the water soluble allelochemicals in Eucalyptus fresh leaf segments inhibited shoot length of radish, onion and tomatoes which is in line with the present study.

The fresh leaf segment mixture of *E. camaldulensis and E. globulus* caused significant reduction in leaf size and area. The highest leaf number and leaf size was observed in control and least in treatment with 200g of fresh leaf segment mixture of *E. camaldulensis* and *E. globulus*. The results agreed with those reported by Djanaguiraman *et al*. (2002) who reported inhibition of seedling growth on green gram, black gram and cowpea as well as reduced leaf size and leaf area index. Leaf expansion was sensitive and responded strongly to increasing concentration of Eucalyptus leaf biomass. The interruption of one plant process by the phenolic compounds released from decomposition of Eucalyptus leaves may have affected other processes like leaf photosynthesis, transpiration, stomatal conductance of leaf and root respiration in cowpea (Djanaguiraman *et al*., 2002, 2003).This may have directly or indirectly reduced size and area of the leaf.

The results showed that the content of chlorophyll were also reduced significantly ($P \leq 0.05$) in all treatments over control (Tables 3 and 6). Moreover, the reduction of chlorophyll content was observed to decrease steadily in treatment containing 150g and 200g of fresh leaf segment mixture of *E. camaldulensis* and *E. globulus*. The results of the present study may be attributed to degradation of chlorophyll pigments as reported by Singh and Rao (2003).They reported thatthe reduction of chlorophyll content in the above concentration of fresh leaf segments

mixture of *E. camaldulensis* and *E. globulus* may be due degradation of chlorophyll pigments or reduction in their synthesis. The action of flavonoids, terpenoids or other phytochemicals present in leaf segments (Djanaguiraman *et al*., 2003).

As the chlorophyll concentration decrease the rate of photosynthesis decreases leading to substantial decrease in all metabolites: total soluble sugar, proteins and soluble amino acids (Singh and Rao, 2003).

Three weeks old seedlings were uprooted and data were recorded and analyzed. The results are presented in tables 4 and 7.The fresh leaf segment mixture of *E. camaldulensis* and *E. globules* decreased shoot and root fresh weights. Treatment with 200g of fresh leaf segments mixture of *E. camaldulensis* and *E. globules* greatly reduced the shoot and root fresh weight of *Sorghum bicolor.* The interaction showed direct relationship between concentration and decrease in fresh weight. At lower level (50g) amounts of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus*, comparatively less reduction in the fresh weights was observed.

This indicate that Sorghum seedlings affected by Eucalyptus extract can tolerate stress up to some extent, but as the amounts increased, significant reduction in fresh weight and growth of the seedlings was observed. The reduction in fresh weight might be due to allelopathic allelochemicals which may have hindered absorption of water leading to less food manufactured as reported by Yang *et al. (*2000) after treatment of rice plant with three allelopathic phenolics.

The data in tables 4 and 7 reveal that different amounts (50g, 100g, 150g and 200g) of fresh leaf segments mixture of *E. camaldulensis* and *E.globulus*significantly ($P \leq 0.05$) reduced shoot and root dry weight of sorghum over control (0g). The slight decrease in dry matter in treatments (50g, 100g, 150g and 200g) of Eucalyptus leaf segments might be attributed to the tolerance of Sorghum to suppression by Eucalyptus leaf biomass. Sasikumar *et al.* (2002) reported that allelochemicals in Eucalyptus reduces most of cell physiological processes such as respiration, photosynthesis which directly reduced food accumulation hence lower dry weight. Therefore, from this study reduction in dry weight may be attributed by reduced physiological processes like photosynthesis and closely agree with findings of Sasikumar *et al*., 2002.

The interaction among sorghum seedlings and concentration was significant because of the inhibitory effect of allelochemicals in uptake of water by seedling and reduction in other physiological processes of sorghum seeding. The reduction in biomass may be due to stunted and reduced seedling growth. Dawar *et al*. (2007) reported that Eucalyptus leaf segments reduced growth and yield of wheat crop as well as sorghum. This harmful effect pointed out that

allelochemicals in any concentration present in soil could decrease the dry weight and yield of sorghum plant.

6.1 **CONCLUSIONS**

The studies provide the evidence that fresh leaf extract mixture and fresh leaf segment mixture of *Eucalyptus camaldulensis* and *Eucalyptus globulus* has allelopathic potential.The present investigation revealed that aqueous leaf extract mixture of *E. camaldulensis* and *E. globulus* at various concentration levels inhibited the germination,coleoptile and radical lengths of *Sorghum bicolor* seedling. Based on the overall findings it can be concluded that allelopathy is a concentration- dependent phenomenon whereby its effect increases as the concentration of the leaf biomass increases.

The treatment with the highest leaf biomass (200g) of fresh leaf segments mixture of *E. camaldulensis* and *E. globulus* reduced significantly the shoot height, leaf size, leaf area, fresh and dry weight and total chlorophyll content as compared to control.Its effectiveness on growth suggest that, leaf segment mixture of *E. camaldulensis* and *E.globulus* may act as a source of allelochemicals after being released into soil or after decomposition. The presence of allelochemicals negatively affects the neighbouring or succesional plants.

The test species (sorghum) responded negatively in a variety of ways to the water extracts of Eucalptus mixture. The results of this investigations support the hypothesis that one or more compounds in water extracts mixture of *E. camaldulensis* and *E. globulus* leaf segments inhibits growth of Sorghum. Further studies are suggested to clarify the possible physiological mechanism related to allelopathic effect of Eucalyptus as well as specific allelochemicals in *E. camaldulensis* and *E.globulus* which inhibit the growth Sorghum (*Sorghum bicolor)* plant.

REFERENCES

An M., Pratley J. and Haig T.(1996).Allelopathy; from concept to reality. Environmental and analytical laboratories and centre for conservation Farming, Charles Stout University, Wagga.

Anglami C. (1998). Sorghum for human food: A review of plant food on Human Nutrition.**52**:88-89.

Awika J.M. and Rooney L.W. (2004). Sorghum phytochemicals and their potential impact on human health. *Phytochemistry*.**65**(**9**):1199-1624.

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Bano S.,Ullah M.A.,Khaliq A., Abbasi K.H. and Khanum S.(2012). Effects of aqueous extract of sundried neem (*Azadirachtaindica*) leaves on wheat and wheat weed(wild oat) in Vitro. *International Research Journal of Plant Science.* **3**(**4**):69-73.

Belium V.S., Kumar A.A. and Sanjaa R.P. (2010).Recent advance in sorghum improvement Research at ICRISAT.*Kasetsart Journal of Natural Science*.**44**: 499-506.

Chen Z.Z., Ho C.K., Ahn I.S. and Chiang V.L.(2006).Eucalyptus methods. Molecular Biology.344:125-134.

Chiapusio G.,Sanchez A.M.,Rrigosa M.J., Gonzalez L. and Pellissier F.(1997).Do germination indices adequately reflect allelochemicals effects on the germination process? *Journal of Chemical Ecology*. 23:2445-2453.

Combs J.L.,Long S.I. and Scurlock J. (1985). Techniques in bioproductivity and photosynthesis.Pergamon Press Oxford, New York, Torontho, Sydney, Frankfurt.

Dawar S., Summira M., Younus Y.M. and Zaki M.J. (2007).Use of Eucalyptus species in the control of root infecting fungi on mungbem and chick-pea.*Pakistan Journal of Botany*.**39**(**3**): 975-979.

Del Moral R., Willis R.J. and Ashton D.H. (1978).Suppression of coastal heath vegetation by *Eucalyptus baxteri*.*Australian Journal of Botany*.**26**:203-219.

Djanaguiraman M.,Ravishankar P. and Bangarusamy U. (2002). Effects of *Eucalyptus globulus* on green gram, black gram and cowpea. *Allelopathy Journal*.**10**:157-162.

Djanaguiraman M., Senthil A. and Ramadass R. (2003). Assessment of rice genotypes for salinity tolerance at germination and seedling stage. *Madras Agricultural Journal*.**90:**506-510

Farrar J.L., Harte D.K., Hargrove J.L. and Greenspan P. (2008). A novel nutraceutical property of selected sorghum (*Sorghum bicolor*) brans: inhibition of protein glycation.*Phytotherapy Research*. **22**(**8**): 1052-1056.

Holmes T.P., Aukema J.E., Von Holle B.,Liebhold A. and Sills E.(2009). Economic impacts of invasive species in forests: past, present and future. *Year in Ecology and conservation biology*.**1162**:18-38.

Iqbal Z., Husain I., Hussain A. and Ashraf M.Y.(2003).Genetic variability to essential oil contents and composition in five species of Eucalyptus.*Pakistan Journal of Botany*.**35**(**5**):843-852.

Jambunathan R. and Subramanlan V. (1988).Grain quality and utilization of sorghum and pearl millet. *In biotechnology in tropical crop improvement.Proceedings of the international biotechnology workshop, Patancheru, India, 12-15 Janvier.**1987***: 133-139.

McWhorter C.G. (1984). Future needs in weed science. *Weed Science*.**32**: 850-855.

Musyimi D.M., Kahihu S.W., Buyela D.K. and Sikuku P.A. (2012). Allelopathic effects of Mexican sunflower {*Tithonia diversifolia (Hemsl*) A. Gray} on germination and growth of Spiderplant (*Cleome gynandra* L.) *Journal of Biodiversity and Environmental Sciences*. 2(8): 26-35.

Nentwing W. (2008).Biological invasion.Springer, Heidelberg, Germany.

Otusanya O.O., Adelusi A.A. and Ilori J.A (2007). Phytotoxicity effects of *Tithoniadiversifolia*on germination and growth of rice. *Research Journal of Botany*. **2**(**1**):23-32.

Pawar K.B. and Chavan P.D. (2004). Influence of leaf leachates of some plant species on free proline content in germinating seeds of *Sorghum bicolor*(L) Moench. *Allelopathy Journal*. **3**:89-92.

Pimentel D., Zinuga R. and Morrison D.(2005).Update on the environmental and economic cost associated with alien-invasive species in the United States. *Ecological Economics*.**52**:273-288.

Rich J.P., Grenier C. and Ejecta G.(2004).Striga resistance in the wild relatives of sorghum. *Crop Science*.**44**:2617-2622.

Romel A., RafiqualHoque A.T. and Hussein K.M. (2008).Allelopathic effects of leaf litters of *Eucalyptus camaldulensis* on some forest and agricultural crops.*Journal of Forest Research.***19**, (**1)**: 19-24.

Sala O.E., Chapin F.S., Armesto J.J.,BerlowE.,Bloomfield J.,Dirzo R. and Huber-Sanwald E. (2001).Global biodiversity scenerios for the year 2100.Science.287:1770-1774.

Samuel O.P., Sennifer A.R. and Keith C. (2005). Invasive plant can inhibit Native tree seedling: Testing potential Allelopatic mechanism. *Plant Ecology*.**18**:153-165.

Sasikumar K., Vijayalakshmi C. and Parthiban K.T. (2002).Allelopathic effects of Eucalyptus on blackgram (*Phaseolusmungo L*) *Allelopathy Journal*.**9**: 205-214.

Seigler D.S.(1996). Chemistry and mechanics of allelopathic interactions.*Argonomy Journal*.**88**:876-885.

Singh D. and Rao Y.B.2003.Allelopathic evaluation of *Andrographis paniculata* aqueous leachets on rice. *Allelopathy Journal*.**11**:71-76

Taiwo L.B. and Makinde J.O. (2005). Influence of water extract of Mexican sunflower (*Tithonia diversifolia*) on growth of cowpea (*Vigna unguicalata*). *Afghanistan Journal of Biotechnology*. **4**: 355-360.

Wendorf F., Clouse A.E., Schild R., Wasylikowa R.K., Hoxely R.A., Harlan R.A. and Krolik H.(1992). *Saharan exploitation of plants 8000 B.P.Nature.* **359**:721-724.

Yang L., Browing J.D. and Awika J.M. (2009). Sorghum 3-deoxyanthocyanins poses strong phase II enzyme induces activity and cancer cell growth inhibition properties, *Journal of Agriculture and Food Chemistry*. **57**(**5**):1798-1804.