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Effect of Root, Stem, Inflorescence Extracts and Root Exudation of *Lepidium Pinnatifidum* Ledeb. on *Triticum Aestivum* L. and *Zea Mays* L. and Few Biological Applications of Its Root Exudates

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ABSTRACT

Plants have the ability to affect the physiology of other plants through allelopathic interactions. The purpose of the present work is to evaluate the allelopathic potential of root, shoot, inflorescence extracts and root exudates of *Lepidium pinnatifidum Ledeb.* on some selected agronomic plants, *Triticum aestivum* L. and *Zea mays* L. And also, to find out few biological applications of its root exudates. The study revealed that *Lepidium pinnatifidum Ledeb.* is highly toxic to the seed germination, root and shoot length, fresh and dry weight and metabolite concentration of *Triticum aestivum* L. and *Zea mays* L. Root, stem and inflorescence extracts of the plant under study showed a complete inhibition of seed germination of wheat and maize. The effect of root exudates on wheat and maize was also inhibitory but less intense as compared to the plant extract. The root exudates of *Lepidium pinnatifidum Ledeb.* also showed insecticidal, phytotoxic, antioxdant activities successfully. Thus, it can be concluded that *Lepidium pinnatifidum Ledeb.* has a strong inhibitory effect on crop plants and also its high toxicity of root exudates suggests the presence of a variety of allelochemicals, thus can be used for medicinal purpose as well. Similarly, the plant can be used as herbicide, insecticide and as an antioxidant.

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INTRODUCTION

Planet earth is the home to all living organisms. All living organisms are categorized into five kingdoms by Whittakar i-e Protists, Monera, Fungi, Plants and animals. All these organisms compete with one another for resources mandatory to life like water, oxygen, CO_2, minerals, food and space. Therefore, survival of one organism definitely affect survival of nearby individual. One unique method of competition is allelopathy.

The phenomenon of allelopathy is defined and explained by many scientists time to time, but still the concept needs more study to be understood. Allelopathy is a form of interaction between two organisms in which the presence of one organism in the vicinity of another organism affect that nearby organism. Usually allelopathy has been studied in plants more than other organisms. Plant has the capacity to release certain chemicals in their environment which can create a positive or negative effect on nearby plant. These chemicals vary greatly in their nature and are called as allelochemicals. Allelopathy is considered as only the negative impact of one plant on other plant by majority of scientists but the concept is not so. The effect may be stimulatory or inhibitory.

Allelopathy has been discussed since long time but the term was coined recently in 1937 by Molish. He is regarded as pioneer of the allelopathy as he described the term in his last publication Der Einfluss einer Pflanza anf die andere- Allelopathie. The term shows Greek origin 'allelon' and 'pathos' meaning suffering each other. The word allelon suggest reciprocal interaction but there is some confusion regarding these interactions as such interactions may not be necessarily harmful which is indicated by the term pathy meaning suffering. Molish described the term as promoting and inhibitory effect of a plant on other by production and release of allelochemicals. As he was aware of the concept that certain substances which shows toxic effect in high concentration can have a stimulatory effect in some lower doses. This concept is more clearly explained by Paracelsus who used the term hormesis and described it in a single phrase ``All the things are poison and are not

poison, only the dose makes things not poison`` (Duke et al., 2006).

Allelopathy is also known as `` Chemical Warfare among plants`` which shows that plants are not always gentle and kind. Allelopathy is not only the negative impact of one plant on other, but it includes the stimulatory influence also therefore broader term allelochemics was applied to generalized the concept to both positive and negative impacts. But allelochemics include all the chemical associations between organisms under this definition allelopathy can be regarded as sub discipline of allelochemics now known as chemical ecology (Whittakar and Feeny, 1971). Allelopathy is explained as any effect whether it is direct effect or indirect effect of one organism on their surrounding organisms by releasing some potential chemicals from their body parts (Rice, 1984). Fitter in 2003 described allelopathy as only the negative or inhibitory effect of one plant on other by release of chemicals. Allelopathy is the influential interaction of plants and animals by the way of release of physiologically active chemicals from plants which has the potential of affecting other plants, nearby micro-organisms and animals too. The term allelopathy is also burrowed by zoology the science of animals. The zoological concept shows the effect of sessile animals like corals on surrounding life. This concept gives a strong support to allelopathy in plants as it has been considered that sessile invertebrates like corals show strong affinities toward plants as they are the host to some of the aquatic plants (Gross, 2003). One of the most allelopathic plants include the American walnut (Juglans nigra) as it inhibits growth of seedlings or plantlets in its near surroundings, creating a toxic effect through release of metabolites from its leaves (Davis, 1928).

Allelopathic variety of rice has been studied broadly due to its strong potential against the growth of aquatic wild plants. These studies are helping the researchers to use the allelopathic genes from this variety into a high yield rice variety. By doing so the need of harmful and expensive herbicides can be reduced up to large extent (Olofsdotter, 2001). The study can be extended up to allelopathic garden grass production which can reduce the growth of weeds in home lawns and public places (Bertin & Weston, 2004). Allelopathic chemicals can be released from roots called root exudates. In soil the first ever organism which interact with these sorts of chemicals are the soil micro-organisms and most probably the soil inhabiting bacteria. Allelochemicals from root can inhibit the growth of soil nitrifying bacteria which will increase ammonium to nitrate ratio. The climax stage perennials prefer to use ammonium as compared to nitrates due to high energy cost in the absorption of nitrates. Allelopathic root exudates can be used for this purpose. (Rice, 1984).

Allelopathic effect can be shown by plants only if they can synthesize and release specific chemicals which contain ability to show certain special influence on other organisms. These potential agents are called allelochemicals. They are secondary metabolites in their nature and are different from macronutrients or micronutrients. They have been classified into 14 major classes which include open chain aldehydes, amines, alcohols, benzoic acid and cinnamic acid and their derivatives, phenols, anthraquinone, lactones, benzoquinone, large fatty acids, lactones and many more. (Rice, 1964). Growth controlling plant hormones are also considered as allelochemicals e.g. ethylene, Gibberelic acid and salicylic acid. (Han et al., 2013).

Different plants can release their allelochemicals in different ways. Some of the noted methods are described by various scientist from time to time. Plant leaves have the ability to synthesize allelochemicals which are washed away and leached down the soil with rain water e.g. Juglans nigra (Bode, 1958). Some allelochemicals are volatile compounds and are released directly into surroundings in gaseous form. e.g. Salvia leucophylla (Bode, 1958). Decomposing mulch of various crops also produce and release phytotoxic substances and can affect growth of next coming vegetation. Mulch of rye cause intense effect on germination of next season crop. (Barnes, 1986). Plant roots have the potential to produce and excrete their own chemicals that have been proven phytotoxic to the surroundings plants. These chemicals are generally called as root exudates. Rice have been proven to create high allelopathic influence through their root exudates on growth of weeds specifically (Olofsdotter, 2001).

Root Exudates

Underground root system of plants can secrete certain chemicals which can affect the germination and growth rate of neighboring plant and also affect activity of soil microbes. These root exudates have significant effect on soil flora, fauna and soil nutrients system. Amount of root exudates secreted by a plant depends on length and proliferation of root into the soil. The larger and deeply buried roots can produce large amount of root exudates as compared to weaker root system. The concentration of root exudates is also affected by many other biotic factors like age of the plant, species, cultivar. Also, abiotic factors have great influence on root exudates concentration like soil substrates and other environmental stresses. Chemically root exudates are a mixture of many different groups of some primary metabolites and also secondary metabolites (Rovira, 1969).

Allelopathy has been used for improvements of agricultural lands and crops since long times (Zeng, 2008). Since allelochemicals can affect plants growth and specifically can compete with herbs so can be used as natural herbicide, thus preventing the use of high chemical pesticides or herbicide. Such practice can prevent agricultural land from soil pollution due to addition of chemicals and also can reduce water pollution efficiently and as result can reduce overall environmental pollution a step toward conservation of ecosystem. (Li et al., 2010; Macias et al., 2003). In Pakistan the use of allelopathic practices has gained importance in recent years. (Cheema et al., 2013). The possibility towards use of allelopathy as an economic solution for many agricultural problems like pollution free control of pests and weeds, control of various diseases of plants, nitrogen conservation in soil, and the production of new agriproducts from allelochemicals has fascinated researchers' interest in the field of allelopathy. (Wezel et al., 2014). An angiospermic plant is selected for investigating allelopathy belonging to family Cruciferae or Brassicaceae named as Lepidium

pinnatifidum Ledeb. (Tutin et al., 1964). The plant is found in both hilly and plain areas of Pakistan and central Asia. Inside Pakistan plant has been reported from Abottabad, Muree, Punjab and Khyber pakhtunkhwa plains. In plains of KhyberPukhtunkhwa plains we reported the plant from district Nowshera, Mardan and Peshawar. Our study focussed on the plant loacated in district Nowshera and District Mardan where L.pinnatifidum was found in lands of Manki sharif, Nowshera cantonment along the road side while growing lavishly in the lawns of AWKUM Garden campus. Lepidium pinnatifidum is an annual herb. Its seed bank start germination in spring after the mid of March. Depending upon the condition of soil, moisture availability and temperature of the area growing season is altered a little bit. Lepidium growing in dry hilly areas vanishes earlier than the members of same specie growing in fertile lands of plains. Similarly, plants growing under the shade of tress grow healthy up to the mid of July. While in full sunlight and drier soil the plant completes its life duration up to the end of June. Seeds get matured in June requiring a temperature of 40 - 43 ℃ for ripening.

Figure 1 Lepidium pinnatifidum Ledeb. (Captured on 6 April 2022).

MATERIALS AND METHODS

Plant collection and extract preparation**:** Lepidium pinnatifidum Ledeb. was collected in last week of March and first two weeks of April from a variety of locations of District Nowshera including Manki sharif, Nowshera cantonment and Wapda housing society Nowshera. With the help of plant cutter stem, root and inflorescence were separated for the future study of their allelopathic potential. The separated plant parts were kept for drying in shade for 2-3 weeks. . Each part was grind into powder with the help of grinder for 5 minutes. All the apparatus including funnels, flasks, petri dishes, beakers, pipettes were sterilized by washing with water first and then sprayed with absolute ethanol to remove any kind of fungus. For more sterilization apparatus was kept in autoclave for 30 minutes for 99.9°F. Filter paper used in the experiment was also autoclaved for sterilization. Aqueous stem, root and inflorescence extract was prepared by taking 20gm of powdered stem, root and inflorescence and dissolved in 200 ml of distilled water in aluminium coated foils separately to avoid any damage to allelochemicals. Solutions were shaken continuously at 120 rev/min for 24 hrs for complete mixing. Similarly aqueous stem, root and inflorescence extracts for 48 hours and 72 hours were prepared. Methanolic and Acetonic stem, root and inflorescence extracts were prepared by the same method.

Root exudates collection**:** Root exudates were collected by Egle et al., 2003 protocol with slight modifications. Seeds of the plant under study were collected from locality of Manki sharif Nowshera Khyberpakhtunkhwa Pakistan and sterilized with absolute ethanol for 2-3 minutes. The seeds were then washed with distilled water three times to make them free of any impurity. Sterilized seeds were then sown in growth chamber containing a mixture of sand and quartz. After 21-22 days of germination plants were removed from the growth chamber, roots were washed thoroughly with distilled water to remove dirt and sand. In a sterilized beaker 0.05mM solution of $Cacl_2$ was prepared and Lepidium pinnatifidum Ledeb. were placed in the solution for 1hr in such a way that roots of the plants were totally submerged in the solution while stem remain above the solution. After 1hr plants were removed from the solution and transferred into another solution of 0.05mM $Cacl_2$ for 2-4 hrs for rapid collection of root exudates. Plants were removed and the solution containing root exudates was obtained.

Figure 2 Showing experimental set up for collection of root exudates from L. pinnatifidum.

Treatments**:** Seeds of Triticum aestivum L. variety AKBAR-2019 and Zea mays L. variety MERAJ-2019 were bought from Agriculture institute Nowshera khyberpakhtunkhwa Pakistan. Seeds were sterilized with absolute ethanol for 2-3 minutes and then washed with distilled water for three times to remove excess of ethanol. Seeds were kept in distilled water to break their dormancy. Sterilized petri dishes and whattman no. 41 filter papers were used. Two folds of filter paper were placed in each petri dish in aseptic environment. Ten seeds of wheat and maize were kept in each petri dish. 2-3 ml of aqueous, methanolic and acetonic extracts of stem, root and inflorescence were applied on the seeds of wheat and maize. Four replicas were prepared with a control. Distilled water was taken as control.

To study allelopathic potential of root exudates of Lepidium pinnatifidum solution of root exudates of various concentrations were prepared (25%, 50%,75% and 100%). Distilled water was taken as control.

LP-REX 25% 25% Root exudates of Lepidium pinnatifidum

LP-REX 50% 50% Root exudates of Lepidium pinnatifidum
LP-REX 75% 75% Root exudates of Lepidium pinnatifidum
LP-REX 100% 100% Root exudates of Lepidium pinnatifidum

Root exudates of each concentration were applied to the test crop plants in a petri dish bioassay. Seeds of wheat and Mays were soaked in distilled water for 30 minutes to break their dormancy. For every petri dish fitted with double fold whattman,s no 41 filter paper 10 seeds of test plants were taken placed with uniform distance. Four replicas were taken for each treatment of root exudates and a control of distilled water. 4-5 ml of each concentration of root exudates was applied to each petri dish in such manner that the filter paper moisture level reach to completion. Temperature was controlled at 25-30℃. Photoperiod of 12-15 hrs was provided under controlled conditions. Petri dishes were observed for 10 days to record rate of germination under the application of root exudates of various concentrations (25%, 50%, 75% and 100%). Data was collected after every 2 days. Similarly, data about radicle and plumule length, fresh and dry weight and content of some primary and secondary metabolites was recorded.

2.4 Biological applications of root exudates of Lepidium pinnatifidum

Following applications of root exudates of Lepidium pinnatifidum were investigated.

Insecticidal activity of root exudates of Lepidium pinnatifidum: To find out the effect of root exudates of Lepidium pinnatifidum in the control of insects' lab set up was arranged. Insect selected for the study was Tribolium castraneum commonly named as Red beetle of stored wheat grains. Insects were taken in small glass jars (250ml). Four replicas were prepared for each treatment (25%, 50%, 75% & 100%). Ten mL of each concentration was poured in the beakers; distilled water was taken as positive control and waited till the evaporation of root exudates. In each jar ten individuals of Triboluim castraneum were taken along with some wheat grains in each jar so that food supply is available to the insects continuously. Oxygen supply was maintained by keeping mouth of jar open. The whole setup was placed at room temperature. Data regarding the rate of deaths and survival of Tribolium castraneum was recorded after specified time using the following equation.

$$\% \text{ Mortality} = \frac{100 - \text{No of insects in the test} \times 10}{\text{No. of insects in control}}$$

Experiment was performed using method of Alif, 2019 with slight modifications.

Phytotoxic effect of root exudates of Lepidium pennatifidum:

To observe phytotoxic effects of root exudates aquatic plant Lemna minor L. was selected. Lemna minor is the known simplest and smallest angiosperm. It is an aquatic weed growing luxuriously in cold to normal temperature ponds. For this purpose Lemna minor was collected from the ponds water of District Nowshera Pakistan. Fifteen healthy plants were placed in each replicate. Four concentrations of root exudates were prepared in distiiled water, 25%, 50%, 75% and 100%. Twenty milliliters of each concentration of root exudates were applied to the growing aquatic weed took in 250ml glass beakers already sterilized. Four replica were prepared for the treatment. Growth inhibitors were taken as negative control. The whole setup was placed in growth chamber. Growth of the plant was observed for one week. The percentage of inhibition was calculated by using the following equation.

$$\% \text{ Inhibition} = \frac{\text{No of fronds in the test} \times 100}{\text{No. of fronds in control}}$$

Experiment was performed using method of Sultana et al., 2019 with slight modifications.

Antioxidant activity of root exudates of Lepidium pennatifidum:

Antioxidant potential of root exudates of L. pinnatifidum was checked following Shah et al, 2021 protocol by using DPPH (1-diphenyle-2-picrylhydrazyl) solution. First of all 0.004% w/v solution of DPPH was prepared in methanol (99.9%) and was taken as control. Various concentrations of root exudates (50µg/mL, 100µg/mL, 500µg/mL) were prepared marked as C_1, C_2, C_3. Two millilitre of 0.004% DPPH was added to all the concentrations in dark so that final volume reached to 3mL and were incubated at room temperature. After one hour absorbance of each sample was calculated at 517nm using spectrophotometer. Data was recorded and percent absorbance potential for each sample was calculated using the following Equation.

$$\% \text{ antioxidant potential} = \frac{\text{Absorbance of the control (nm)} - \text{Absorbance of test (nm)} \times 100}{\text{Absorbance of control}}$$

Data analysis**:** All the data recorded was analysed using SPSS software version 21 followed by ONE WAY ANOVA and DUNCAN test.

RESULTS AND DISCUSSION

Acetonic, Methanolic and aqueous extracts of root, stem and inflorescences of Lepidium pinnatifidum Ledeb. were applied to the selected crops seeds, wheat and maize after 24hrs, 48hrs, 72hrs. The effect on of crop seeds observed is given as follow.

Allelopathic effect of Lepidium pinnatifidum on Zea mays

Action of Lepidium pinnatifidum root, stem and inflorescence on Zea mays

The action of extract from Lepidium pinnatifidum root, stem and inflorescence was studied on following characters of maize.

Germination

Nine types of root extract of L. pinnatifidum, aqueous (24hrs, 48hrs, 72hrs), methanolic (24hrs, 48hrs, 72hrs) and acetonic (24hrs, 48hrs, 72hrs) were applied to the test plant seeds (Zea mays L.). The extract showed a strong inhibitory effect on the germination of test plant. The results indicate that all the types of the extracts showed a great inhibition on germination of seeds as compared to the control. All the seeds failed to germinate at all other than control. The same results were recorded for all the nine types stem and inflorescence extracts.

Plumule and radical

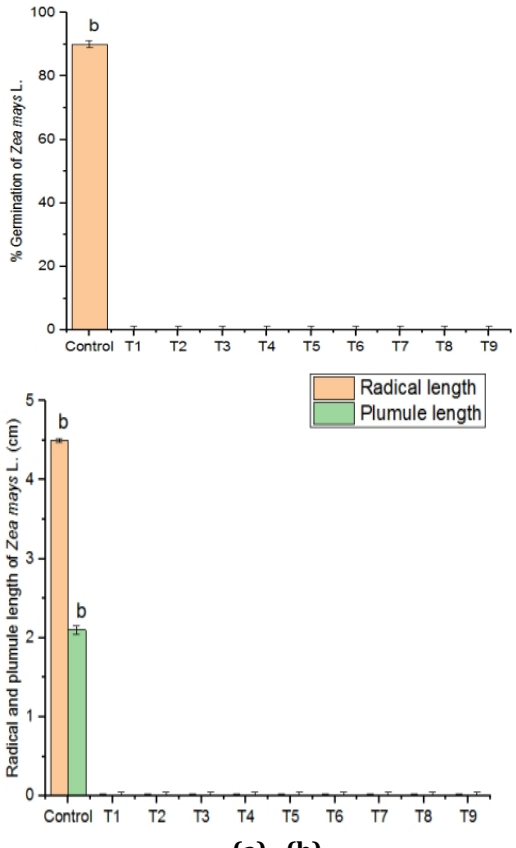
The seeds of the plant under observation (Zea mays L.) were treated with variety of root extract, aqueous root

extract (24hrs, 48hrs, 72hrs), methanolic root extract (24hrs, 48hrs, 72hrs) and acetonic root extract (24hr, 48hrs, 72hrs) of L. pinnatifidum. But neither of the seeds of the test replicates grew at all. Results declared zero length of radical and plumule of Zea mays L. seeds. While that of the seeds of control showed an increase in radical and plumule length. Similar was the case of all types of stem and inflorescence extracts of Lepidium pinnatifidum.

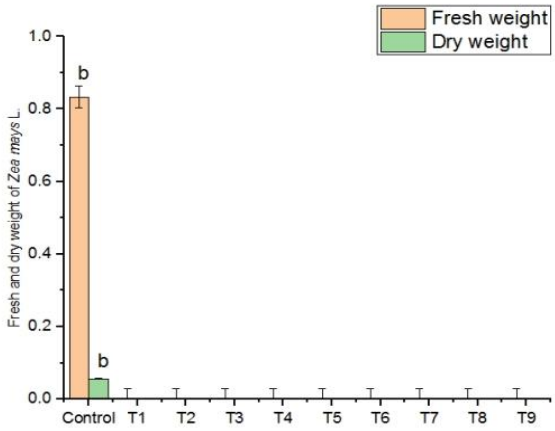
Fresh & dry weight

Aqueous root extract of L. pinnatifidum of different types (24hrs, 48hrs, 72hrs), methanolic root extract of three different types (24hrs, 48hrs, 72hrs) and acetonic root extract (24hrs, 48hrs, 72hrs) were applied on the seeds of test crop plant. But the seeds could not germinate into plantlets in the presence of each type of root extract. Thus the fresh and dry weight was zero as compared to the control. All the stem and inflorescence extracts showed the same results as those of root extracts of Lepidium pinnatifidum. The results match with allelopathic effect of Moringa oleifa Lamk plant extract on crop plant which strongly inhibits the germination, plantlet size and weight of crops (Tahir et al., 2018). Results are also matching with the complete inhibition of, germination and shoot length and root length of lettuce by the leaf extract of Dischidia imbricate (Blume) Steud. (Krumsri et al., 2019). The results match with the inhibitory action of Artimisia argyi on the germination, growth of seedlings and fresh and dry weight of Brassica pekinensis, Oxalis corniculata, Oryza sativa and Setaria virdis. (Li et al., 2021).

Fig 3. Showing the allelopathic action of extract from root of L. pinnatifidum on rate of germination (a) growth of shoot and root (b). fresh & dry weight of maize (c).

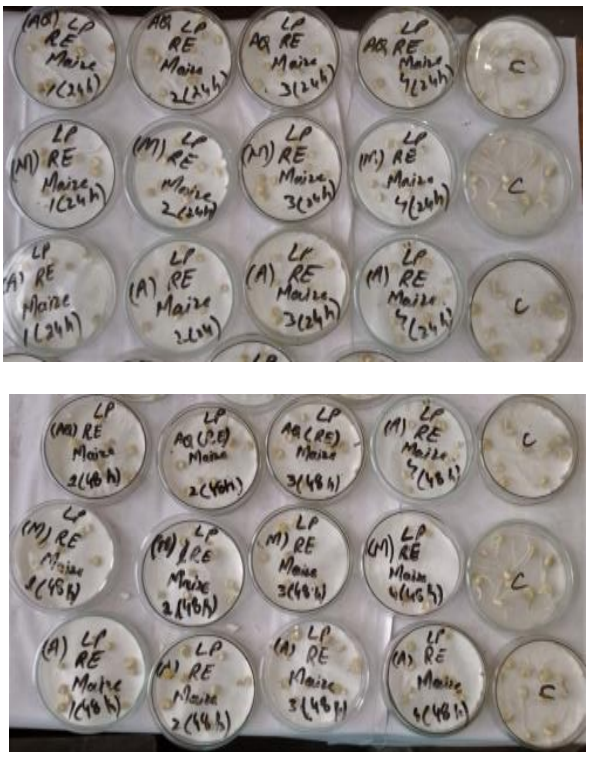


(a) (b)



(c)

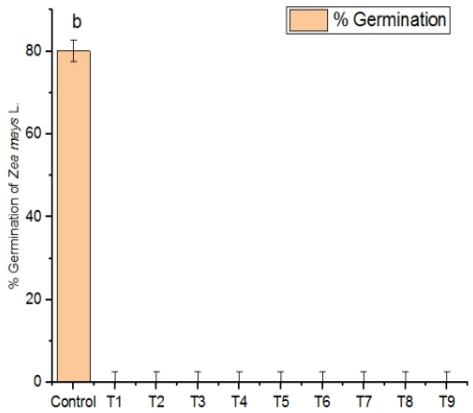
Fig 4 Effect of Lepidium pinnatifidum root extract (aqueous, methanolic, acetonic), 24hrs (a), 48hrs (b), 72hrs (c) on germination of Zea mays.

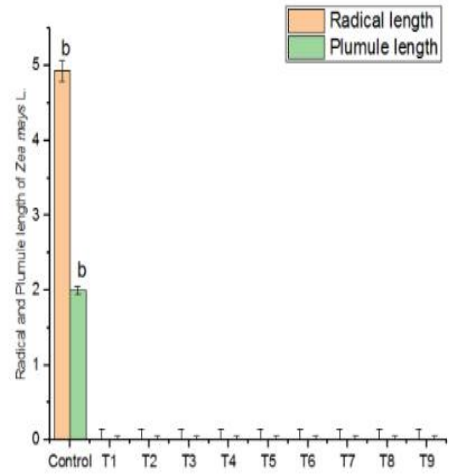


(a) (b)

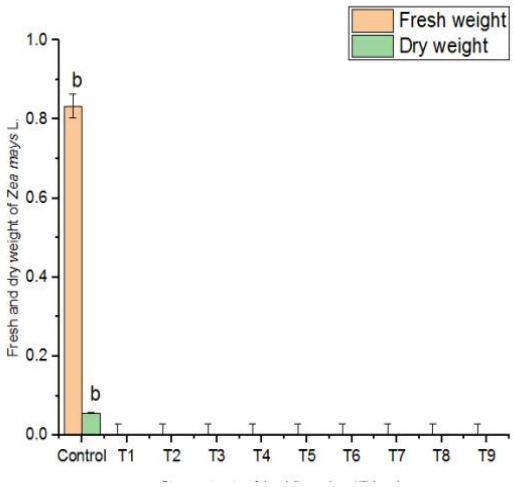
(c)

Fig 5 Allelopathic action of Lepidium pinnatifidum stem on germination (a), radicle and plumule length (b) and fresh &dry weight of Zea mays (c)



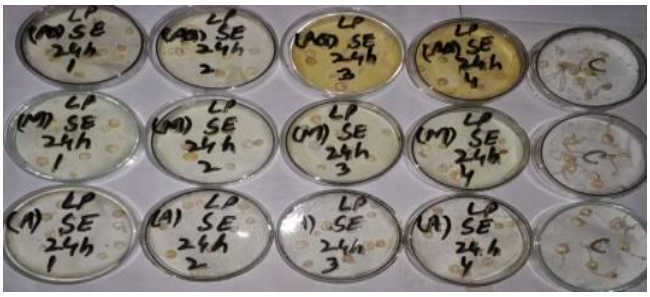


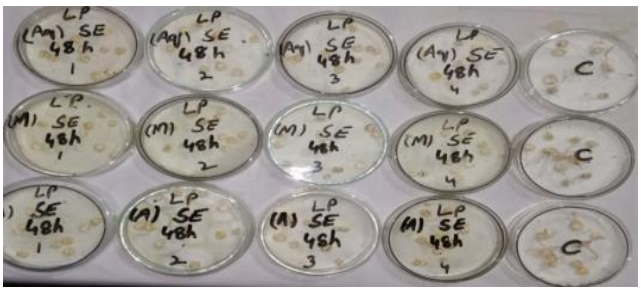
(a) (B)



(c)

Fig 6 Effect of Lepidium pinnatifidum stem extract (aqueous, methanolic, acetonic), 24hrs (a), 48hrs (b), 72hrs (c) on germination of Zea mays.

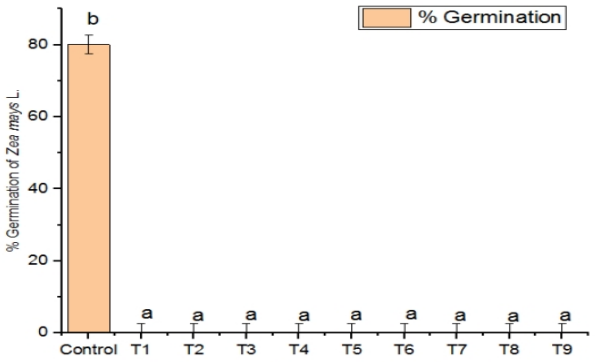


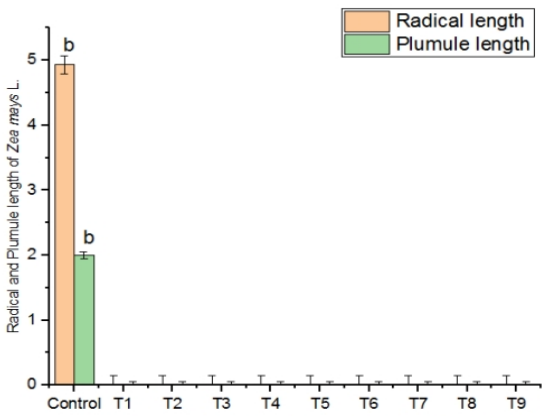


(a) (b)

(c)

Fig 7 Allelopathic activity of Lepidium pennatifidum inflorescence on rate of germination (a), growth of shoot and root (b) and fresh & dry weight in Zea mays.





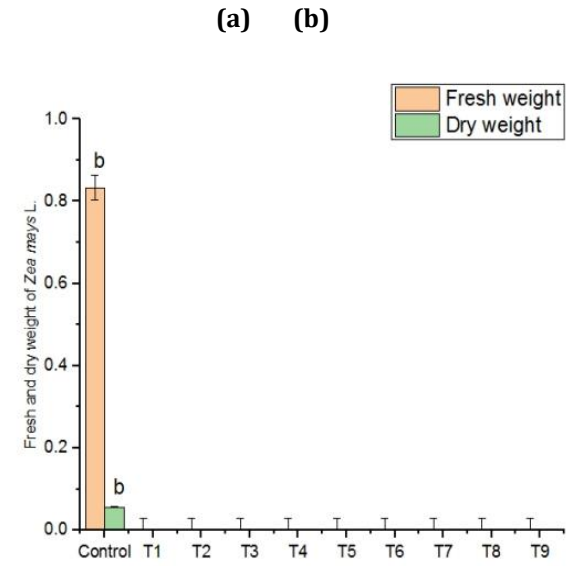
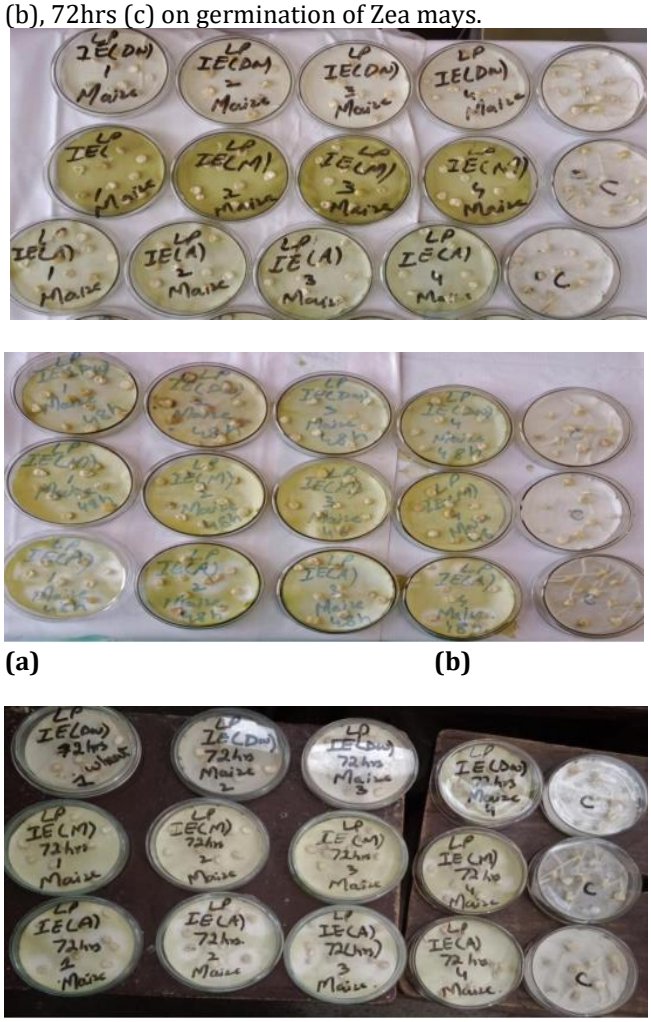


Fig 8 Effect of Lepidium pinnatifidum inflorescence extract (aqueous, methanolic, acetonic), 24hrs (a), 48hrs (b), 72hrs (c) on germination of Zea mays.



Action of root exudates from L. pinnatifidum on Maize Germination

Root exudates obtained from Lepidium pinnatifidum of varying concentration, 25%, 50%, 75% and 100% were applied to the maize seeds to check the effect of root exudates upon seed germination. Results indicated that seed germinate under 25% root exudates while a descending trend was seen in 50%, 75% while lowest germination under 100%. Highest rate of germination was observed under control.

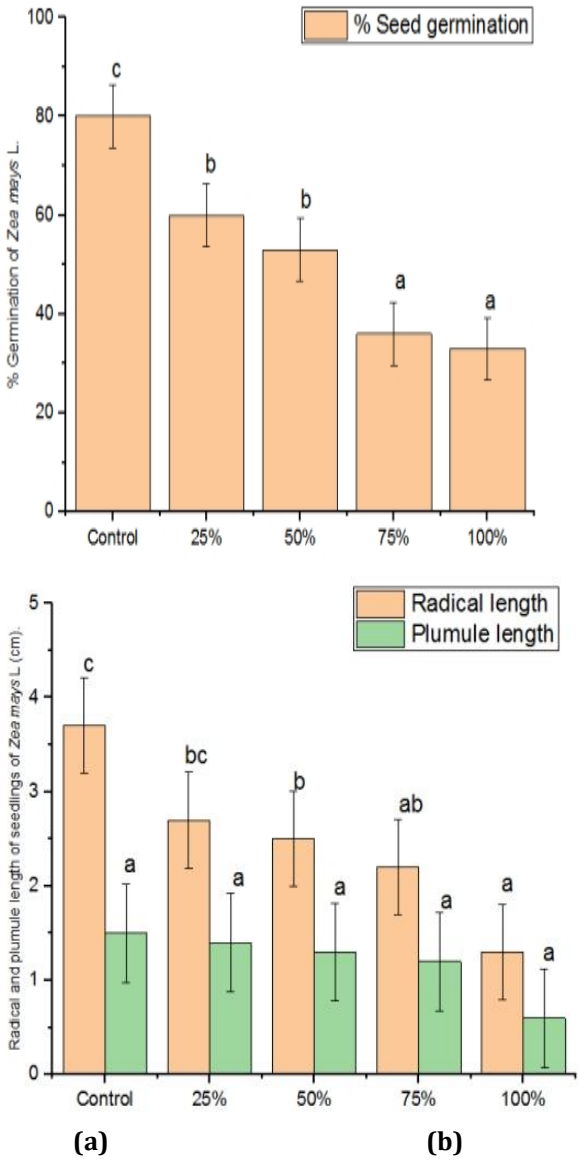
Radical & plumule

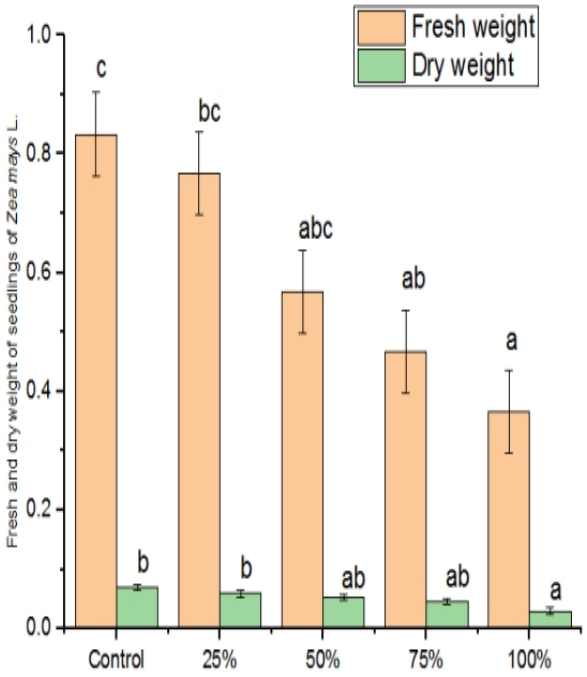
A variety of root exudates of Lepidium pinnatifidum (25%, 50%, 75%, 100%) were applied on maize seeds to check the allelopathic effect of root exudates of Lepidium pinnatifidum. Results declared a decreasing trend in radical and plumule length of test seeds under the effect of increasing concentration of root exudates. Highest growth was recorded in control while lowest was observed in 100% root exudates.

Fresh & dry weight

Maize seeds were treated against root exudates of Lepidium pinnatifidum of various concentration, 25%, 50%, 75%, 100% for their fresh and dry weight. Highest fresh weight was recorded in control, a continuous decreased at 50% and 75% was observed while fresh weight was highly affected under 100% root exudates.

Fig 9 shows the action of exudates from roots of Lepidium pinnatifidum on germination (a), shoot and root length (cm) (b) and fresh & dry weight (c) in maize.





(c)

Exudates of Lepidium pinnatifidum and phytochemicals in Zea mays L.

a Sugar

Sugar content of seedlings in Zea mays is affected by the various concentration of root exudates. Sugar concentration was highest at lowest concentration of root exudates (25%). While lowest sugar concentration was calculated at highest concentration of root exudates (100%).

b Chlorophyll

Root exudates exert inhibitory affect on concentration of Chlorophyll of seedlings of Zea mays With the increase in concentration of root exudates of Lepidium pinnatifidum., the amount of chlrorphyll a decreased in the seedlings.

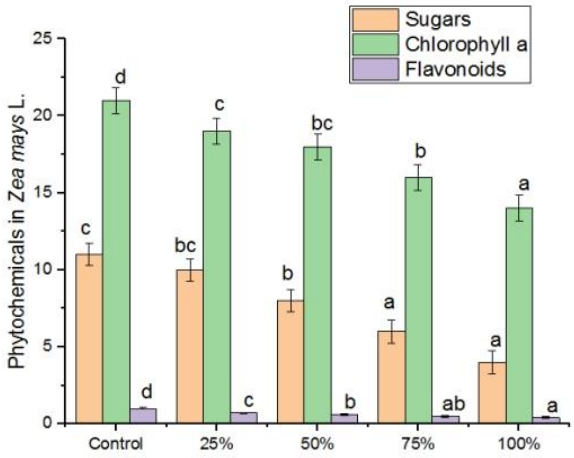
c Flavonoids

Flavonoids content in seedlings of maize was also affected by root exudates of Lepidium pinnatifidum. Highest concentration of flavonoids was found in control continuously decreased with increasing concentration of root exudates from 25%, 50%, 75%, 100%.

The results of evaluation of effects of root exudates collected from Lepidium pinnatifidum on seed germination, growth, fresh and dry weight and on phytochemicals contents of crop plants are similar with the allelopathic potential of root exudates of Sorghum bicolor L. on wheat and weeds growth and chlorophyll content showing continuous decrease in growth in continuously increasing concentration of root exudates of Sorghum bicolor L. (Naby, et al., 2021).

The results are also in comparison with the results of root exudates of Tithonia diversifolia on the germination rate of seeds, growth of seedlings, shoot and root length of crops. The content of metabolites in test crops was also affected negatively. The overall effect of root exudates was inhibitory. (Noguchi, 2020).

Figure 10 Determination of Sugars, Chl a and Flavonoids in Zea mays L.



Action of Lepidium pinnatifidum on Triticum aestivum L.

To investigate the allelopathic activity of Lepidium pinnatifidum on Triticum aestivum following charaters of wheat were brought under study.

a Germination

The seeds of wheat were treated with various treatments of root, stem and inflorescence extracts, methanolic (24hrs, 48hrs, 72hrs), acetonic (24hrs, 48hrs, 72hrs) and aqueous (24hrs, 48hrs, 72hrs). Results showed that all the treatments of extracts (stem, root, inflorescence) exerted an inhibitory action on the seeds germination as all the seeds fail to germinate except in control.

b Plumule & radical size

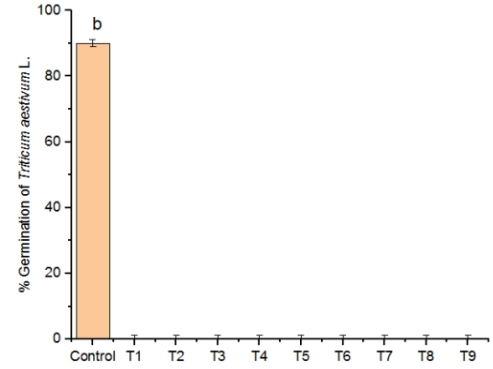
Triticum aestivum seeds were treated with variety of root extract, aqueous root extract (24hrs, 48hrs, 72hrs), methanolic root extract (24hrs, 48hrs, 72hrs) and acetonic root extract (24hr, 48hrs, 72hrs) of L. pinnatifidum. But neither of the seeds of the test plant grew at all. Results confirmed zero length of radical and plumule of Triticum aestivum L. seeds. While that of the seeds of control grew into plantlets. Same results were found in case of stem and inflorescence extracts.

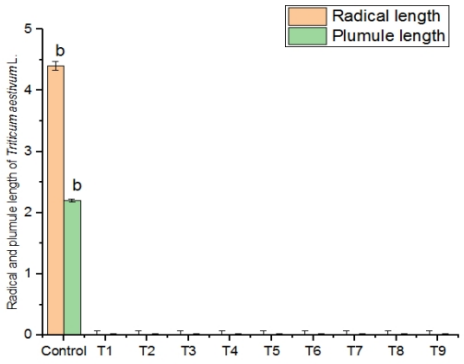
c Fresh & dry weight

Aqueous root, stem and inflorescence extracts of L. pinnatifidum (24hrs, 48hrs, 72hrs), were applied on the seeds of test crop plant. But the seeds could not germinate into plantlets under the influence of each type of extract. The fresh and dry weight was zero as compared to the control. The same results were found for metahnolic and acetonic root, stem and inflorescence of Lepidium pinnatifidum.

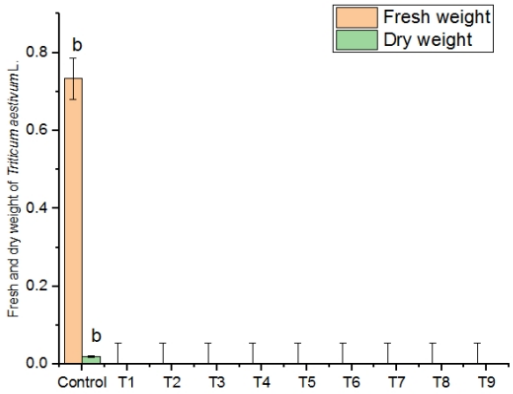
The results are similar with the results of allelopathic potential of tree of heaven (Ailanthus altissimia) on oil seed rape in which the germination was inhibited to the large extent and radical length was inhibited by 94.88% while plumule length was inhibited by 98.99%.(Novak et al., 2018). The strong hostile behavior of plants extracts confirms the findings of Hashmi, 2021 which explained a large variety of phytochemicals in this plant after phytochemicals screening. The extreme negative impact of all types of extracts of L. pinnatifidum on wheat (T.aestivum L.) and maize (Zea mays L.) may be due to the presence of strong allelochemicals present in the donor plant which has been confirmed by the work of Rabab, et al., 2020.

Fig 11 Allelopathic action of Lepidium pinnatifiudm root on germination (a), radicle & plumule length (b) and fresh & dry weight (c) in wheat.





(a) **(b)**



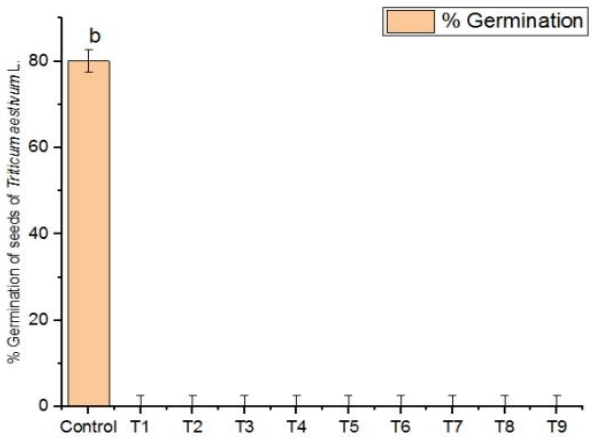
(c)

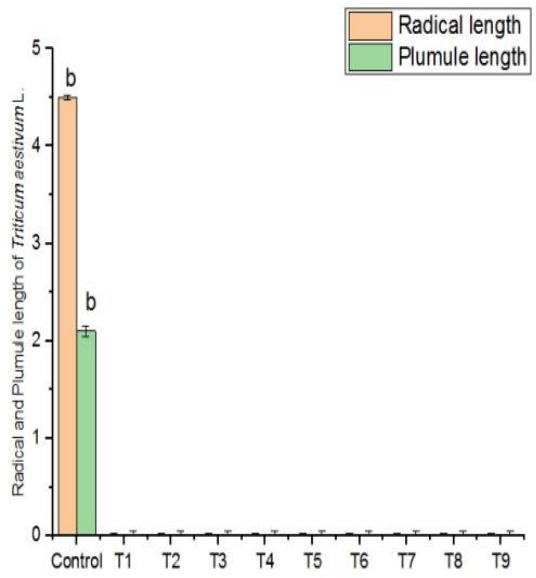
Fig 12 Effect of Lepidium pinnatifidum root extract (aqueous, methanolic, acetonic), 24hrs (a), 48hrs (b), 72hrs (c) on germination of Zea mays.

(a) **(b)**

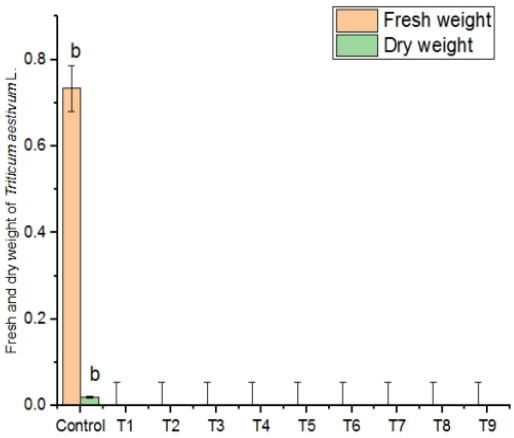
(c)

Fig 13 Allelopathic effect of Lepidium pennatifidum stem on germination (a) radicle & plumule length (b) and fresh & dry weight of wheat.





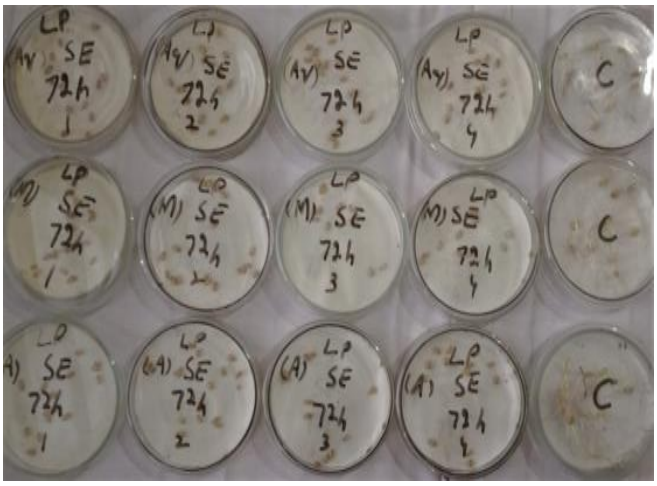
(a) (b)



(c)

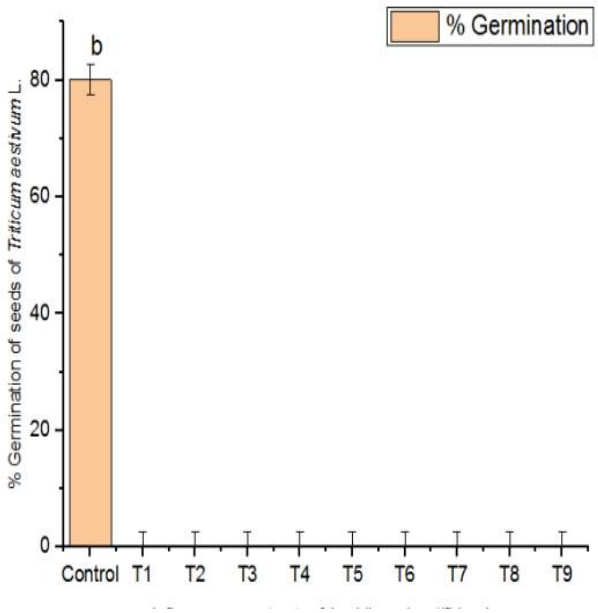
Fig 14 Effect of Lepidium pinnatifidum stem extract (aqueous, methanolic, acetonic), 24hrs (a), 48hrs (b), 72hrs (c) on germination of Zea mays.

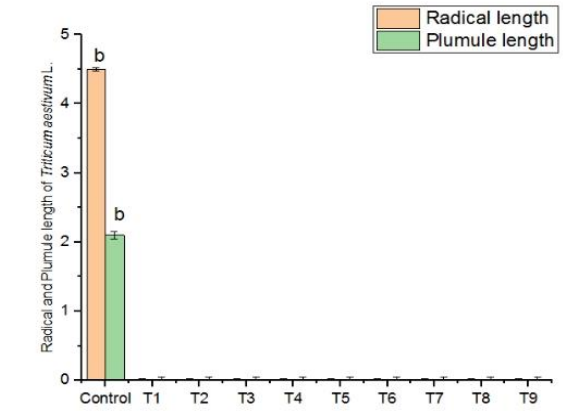
(a) **(b)**



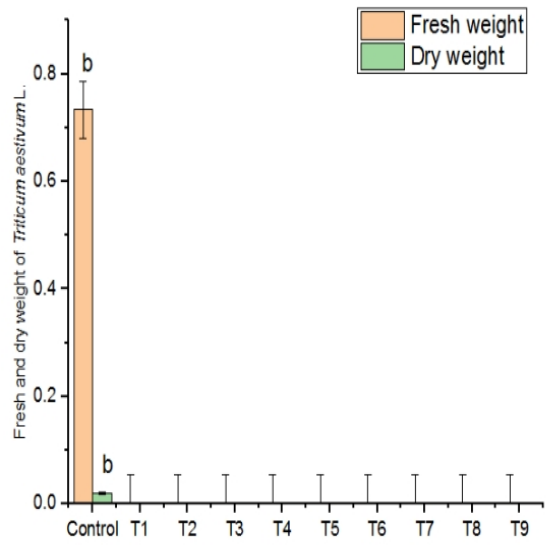
(c)

Fig 15 Allelopathic action of Lepidium pinnatifiudm inflorescence on germination (a), radicle & plumule (b) and fresh & dry (c) weight of wheat



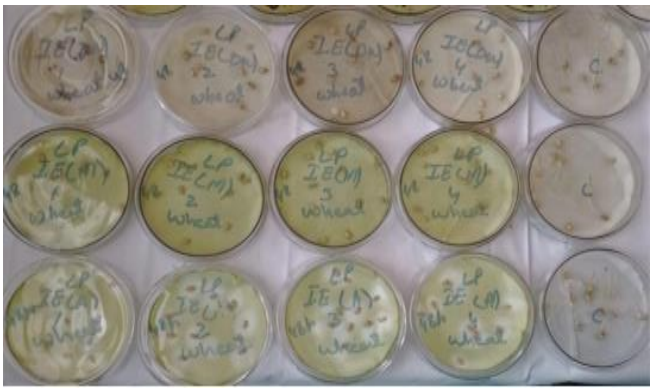


(a) (b)



(c)

Fig 16 Effect of Lepidium pinnatifidum inflorescence extract (aqueous, methanolic, acetonic), 24hrs (a), 48hrs (b), 72hrs (c) on germination of Zea mays.



(a) (b)

(c)

Exudates obtained from L. pinnatifidum root and wheat: Following charaters of wheat were observed under the effect of root exudates of Lepidium pinnatifidum.

Germination:

Root exudates of L. pinnatifidum of four different concentration, 25%, 50%, 75% and 100% were applied to the wheat seeds to check the allelopathic effect of root exudates on seed germination. Results indicated that seed germinated in 25% root exudates while the rate of germination decreased in 50% and 75% while lowest germination under 100%. Highest rate of germination was recorded under control.

Radical & plumule:

Root exudates from L. pinnatifidum (25%, 50%, 75%, 100%) were applied on wheat seeds. Results declared a decreasing trend in length of radical and plumule under the effect of increasing concentration of root exudates. Highest growth was observed in control while lowest growth in 100% root exudates.

Fresh & dry weight:

Seeds of Triticum aestivum were examined against root exudates from Lepidium pinnatifidum of various concentration, 25%, 50%, 75%, 100% for fresh and dry weight. Highest fresh weight was calculated in control, decreased by 10% in 25% root exudates, a continuous decreased at 50% and 75% was observed while lowest values were seen under 100%.

Fig 17 Allelopathic activity of exudates from Lepidium pinnatifiudm root on germination of Triticum aestivum.

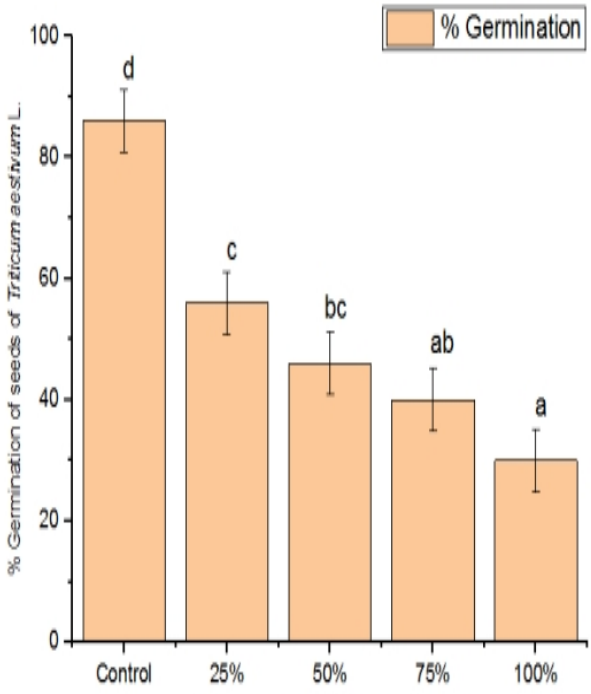


Fig 18 Allelopathic potential of exudates from L. pinnatifidum root on shoot & root length of Triticum aestivum.

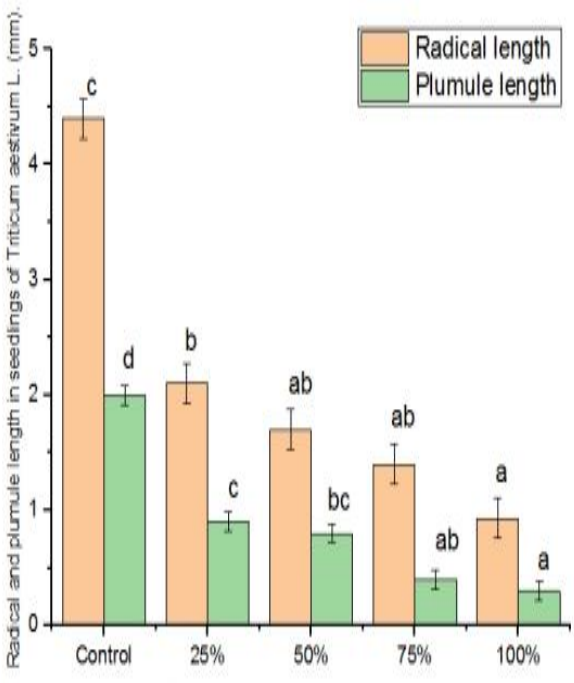
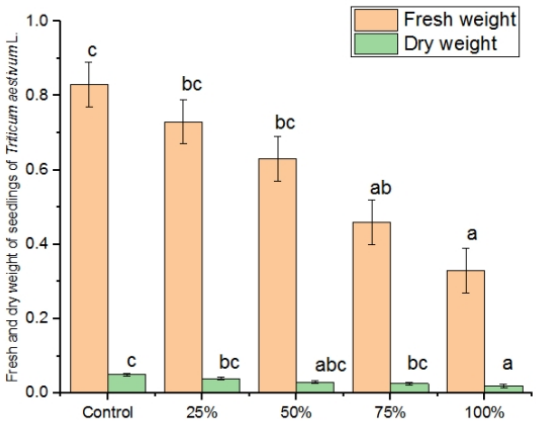


Fig 19 Allelopathic action of exudates from L.pinnatifidum root on fresh & dry weight of Triticum aestivum



Potential of exudates from L. pinnatifidum root on phtyochemicals in wheat

a Sugar:

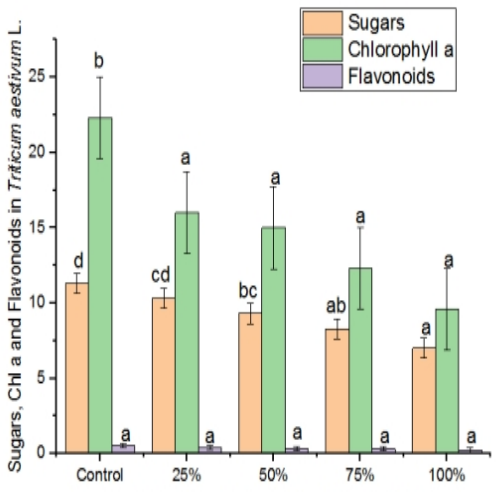
The result showed that concentration of sugars is affected by the concentration of root exudates of L. pinnatifidum. Lowest concentration of sugar is noted in seedlings of Triticum treated with 100% root exudates. While highest sugar content is recorded in control.

b Chlorophyll :

Results indicate that like the sugars, chlorophyll content was also affected by treating the seedlings of Triticum with various concentrations. As concentration of root exudates increased the amount of chlorophyll decreased.

c Flavonoids:

Results indicate that like the sugars and chlorophyll, the flavonoids were also affected by treating the seedlings of Triticum with varying concentrations exudates of Lepidium pinnatifidum Lowest concentration of flavonoids was noted in seedlings of wheat examined with 100% . While highest sugar content was noticed in control. Results confirmes the previous work about the effects of root exudates of Lantana camara on the seed germination and growth, primary and secondary metabolites concentration in T. aestivum, Cicer arietinum. Root exudates lowers down the concentration of sugar and chlorophyll. Similar was the case with flavonoids and catalase content decreased with the increase in concentration of root exudates from, 25%, 50%, 75% to 100%. (kato-Noguchi & Kurniadie, 2021). **Fig 20** Determination of Sugars, Chl a anf Flavonoids in Triticum aestivum L.

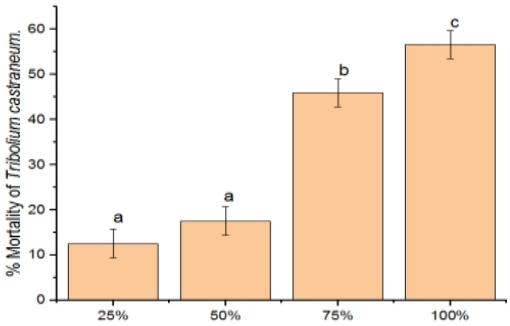


Biological applications of root exudates.

Role of root exudates of L. pinnatifidum as insecticide:

Root exudates of L.pinnatifidum were tested for insecticidal activity against Tribolium castraneum. Mortality rate of Tribolium was observed under the effect of varying concentrations of root exudates of L. pinnatifidum (25%, 50%, 75% and 100%). Results showed that 100% root exudates has a strongest insecticidal action affecting 56% insects following a significant ($p < 0.05$) decreasing trend in mortality by 75% root exudates showing a significant ($p < 0.05$) motility rate of 44.5%, while the mortality rate of 50% root exudates was 18% and 25% root exudates affected 12% insects accordingly. Thus it was concluded that root exudates of Lepidium pinnatifidum Ledeb. shows insecticidal potential. Results are matching with the works of Johnson and Gregory, 2006 which suggest that root exudates of plant have an insect repellent potential, thus root exudates are also a form of biological defense against soil insects.

Fig 21 Effect of root exudates of L. pinnatifidum as insecticide on Tribolium

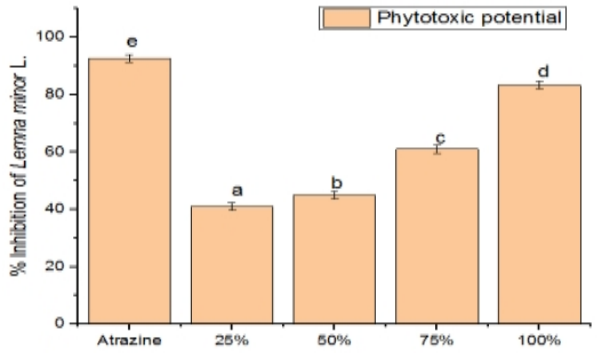


Role of root exudates of L. pinnatifidum in phytotoxicity:

Root exudates were investigated for their phytotoxic activity on duck weed (Lemna minor). The host plant was grown under the effect of 25%, 50%, 75% and 100% root exudates of L. pinnatifidum. It was observed that root exudates exhibit a prominent phytotoxic influence on tested plant. Lemna minor was most significantly affected by 100% root exudates with a significant ($p < 0.001$) inhibition rate of 83% following 75% root exudates with significant inhibition rate of 61.2% while 50% root exudates exerta inhibition growth rate of 53.7% and 25% root exudates inhibit 42.5% Lemna minor respectively as compared with the control. Phytotoxicity was confirmed by cytotoxicity observed under microscope. No significant inhibition was noticed in distilled water. While highest rate of inhibition was observed in negative control Atrazine.Thus it can be concluded that root exudates of L. pinnatifidum can be used as herbicides after proper formulation.

Phytotoxic effect of root exudates is confirmed by the experiment performed on Solidago canadensis whose root exudates exert an inhibitory action on the growth of other invasive and native plants. But the effect on the native plants was more than invasive plants with increasing concentration of root exudates. (Yang and Li, 2022).

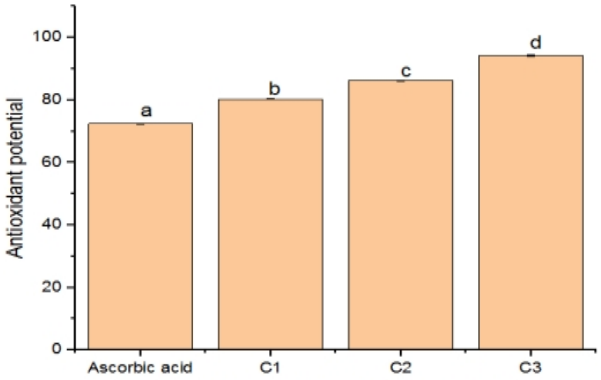
Figure 22 Root secretion of Lepidium. pinnatifidum and Lemna minor.



Antioxidant activity of root exudates:

The highest rate of DPPH scavenging ability was observed to be significant ($p < 0.0001$) in concentration three labeled as C_3 of root exudates with 94.1% inhibition at 500µg/mL of root exudates of L. pinnatifidum, followed by C_2 which revealed a significant ($p < 0.0001$) inhibition rate of 86.1% at 100µg/mL. While the C_1 showed lowest value of absorption 80.6% at 50µg/mL. The lowest value of absorption was attributed to the high degree of antioxidant potential. Thus root exudates showed a significant antioxidant activity. Results were confirmed by Kasheri et al., 2020 who investigated antioxidant activity of Cestrum nocturnum. From the above study it can be concluded that root exudates of Lepidium pinnatifidum Ledeb. can be use as antioxidant in future.

Figure 23 Antioxidant potential of root exudates of Lepidium pinnatifidum Ledeb.



CONCLUSION

Lepidium pinnatifidum Ledeb. is a wild annual herb, a member of the family Brassicaceae. Much is known about its morphological properties but little is known about its therapeutic potential. Overall the plant is known for its weedy nature. The present work add some information to the allelopathic nature of the plant on some selected crops, Triticum aestivum L. and Zea mays L. The study revealed that all types of extracts of root, shoot and inflorescence of Lepidium pinnatifidum Ledeb. are highly allelopathic to both the crops. Aqueous, acetonic and methanolic extracts each of 24hrs, 48hrs and 72hrs of root, stem and inflorescence of the plant was equally inhibitory to the seed germination, radical and plumule growth and dry and fresh weight of the tested crops (Triticum aestivum L. & Zea mays L.). Similarly root exudation of Lepidium pinnatifidum Ledeb. also exerts inhibitory effect on both

the crops. The inhibitory potential of root exudates increased along with increasing concentration of root. In addition the phytochemical analysis of wheat and maize after treating with a variety of root exudates of L.pinnatifidum Ledeb., 25%, 50%, 75% and 100% revealed that content of sugar, chlorophyll and flavonoids in test crop decreased along with increasing concentration of root exudates. Therefore it is concluded that L. pinnatifidum Ledeb. is a highly toxic plant and should be immediately uprooted from an agriculture field. Our study also focused on biological applications of root exudates of L. pinnatifidum Ledeb. as insecticide, phytotoxic, and antioxidant. The present study suggests that as the concentration of root exudates increased, its phytotoxic effect, insecticidal and antioxidant potential also increased. The study also confirms that the toxicity of root exudates of Lepidium pinnatifidum Ledeb is less as compared to that of plant extracts. As the present work suggests Lepidium pinnatifidum Ledeb. a highly toxic plant, high scale lab work is needed to find out the phytochemicals responsible for its high level toxicity. The root exudation of the plant showed insecticidal activity and phytotoxic potential so in future efforts are required to actually use its root exudates as insecticide and weedicide to solve problem of synthetic fertilizers soil pollution. Research is needed to explore the phytotoxic chemicals in its root exudates which could be used as natural herbicide. In addition the high level of toxicity is an indication of strong phytochemicals thus research is required to explore the medicinal values of Lepidium pinnatifidum Ledeb. in future.

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