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Allelopathic effects of bark, leaf and root exudate of *Guaiacum Officinale* (Lignum) on seed germination, root length, shoot length, dry weight of *Pennisetum Glaucum* (Millet)

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Abstract

The allelopathic effect of leaf, root and bark extract of *Guaiacum officinale* on seed germination of *Pennisetum glaucum* (millet) were studied by applying three treatments (10, 25 and 50% extracts of each). Maximum effect on germination was observed in 50% leaf extract of *Guaiacum officinale*, in which only 10% seeds were germinated, compared to bark 70% and root 70.33% respectively. It was found that the seedlings grown in 50% leaf extract have the lowest dry weight of 0.016 g compared to seeds grown in bark and root extracts of *Guaiacum officinale* (0.06 and 0.68 grams respectively). The average root length was observed to decrease with the increasing concentration of root and leaves extract of the plant, the lowest root length was observed in root extract (0.59 cm) as compared to bark and leaves extract (3.43 and 1.33 cm respectively). The lowest shoot lengths were observed in root extract (1.96 cm) as compared to bark and leaves extracts of the plant (1.96 and 4.0 respectively).

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Introduction

Allelopathy is derived from two words 'Allelon' and 'Pathos', Allelon means each other and pathos means suffer, it means the harmful effect of one plant on another plant (Roger Manuel, 2006). In 1937, Molishan Austria nprofessorf irst expressed the word Allelopathy by defining the term as the harmful effect of one plant species on the another plant species due to the release of certain types of allelo chemicals which are invisible in the environment but have significant effect on the plants which grows in their vicinity or grows nearby, these allelochemicals have the ability to inhibit the growth as well as the ability to retard the growth of other plant species. The concept of Allelopathy was further elaborated by different scientist with the passage of time i.e. (Bonner, 1950; Grummer and Beyer, 1960; Evenari, 1961; Whittaker, 1970; Pitman and Duke, 1978; Fischer *et al.*, 1978). Allelochemicals are produced by various parts of plant body such as stem, leaves, roots, flowers, inflorescence, fruits and seeds and these chemicals are produced as a byproduct or end product (Lavabre, 1991).

There are four ways by which allelochemicals are released in an environment 1. By leaching it occurs when fallen part of any plant such as bark and leaves get mixes with water and penetrate through the soil. 2. Decomposition: it occurs when plants die and get decomposed to the soil. 3. Exudation in this process root of a plant release different types of organic and inorganic substances which becomes harmful for those plants which grow nearby. 4. In this process allelochemicals are released from the leaves of certain types of plants known asterpenes (Qin).

One of the toxic chemicals known as Juglone has the capability to inhibit the growth of many trees such as pine, birch, blueberry and tomato. These chemicals have positive as well negative effects on other plants species. Allelochemicals are also known as a tool of biological warfare which a plant species uses to defend its territory by defending the growth of other plants (Qin).

Materials and methods

The leaves, barks and live roots were collected from the Guaiacum officinal (Lignum) trees of Institute of Environmental studies, University of Karachi at (24.9400° N, 67.1200° E) in the month of November 2013.

Leaves barks and roots were collected from the plant in vegetative form and washed with tap water before placing them in an oven for drying at 70 C after drying all the materials were grinded to form a power to get easily an aqueous solution.

Powders of each (barks, leaves and roots) were mixed in 100 ml distilled water separately. Aqueous extract was obtained as filtrate of the mixture and final volume was adjusted to 100 ml; this gave 10% aqueous extract. The extract was considered as stock solution and a series of dilutions with different strengths (50, 25, and 10%) were prepared.

The seeds of *Pennisetum glauccum* (millet) were used as test specie ten seeds were placed in each sterile petri dish (9 cm diameter) lined with whatman's filter papers and 5 ml of extracts of each concentration were added to each petri dish as per treatments (Gill *et al.*, 1993). The distilled water was used as control. Three replicates were used for each treatment and control and the experiment done using complete randomized design. The experiment was conducted at room temperature (25°C), diffused light (during the day) was made available for the experiment.

Germination rate was observed on the second day, while shoot and root length were registered on the 7th day, counted from the beginning of the experiment. After one week, percentages of germinated seeds were recorded and with the help of scale the length of roots and shoots was measured in cm (Devi and Dutta, 2012).

Results and discussions

Percentage Germination of *Pennisetum glauccum* Grown in Leaves Extract of *Guaiacum officinale*

In aqueous leaves extract of lignum the rate of germination declined as compared to root and bark of lignum. It was found that $56.66 \pm 4.30\%$ of seeds were germinated in control. The rate of germination started to decline with the increase in the concentration of the extract of leaves of lignum. The percentage of grown seeds in 10% extract of leaves of lignum was recorded to be $43.33 \pm 3.33\%$ while in 25% it was observed to be $33.33 \pm 2.45\%$ and in 50% leaves extract it declined to $10 \pm 3.98\%$ which is the lowest as compared to other treatments.

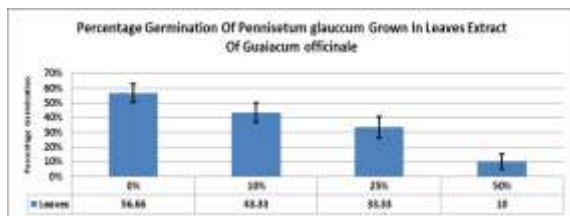


Fig. 1. Percentage of Germination of *Pennisetum glauccum* grown in leaves extract of *Guaiacum officinale*.

Percentage Germination of *Pennisetum glauccum* Grown in Root Extract of *Guaiacum officinale*

In aqueous root extract of lignum, it was found that the percentage germination of *Pennisetum glauccum* seeds declined in treatments as compared to control. The percentage of seed germination in control was $80 \pm 6.85\%$ while it was found that in 10% extract, it decreased to $76.66 \pm 5.20\%$ it further decreased to $73.66 \pm 5.01\%$ in 25% root extract of lignum, while in 50 % root extract the percentage of seeds germination was observed to be $70.33 \pm 5.77\%$.

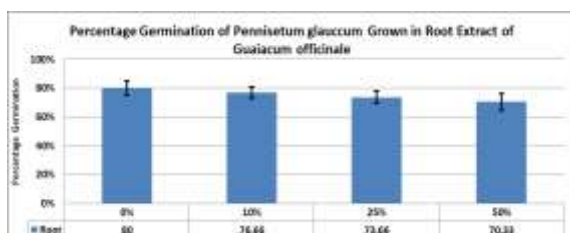


Fig. 2. Percentage of *Pennisetum glauccum* grown in root extract of *Guaiacum officinale*.

Percentage Germination of *Pennisetum glauccum* Grown in Bark Extract of *Guaiacum officinale*

In bark extract the percentage of germination was less in all the concentrations, compared to control. As the concentration increased, the rate of seed germination started to decline, higher inhibition was observed in 50 percent concentrations of bark of lignum $70 \pm 5.2\%$. The percentages which were observed in various concentration of bark extract of lignum are as follow. In control, the germination of seeds was $83.33 \pm 6.77\%$, in 10% bark extract it was found to be $80 \pm 6.87\%$. In 25% bark extract the percentage of seeds germination was observed to be $76.66 \pm 5.24\%$ while in 50 % bark extract it was observed to be $70 \pm 4.98\%$ which was least as compared to other treatments.

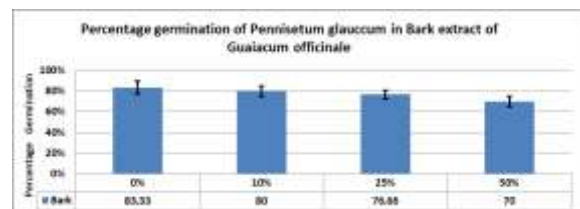


Fig.3. Percentage of germination of *Pennisetum glauccum* in bark extract of *Guaiacum officinale*.

Determining Average Dry Weights of Seedlings Grown in Leaves Extract of *Guaiacum officinale*

The results of dry weights observed in different concentration of leaves extract of lignum are as follow: it was observed that the dry weight start to decline very rapidly when the concentrations of leaves extract started to increase.

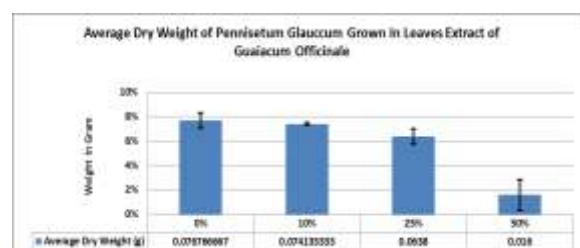


Fig. 4. Average dry weight of *Pennisetum glauccum* grown in leaves extract of *Guaiacum officinale*.

The highest dry weight was observed in 10% leaves extract of lignum 0.074 ± 0.0009 grams when compared to other treatments, in control the dry

weight was observed to be 0.076 ± 0.0063 grams and it started to decrease to 0.063 ± 0.0064 grams in 25% and 0.016 ± 0.012 grams in 50% root extract of lignum was observed.

Determining Dry weights of Seedlings Grown in Root Extract of *Guaiacum officinale*

In different concentration of root extract of lignum the results are as follow. It was observed after weighing that the dry weights start to decrease when the percentage of extracts of lignum root starts to increase it was seen that the lowest concentration was observed in 50% root extract 0.687 ± 0.019 gram while the highest dry weight was observes in the control 0.77 ± 0.0331 grams.



Fig. 5. Average dry weight of *Pennisetum glaucum* grown in root extract of *Guaiacum officinale*.

Determining Dry weights of Seedlings Grown in Bark Extract of *Guaiacum officinale*

Dry weight determination is one of the important parameter of allelopathy. When the dry weights of seedlings of *Pennisetum Glaucum* were determined in bark extract of lignum it was observed to be decreasing with the increasing concentrations.



Fig. 6. Average dry weight of *Pennisetum glaucum* grown in bark extract of *Guaiacum officinale*.

It was observed that in control the dry weights of seedlings were 0.75 ± 0.1 grams and it reduced 0.70 ± 0.02 grams in 10%. In 25% bark extract of *Guaiacum officinale* the dry weight reduced to 0.27 ± 0.02

grams and it further reduced to 0.06 ± 0.01 grams in 50 % bark extract of lignum.

Determining Average Root and Shoot Lengths of *Pennisetum Glaucum* seeds Grown in Bark, Root and Leaves Extract of *Guaiacum officinale* (Lignum) Observed Root lengths and shoot lengths in Leaves extract

Measuring of root lengths is one of the important parameters which are used to determine the allelopathic effects of different allelochemicals on root and shoot lengths. When determined the average root lengths of *Pennisetum glaucum* seeds grown in leaves extract of lignum it was observed that the average root lengths of *Pennisetum glaucum* seeds decreased with the increasing concentration, it was observed that the average root lengths in 0% was highest and it was 10.51 ± 1.16 cm, which decreased to 4.54 ± 0.46 in 10% and 1.55 ± 0.24 cm in 25% and 1.33 ± 0.11 cm in 50% leaves extract Of lignum was observed. When calculated the average shoot lengths of *Pennisetum Glaucum* seeds grown in leaves extract of lignum it was observed that the average shoot length of seedlings of *Pennisetum glaucum* in 10% leaves extract of lignum has the highest average shoot length of 4.81 ± 0.41 cm compared to other treatments. The average shoot length decrease to 3 ± 0.26 cm in 25% leaves extract and it was observed that in 50% leaves extract the average shoot length increased to 4 ± 0.4 cm. In control the average shoot length was observed to be 4.48 ± 0.44 cm which is lower as compared to 10% leaves extract lignum.

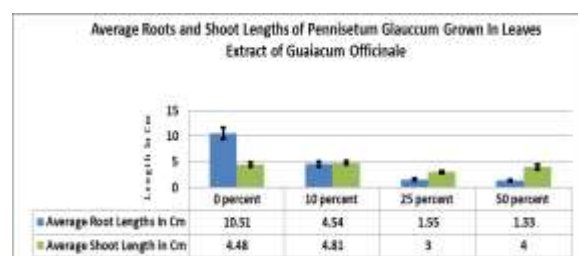


Fig. 7. Average roots and shoot lengths of *Pennisetum glaucum* grown in leaves extract of *Guaiacum officinale*.

Observed Root lengths and shoot lengths in Root extract

When determined the root lengths of seedlings grown in root extract of lignum it was observed that the average root length decreased with the increasing concentration. The highest average root lengths were observed in control which was 6.3 ± 0.86 cm, the average root lengths decreased to 2.07 ± 0.22 cm in 10% root extract, 1.16 ± 0.22 cm in 25% root extract and 0.59 ± 0.1 cm in 50% root extract of lignum was observed.

When measured the shoot lengths of *Pennisetum glauccum* seeds grown in root extract of lignum different variation has been observed with the increasing concentration, it was observed that in 0 % (control) the shoot length was highest as 5.3 ± 0.72 cm which started to decrease in 10% to 1.74 ± 0.14 cm and 1.51 ± 0.16 cm in 25%, the average shoot length increased to 1.96 ± 0.12 cm in 50% root extract of lignum.



Fig. 8. Average roots and shoot lengths of *Pennisetum glauccum* grown in root extract of *Guaiacum officinale*.

Observed Root lengths and shoot lengths in Bark extract

When observed the average root lengths in different concentrations of bark extract of lignum it was observed that 25% bark extract has the highest average length of 6.86 ± 0.82 cm (mean $\pm$ SE) while the average root length in 10% extract was nearer to 25% it was observed to be 6.62 ± 0.49 cm. The average root length decreased to 3.43 ± 0.39 cm in 50 % extract and 1.66 ± 0.35 cm in control treated with distilled water. When measured the average shoot lengths of seedlings of *Pennisetum glauccum* grown in bark extract of lignum it was found that the average shoot lengths of seedlings decreased when there is

increase in the concentration of bark extract. It was observed that the control has the lowest shoot lengths of 1.99 ± 0.22 cm it increased to 3.38 ± 0.29 cm when the concentration increased to 10% further the shoot length reduced to 3.27 ± 0.35 cm in 25% with and 2.56 ± 0.28 cm was observed in 50% bark extract of *Guaiacum officinale*.

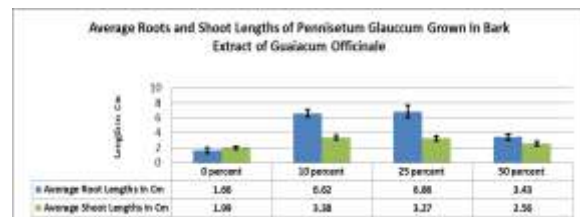


Fig. 9. Average root and shoot length of *Pennisetum glauccum* grown in bark extract of *Guaiacum officinale*.

Discussions

From the results it was found that leaf extract had the strongest allelopathic effect on seed germination. It was also found that the inhibitory allelopathic impact of leaf extract was more powerful than other vegetative parts. It is also observed that the leaves of *bunburyana* and *A. codonocarpa*, *M. georgei* and *E. tomentosa* leaves, have significantly inhibited the growth of seeds, a greater inhibition is observed when *bunburyana* seeds were treated with its own leaves (Jefferson and Pennacchio, 2003). It's also observed that when the seeds of *Zea mays* were treated with the leaf extract of *Partheniumhysterophorus* and *Chromolaenaodorata* a greater inhibition was observed (Devi and Dutta, 2012).

It is reported that the leaves of *Chromolaenaodorata* contains allelochemicals which inhibit the growth of many plants (Gill *et al.*, 1993). It's also observed that the extracts of *Partheniumhysterophorus* reduces the root and shoot lengths of *Oryza sativa* and wheat (Devi and Dutta, 2012).

When compared the roots and shoots lengths of *Pennisetum glauccum* seeds grown in leaves extract it was observed that the shoot lengths were higher as

compared to root lengths. In control the root length was higher than the shoot lengths.

In root, extract of 25 and 50% the shoot lengths was longer when compared to the root lengths but a difference was observed in 0% (control) and 10% root extract of *Guaiacum officinale*. In both of the concentrations, the root lengths were longer as compared to shoot lengths.

When compared the root and shoot lengths in bark extract it was observed that the root lengths were higher as compared to the shoot lengths in 10, 25, and 50% respectively but in control the shoot length was higher than the root length.

The dry weights decreased significantly with the increasing concentration of both bark and leaves extract of *Guaiacum officinale*, a very little difference was observed in the dry weight of seeds grown in root extract.

The percentage of seeds germination decreased in bark and root extract very slowly with the increasing concentration while the rate of germination started to decrease very rapidly in leaves extract with the increasing concentration.

The study demonstrated that aqueous leaf extracts of *Guaiacum officinale* exhibited significant inhibitory effects on seed germination and seedling growth of all test species while the root and bark extract of *Guaiacum officinale* doesn't show as much inhibition as compared to leaves extract.

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