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## EFFECT OF DIFFERENT CONCENTRATIONS OF AQUEOUS LEAF EXTRACTS OF SOME PLANTS ON THE GERMINATION AND SEEDLING GROWTH OF MAIZE, Z. mays L. AND WHEAT (T. aestivum L.)

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#### Abstract

Allelopathic effect of aqueous leaf extract of three different species (eucalyptus, guava, and litchi) were used to investigate their effect on germination, shoot and root lengths of two food crops *viz. Zea mays* L. (Maize) and *Triticum aestivum* (Wheat). Mature fresh leaves of three species were crushed and soaked for 24h; the filtrates were diluted to make different concentrations (T1 = 0.5%, T2 = 1.0%, T3 = 1.5%, T4 = 2.0%, and T5 = 0.0%), and were used to investigate their effect on the tested crops. The aqueous leaf leachate of three species was found to have inhibitory effect on germination, shoot, and root elongation on the tested crops. Current study indicates that inhibitory effect was much more pronounced at higher concentrations, and the effect increased with the increase in concentration. Among trees, *Eucalyptus* has much more effect on the tested crops than other species.

Key words: Aqueous Leaf extracts, germination, seedling growth, Maize, wheat

#### Introduction

Allelopathy is a biological phenomenon by which an organism produces one or more chemicals that influence the growth, survival and reproduction of nearby species. The allelopathic action of various natural compounds on the growth and development of many plants may be inhibitory or stimulatory depending on their concentrations in the surrounding medium and on their physiological activity within plants (El-Daly and Soliman, 1997). Allelochemicals inhibit seed germination by blocking hydrolysis of nutrients reserve and cell division (Irshad and Cheema, 2004), and cause significant reductions in the growth of plume and radical of various crops (Ogbe *et al.*, 1994). Einhellig (1995) mentioned that allelochmeicals may be selective in their action or plants may be selective in their responses. Moreover, allelochemicals which inhibit the growth of some species at certain concentrations may stimulate the growth of same or different species at different concentrations (Narwal, 1994). Allelochemicals once released, are short lived in the environment and therefore do not disastrously upset the balance as the chemicals would do (Einhellig, 2004).

Zea mays L. (Maize) is a member of the grass family Poaceae. It is a cereal grain that was first grown by people in ancient Central America. Wheat (*Triticum* spp.) is a cereal grain, originally from the Levant region of the Near East and Ethiopian Highlands, but now cultivated worldwide.

Guava (*Psidium guajava* L.) is a member of the family Myrtaceae, it is a single source of many beneficial components of herbal remedies, which are edible without any known detrimental effect (Gutierrez *et al.*, 2008). Guava leaf contains volatile oil as quercetin, avicularin, guaijaverin, etc (Morant *et al.*, 2008). Previous studies on the chemical composition of guava leaves have identified chemical products with allelopathic properties (Monteiro and Vieira, 2002) such as terpenoids, flavonoids, coumarins, cyanogenic acids (Begum *et al.*, 2002; Gutiérrez *et al.*, 2008).

Eucalyptus species are indigenous to Australia; they have been widely introduced into countries throughout the world because of their rapid growth and the rising demand for paper and plywood (Turnbull, 1999; Cossalter and Pye-Smith, 2003). Some 170 species, varieties and provenances of eucalyptus were tried in India (Bhatia, 1984), out of which the most outstanding and favoured has been the *E*. hybrid, a form of *E. tereticornis* known as Mysore gum. The allelopathic effects of eucalyptus have been studied extensively (Del Moral and Muller, 1969;

Willis, 1999; Sasikumar *et al.*, 2002; Bajwa and Nazi, 2005; El-Khawas and Shehata, 2005). Phenolic acids and volatile oils released from the leaves, bark and roots of certain Eucalyptus species have deleterious effects on other plant species (Sasikumar *et al.*, 2002; Florentine and Fox, 2003).

The Lychee (*Litchi chinensis*) is the sole member of the genus *Litchi* in the soapberry family, Sapindaceae. It is a tropical and subtropical fruit tree native to the Guangdong and Fujian provinces of China, and now cultivated in many parts of the world.

However, very few studies have been conducted to know the allelopathic effect of litchi on agricultural crops, so present study was carried out by keeping in mind the present scenario of having little knowledge of allelopathic effect of litchi. Present study was to elucidate the allelopathic potential of different concentrations of leaf extract of three tree species used by local of Doon valley (litchi, guava and eucalyptus) on maize and wheat. Such information should be beneficial when planning for sowing maize and wheat near or beneath of these respective trees.

# Materials and Methods

**Plant Extract:** Fresh leaves of field grown mature plants of three tree species viz, *Eucalyptus hybrid* (eucalyptus), *Psidium guajava* (guava), *Litchi chinensis* (litchi) were collected from agricultural land near Selakui Dehradun, India. The aqueous extracts were prepared from fresh leaves and were left for dry till complete moisture is lost and grinded into a fine powder separately in an electrical grinder. The powder was passed through 1.5 mm mesh and weighed to 2.5 g, 5 g, 7.5 and 10 g (leaf). These extracts were diluted and filtered through Whatman No. 1 filter paper to get different concentrations of aqueous extracts of 0.5 %, 1.0%, 1.5% and 2% solutions and the flasks were left for 48 hours at room temperature ( $25^{\circ}C \pm 35^{\circ}C$ ). Same procedure was repeated in other tree species.

**Bioassays:** 10 seeds of each food crop were placed in sterilized Petri-plates containing absorbent cotton which was distributed evenly on the surface and saturated with the respective concentration. The treatments were replicated 3 times and 3 replicates of control treatment with distilled water were also prepared. The seeds were observed every day and number of germinated seeds was recorded. Extract/distilled water was added just to moisten the seeds whenever required. The Petri plates were kept under natural light dark cycle (Rice, 1984) with temperature ranging from 30° C to  $35^{0}$ C. The following nomenclatures were used as  $T_{1} = 0.5\%$ aqueous extract;  $T_{2} = 1.0\%$  aqueous extract;  $T_{3} = 1.5\%$  aqueous extract,  $T_{4} = 2.0\%$  aqueous extract, and  $T_5 = Control$  (distilled water). The emergence of the radical from the seed was regarded as germinated and germination was recorded every day till the  $10^{th}$  day. The root length and shoot length of the seedlings were recorded on the 15 day of the experiment. Percentage of inhibition/stimulation effect on germination over control (T) was calculated the using the formula given by Surendra and Pota, (1978): I =  $100 - (E_2 \times 100/E_1)$ , where, I is % of inhibition/stimulation,  $E_1$  the response of control and  $E_2$  the response of treatment. Ratio of elongation was calculated as (Rho and Kil, 1986).

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

#### Results

#### 1- Effects of aqueous leaf extracts on germination:

The highest inhibitory effect (56.81%) was recorded in wheat in T4 treatment of *eucalyptus* while the lowest (5.66%) was in maize (T1) by litchi. All the tree species have inhibitory effect on the tested crops. Stimulatory effect was not observed for both maize and wheat leading to the conclusion that all the tree species have inhibitory effect on the germination of the tested crops. It has been also observed that as the concentration of the leaf leachate increases, and inhibitory effect also increases (Table 1, Figure 2). However, higher concentration level of leachates showed maximum reduction in germination.

| Table 1: Germination    | percentage     | of whea  | t and | maize | treated | with | different | concentration | ns |
|-------------------------|----------------|----------|-------|-------|---------|------|-----------|---------------|----|
| of aqueous leaf extract | t of three tre | e specie | s.    |       |         |      |           |               |    |

| Tree         | Litchi       |              | Gu           | ava          | Eucalyptus   |              |  |  |
|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--|--|
| species      |              |              |              |              |              |              |  |  |
| Crops        | Wheat        | Maize        | Wheat        | Maize        | Wheat        | Maize        |  |  |
| Treatment    | Mean         |              |              |              |              |              |  |  |
| T1           | 71(- 19.31)  | 50 (- 5.66)  | 74 (-16.85)  | 49 (- 23.43) | 73 (-17.04)  | 42 (- 6.66)  |  |  |
| T2           | 63 (- 28.40) | 48 (- 9.43)  | 69 (- 22.47) | 46 (- 28.12) | 58 (- 34.09) | 33 (- 26.66) |  |  |
| T3           | 60 (-31.81)  | 32 (- 39.62) | 64 (- 28.08) | 33 (- 48.43) | 44 (- 50)    | 27 (- 40)    |  |  |
| T4           | 56 (-36.36)  | 31 (- 41.50) | 45 (- 49.43) | 30 (- 53.12) | 38 (-56.81)  | 21 (- 53.33) |  |  |
| T5 (Control) | 88           | 53           | 89           | 64           | 88           | 45           |  |  |

Values in the parenthesis indicates the inhibitory (-) or stimulatory (+) effects in comparison to control (T5).

#### 2- Effects of aqueous leaf extracts on shoot and root elongation of maize:

The shoot and root length of maize were measured and compared with those of control are presented in table 2. The higher concentration of aqueous leaf extracts caused severe inhibition in comparison to control (T5). The highest shoot length (8.4cm) and root length (12.5cm) were recorded at T1 by litchi and lowest shoot (0.99cm) and root (1.4) length were recorded at T4 for *eucalyptus*. The maximum inhibitory effect on root and shoot length was found by *eucalyptus* in treatment T4 and minimum inhibitory effect in T4 was observed by litchi. Maximum root (88.65%) and shoot (79.39) elongation ratio was found in litchi at T1 treatment and minimum root (10.14%) and shoot (11.59%) elongation ratio were found in *eucalyptus* at T5 treatment (Fig 1 and 2).

 Table 2: Effects of aqueous leaf extracts of three tree species on shoot length (SL) and root
 length (RL) of maize.

| Tree species | Litchi       |               | Gua            | iva          | Eucalyptus    |              |  |
|--------------|--------------|---------------|----------------|--------------|---------------|--------------|--|
| Treatment    | SL (cm)      | RL(cm)        | SL (cm)        | RL(cm)       | SL (cm)       | RL (cm)      |  |
| T1           | 8.4 (-20.60) | 12.5 (-11.34) | 3.63 (-57.49)  | 4.1 (-61.32) | 4.95 (-42.03) | 3.2 (-78.26) |  |
| T2           | 6.8 (-35.72) | 10.7 (-24.11) | 3.44 (-59.71 ) | 3 (-71.69)   | 3.05 (-64.28) | 2.4 (-82.60) |  |
| T3           | 4.8 (-53.63) | 5.3 (-62.41)  | 2.18 (-74.47)  | 1.8 (-83.01) | 2.47 (-71.07) | 2 (-85.50)   |  |
| T4           | 3 (-71.64)   | 3.5 (-75.17)  | 1.12 (-86.88)  | 1.3 (-87.73) | 0.99 (-88.04) | 1.4 (-89.85) |  |
| T5           | 10.58        | 14.1          | 8.54           | 10.6         | 8.54          | 13.8         |  |

Values in the parenthesis indicates the inhibitory (-) or stimulatory (+) effects in comparison to control (T5).

#### 3-Effects of aqueous leaf extracts on shoot and root elongation of wheat:

The shoot and root length of wheat was greatly influenced with increasing concentration and caused severe inhibition in comparison to control (T5), the root and shoot lengths were measured and compared with those of control and are presented in table 3. The highest shoot length (5.24 cm) and root length (12.5 cm) were recorded in T1 for litchi and lowest shoot (0.33 cm) and root (0.75 cm) length were recorded in T4 for *eucalyptus*. The maximum inhibitory effect on root and shoot length was observed by *eucalyptus* in treatment T4 and minimum inhibitory effect in T4 was observed by litchi. Maximum root (80.65%) and shoot (63.98) elongation ratio were found in litchi at T1 treatment and minimum root (4.97%) and shoot (3.89%) were found in eucalyptus in T5 treatment (Fig 3 and 4).

| Tree<br>species | Litchi          |                | Guava          |               | Eucalyptus     |                |  |
|-----------------|-----------------|----------------|----------------|---------------|----------------|----------------|--|
| Treatment       | SL (cm)         | RL (cm)        | SL (cm)        | RL (cm)       | SL (cm)        | RL (cm)        |  |
| T1              | 5.24 (- 36.01 ) | 12.5 (- 19.35) | 3.51 (- 58.46) | 9 (- 37.93)   | 4.38 (- 48.16) | 9.4 (-37.74)   |  |
| T2              | 4.41 (- 46.15)  | 8.1 (- 47.74)  | 1.71 (- 79.76) | 5.3 (- 63.44) | 1.78 (- 78.93) | 4.2 (- 72.18)  |  |
| T3              | 5 (- 38.94)     | 5.58 (-64.00)  | 1.17 (- 86.15) | 3 (- 79.31)   | 0.43 (- 94.91) | 1.8 (- 88.07)  |  |
| T4              | 1.53 (-18.68)   | 3.09 (-80.06)  | 0.56 (- 93.37) | 2.1 (-85.51)  | 0.33 (- 96.09) | 0.75 (- 95.03) |  |
| T5              | 8.19            | 15.5           | 8.45           | 14.5          | 8.49           | 15.1           |  |

Table 3: Effects of aqueous leaf extracts of three tree species on root length (RL) of wheat.

Values in the parenthesis indicates the inhibitory (-) or stimulatory (+) effects in





Fig.1: Relative elongation ratio (RER) of shoot of Maize grown in petri dishes at different concentrations of three different species



Fig.2: Relative elongation ratio of (RER) Root of Maize grown in petri-dishes at different concentrations of three different species



Fig.3: Relative elongation ratio (RER) of shoot of wheat grown in petri-dishes at different concentrations of three different species



Fig.4: Relative elongation ratio of (RER) root of wheat grown in petridishes at different concentrations of three different species

#### Discussion

Most researchers have revealed that foliage leachates are a potent source of toxic metabolites and their toxic effects are species specific (May & Ash, 1990; Bhatt *et al.*, 1993; Todaria *et al.*, 2005). In our study the acceptability of the trees on tested crops decreased in the order *eucalyptus* > guava > litchi. Our study revealed that leaf leachates of the tree species tested influenced the germination, root and shoot length of the tested crops. The study revealed that the inhibitory effect of leaf leachate on the seed germination is a concentration dependent phenomenon and different species varied in their response to different leachates, our results was supported by Assaeed and Al Doss., 1997 and Mousawi and Al-Naib., 1975. Generally, in

studies with aqueous extracts, the observed inhibitory effects are attributed to change in pH raising concern about allelopathy and its ecological existence and relevance (Conway *et al.*, 2002, Harper 1977, and Sisodia, 2008). After making these observations, it could be concluded that extract of three tree species might possess some growth inhibitors which inhibit growth and germination of the tested crops. In our study, highest concentration level of leaf leachates showed maximum reduction in germination in the two tested crops. Similar observation was also found by Mousawi and Al Niab *et al.*, (1975), Sazada *et al.*, (2009), Siddiqui, *et al.*, 2009, Kalitha, *et al.*, (1996). It was well documented that, release of allelochemicals occurs at the time of germination or at the early developmental stage, as the plants are more susceptible in terms of competition with their neighboring plants for light, nutrients and water (Chou, 1992). An indirect relation between lower germination rate and allelopathic inhibition may be the consequence of inhibition of water uptake and alteration in the synthesis or activity of Gibberellic acid (GA<sub>3</sub>) (Neon, 1984).

In present study, it was observed that the growth and germination of two tested crops were mostly inhibited by leaf extract of *eucalyptus*; similar results were obtained by Singh and Bawa, (1982) while working on leaf leachates of *Eucalyptus globulus*. These inhibitory effects may be attributed to the allelochemicals such as phenolic acids, tannins and flavonides present in *eucalyptus* leaves (Shiva and Bandyopandhyay, 1985). Different inhibitory or stimulatory effects of various parts of the same plant are likely due to variability in the amount of phytotoxic compounds in different plant tissues (Rice, 1974; Nishimura *et al.*, 1982; May & Ash, 1990). The results of this study revealed that allelopathic influences are (i) species specific (ii) have different effects on germination and shoot and root growth, and (iii) the toxicity also depends on the concentration of allelochemicals in the medium. On an average, the leaf leachate of *eucalyptus* was more toxic to the germination and roots and shoot on both tested crops, while the leaf leachate of litchi was toxic to least toxic on the growth of the tested crops.

#### Conclusion

The inhibitory effect of leaf leachate on seed germination was concentration dependent phenomenon i.e. increases in concentration exerted more inhibition. This affirmed the fact that the response of the target crops was concentration dependent. The effectiveness of these extracts on the germination and growth of the crops showed that although presence of three studied trees have inhibitory effect on the tested crops but among them *Eucalyptus* was having most inhibitory effect, this effect would negatively affect the neighboring or successional crop plants mainly, wheat and maize.

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