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Allelopathic activity of hexane and ethyl acetate fractions of crude extract of leaves, bark and seeds of *Albizia-lebbek* L. (Benth.)

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Abstract
Allelopathy is resultant of competition between two neighbouring plant species for resources and other needs. Such competitions often enhance allelochemical production and accentuate their growth inhibitory tendency. Hexane and ethyl acetate fractions of crude extract of different parts viz. leaves bark and seeds of Albizia lebbek were tested for their allelopathic activity against germination of radish seeds. The percent growth inhibition of radish seeds by hexane and ethyl acetate fractions are being reported in this paper.

Keywords: allelochemical, fractions, albizia lebbek, allelopathic activity

1. Introduction
The plant systems are just like a vast natural laboratory and every plant is a unique chemist who synthesizes various photochemical with different specific geometry having different chemical and physical properties with their unique role in nature. Such phytochemicals may have stimulation or suppression effect on germination of other neighbouring plants. These compounds show biochemical interactions with living organisms i.e. plants or animals in a complex way. So the study of such phytochemicals which suppress or enhance the germination of other neighbouring plant is an important research task to understand the biochemical interactions of one plant to others. Allelopathy is resultant of competition between two neighbouring plant species for resources and other needs. Such competitions often enhance allelochemical production and accentuate their growth inhibitory tendency. Allelopathic inhibition typically results from a combination of allelochemicals which interfere with several biochemical and physiological processes in rhizosphere. "Allelopathy" is a Greek word meaning to suffer from each other. Allelopathy has been considered an important attribute to the success of a weed in natural ecosystems (Callaway and Ridenour 2004; Kimura *et al*. 2015; Lorenzo *et al*. 2010) [5, 2, 4]. Allelopathic activities of any weed has tendency to affect seed germination, seedling growth of neighbouring plant species, as well as of the same species, in both natural and agricultural systems (Dorning and Cipollini 2006; Bich and Kato-Noguchi 2014; Lara-Núñez *et al*. 2006) [3, 7, 1]. The study of allelochemicals is unanimous choice to control the germination of several herbaceous plants or weeds. Although the complete research of allelochemicals and their mechanism is lacking due to different concentration and interactions with different plants (Parvin *et al*., 2011) [6].

2. Materials and methods
1. Extraction and Preparation of fractions/extract from leaves and test solutions for screening of allelopathic activity
The shadow dried crushed pieces of leaves of *Albizia-lebbek* (3.0 kg) were taken into round bottom flask (5 lit.) and extracted with hot methanol for six hours. The solvent was removed to get extractives. The procedure was repeated thrice. The extractives were concentrated over water bath under reduced pressure to obtain the viscous mass. This viscous crude extract of leaves of *Albizia-lebbek* was mixed with silica gel (60 – 120 mesh) and fractionated successively (the solvent was mixed with extract and kept for six hours) with different solvent viz. hexane and ethyl acetate. The procedure was repeated four times. The fractionated solvent concentrated on water bath under reduced pressure to obtain the viscous mass. The viscous mass thus obtained was the fraction of respective solvent.

Crude extract and all obtained fractions were evaluated for their allelopathic activity.

2. Extraction and Preparation of fractions/extract from bark and test solutions for screening of allelopathic activity

The shadow dried chopped pieces of bark of *Albizia-lebbek* (3.0 kg) were taken into round bottom flask (5 lit.) and extracted with hot methanol for six hours. The solvent was removed to get extractives. The procedure was repeated thrice. The extractives were concentrated over water bath under reduced pressure to obtain the viscous mass. This viscous crude extract of bark of *Albizia-lebbek* was mixed with silica gel (60 – 120 mesh) and fractionated successively (the solvent was mixed with extract and kept for six hours) with different solvent viz. hexane and ethyl acetate. The procedure was repeated four times. The fractionated solvent concentrated on water bath under reduced pressure to obtain the viscous mass. The viscous mass thus obtained was the fraction of respective solvent. Crude extract and all obtained fractions were evaluated for their allelopathic activity.

3. Extraction and Preparation of fractions/extract from seeds and test solutions for screening of allelopathic activity

The shadow dried chopped pieces of seeds of *Albizia-lebbek* (3.0 kg) were taken into round bottom flask (5 lit.) and extracted with hot methanol for six hours. The solvent was removed to get extractives. The procedure was repeated thrice. The extractives were concentrated over water bath under reduced pressure to obtain the viscous mass. This viscous crude extract of leaves of *Albizia-lebbek* was mixed with silica gel (60 – 120 mesh) and fractionated successively (the solvent was mixed with extract and kept for six hours) with different solvent viz. hexane and ethyl acetate. The procedure was repeated four times. The fractionated solvent concentrated on water bath under reduced pressure to obtain the viscous mass. The viscous mass thus obtained was the fraction of respective solvent. Crude extract and all obtained fractions were evaluated for their allelopathic activity.

A stock solution of 2000ppm of each fraction / extract were prepared in minimum amount of acetone and made up with distilled water. Further dilutions of 1500ppm, 1000ppm, 500ppm and 100ppm were made up from that stock solution for analyzing their allelopathic activity.

3. Experimental section

Allelopathy Test

The seeds were surface sterilize in 95% ethanol for 15 seconds and sown in Petri plates of 90 mm diameter. In each Petri plate, ten seeds were taken. The Petri plates were layered with two ordinary filter papers each on with 7 ml of test solutions of different compounds of varying concentrations (100ppm, 500ppm, 1000ppm, 1500ppm and 2000ppm) were poured. A mixture of distilled water: ethanol (30: 1, 7 ml) was taken as control. Three replicates of each concentration were taken.

The radish (*Raphanus sativus* L.) seeds selected for activity analysis were collected from Kisan Seva Kender CCSHAU, Hisar. The variety of the seeds used was spheda. The investigations were carried out in the Department of Chemistry. The radish seeds were allowed to germinate at 25 ^{0}C in incubator with 12 hours of photoperiod. After 120 hours, the number of seeds germinated in each Petri plate were counted and percent seed germination inhibition values were calculated (Feo, 2003) [8].

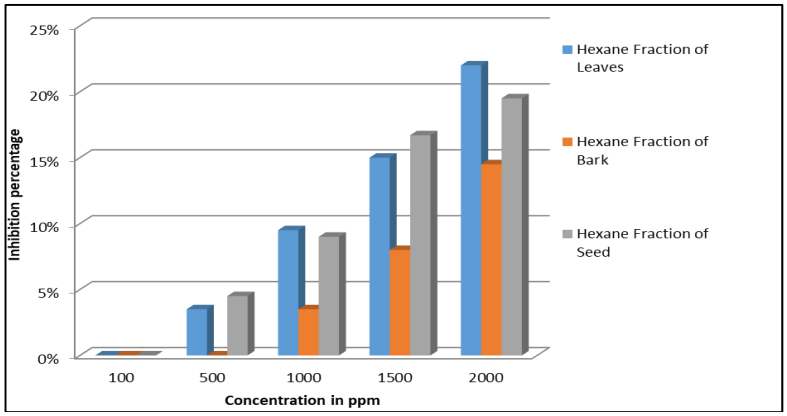

Fig 1.1: Allelopathic activity of Hexane fraction of leaves, bark and seeds of *Albizia-lebbek* (L.) Benth.

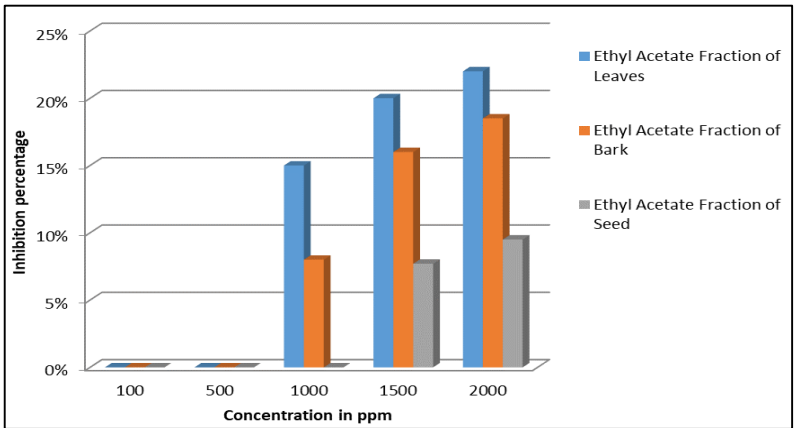

Fig 1.2: Allelopathic activity of Ethyl Acetate fraction of leaves, bark and seeds of *Albizia-lebbek* (L.) Benth.

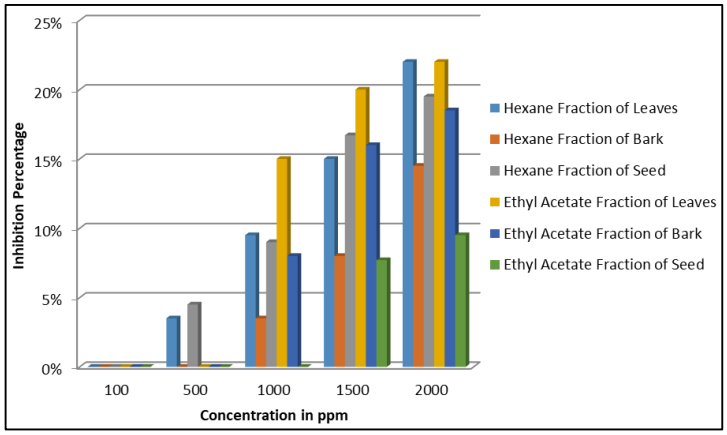

Fig 1.3: Allelopathic activity of Hexane and Ethyl Acetate fraction of leaves, bark and seeds of *Albizia-lebbek* (L.) Benth.

4. Result and Discussion

Allelopathy Activity

The data of activity presented in figure 1.1 revealed that maximum allelopathic effect i.e. 22% was shown by hexane fraction of leaves at 2000ppm followed by same fraction of seeds at 2000ppm i.e. 19.5%. Hexane fraction of seeds showed 16.7% inhibition at 1500ppm concentration followed by hexane fraction of leaves i.e. 15% at same concentration.

The data of activity presented in figure 1.2 revealed that maximum allelopathic effect i.e. 22% was shown by ethyl acetate fraction of leaves at 2000ppm followed by same fraction of leaves at 1500ppm i.e. 20%. Ethyl acetate fraction of bark showed 18.5% inhibition at 2000ppm concentration followed by similar fraction of bark i.e. 16% at 1500ppm concentration. Ethyl acetate fraction of seeds followed by similar fractions of bark and leaves at low concentration i.e.100ppm and 500ppm were found least active against germination of seeds of radish.

The data of activity presented in figure 1.3 revealed that maximum allelopathic effect i.e. 22% was shown by both i.e. ethyl acetate and hexane fraction of leaves at 2000ppm followed by ethyl acetate fraction of leaves at 1500ppm i.e. 20%. Hexane fraction of seeds showed 19.5% inhibition at 2000ppm concentration followed by ethyl acetate fraction of bark i.e. 18.5% at 2000ppm concentration. Ethyl acetate fraction of seeds followed by both i.e. hexane and ethyl acetate fractions of bark and leaves at low concentration i.e.100ppm and 500ppm were found least active against germination of seeds of radish. The comparison has been made between hexane and ethyl acetate fractions of different plant parts viz. leaves, bark and seeds of *Albizia-lebbek*. Leaves of *Albizia-lebbek* were found most effective allelopathic at higher concentration i.e. 2000ppm followed by hexane fraction of seeds and ethyl acetate fraction of bark. Besides the aetiological study of growth inhibition activity of specific species, there are persistent challenges in allelopathy to determine the mechanism of action of compounds and their metabolites. New frontiers will focus on allelopathy to enhance crop yield and develop a more sustainable weed and pest control.

5. References

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