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# Allelopathic effect of *Cymbopogon citratus* aqueous leaves extract on the morphology and physiology of plants

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## ABSTRACT

The allelopathic potential of *Cymbopogon citratus* on the morphology and physiology of plants was investigated under laboratory conditions. Seeds of a dicot plant (*Cicer arietinum*), a monocot plant (*Zea mays*) and a weed (*Amaranthus retroflexus*) were treated with aqueous leaves extract of *Cymbopogon citratus* at different concentrations (25%, 50% and 100%, distilled water being used as control). Results recorded showed complete inhibition of germination of *Amaranthus retroflexus* seeds at all concentrations while the percentage of germination of *Cicer arietinum* decreased with the increasing concentration of *Cymbopogon citratus* aqueous leaf extract. *Zea mays* showed the least effect of *Cymbopogon citratus* leaf extract and sustained their germination and growth. Analysis of the morphological and physiological responses of the experimental data obtained revealed that the aqueous extract of the leaves of *Cymbopogon citratus* has a great negative allelopathic potential with respect to all three plants.

**Key Words -** Allelopathy, Germination, Allelochemicals, Biocontrol, Weeds

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## INTRODUCTION

The interaction among plants, animals and microbes is a peripheral that is always being carried out in the environment (Mukherjee & Dalal, 2015). Many species tend to regulate one another through the production and release of chemicals, stimulants and inhibitors (Putnam & Tang, 1986). Theophastrus 'the father of botany', observed the earliest interventions of weed and crop allelopathy on other plants while discussing how chickpea "exhausted" the soil and had adverse effects on weeds. Cato the Elder (234-140 B.C.) wrote in his book how barley "scorch up" corn land and how walnut trees were harmful to other plant (Zeng, *et al.* 2001). All these findings and literature that has been reported with respect to plant interference for over 2000 years fall under the umbrella term "allelopathy" (Weston & Duke, 2003). The term 'allelopathy' is derived from the amalgamation of the Greek words 'allelon' and 'pathos' which means 'of each other' and 'to suffer' respectively (Rizvi, H, & Rizvi, 1992). Rice (1984) defines allelopathy as a phenomenon involving direct or indirect, beneficial or harmful effects of a plant (including microorganism) on another plant through the release of various chemicals in the environment (Rice, 1984).

India being an agriculture-based country has huge bearings in seeking sophistication and stabilization in crop production and the related industries. A substantial shift towards synthetic agrochemicals in order to increase crop production has taken a toll on the environment as well as human health in the past few centuries. The conservative approaches to plant protection for controlling diseases, pests and weeds have been shadowed with the increasing use of synthetic pesticides in agriculture (Sharma, *et al.* 2018). According to the reports pertaining to specifically India, despite being banned for use and manufacture, around 104 pesticides out of 293 registered ones still continued to run the show without any hindrance (Gol, 2021). Toxic as they are, they are absorbed by plants and animals and may lead to bioaccumulation in humans if consumed (Kaur, *et al.* 2024). Thus, there is a need to devise a sustainable solution for the problem so that there is an impactful shift from the overexploitation of agrochemicals to a greener alternative that has lesser detrimental effect on the environment.

Allelopathy could be a potential way out of the issues if the traditional knowledge is applied in a suggestive way. The effect of weeds on weeds, weeds on crops and crops on crops have been studied invariably since ages and the results obtained clearly portray that allelopathic control of weeds, exploitation of beneficial interactions in crop rotation and mixed cropping as well as elimination of deleterious effects of crops on crops have a direct role in elevating crop production (Rizvi, H, & Rizvi, 1992). Weeds stubbornly compete with crops causing a considerable decline in yield (Mushtaq & Siddiqui, 2018). They are also responsible for far more losses than that caused by any other category of agronomic pests such as insects, pathogens, nematodes and herbivores etc. (Abouziena & Haggag, 2016). Allelopathy has profound implications on weed suppression and a better tool for significant weed control measures (Mushtaq & Siddiqui, 2018). Allelochemicals are suitable alternative to synthetic herbicides because of their safer toxicology and environmental profiles (Bednarz, *et al.* 2023). Natural compounds deliver a wide choice of potential new sustainable and safe herbicides called the bioherbicides, which are based chemicals released by living organisms (Soltys, *et al.* 2013). Allelopathy may be a potential solution to the problem of evolution of herbicide resistance and environmental pollution (Jabran, *et al.* 2015).

Aromatic plants are economically important for the essential oils that are found in them. Several reports suggest that these essential oils show allelopathic activities against weed growth (Maurya, *et al.* 2022). The main constituent of essential oils are terpenoids which are responsible for their plant inhibiting effect. Some aromatic plants like *Artemisia* sp., *Tagetes minuta, Mentha* species, *Ocimum* species, *Thuja occidentalis*, etc. are known to show such effects (Maurya, *et al.* 2022). *Cymbopogon citratus*, being an aromatic plant, can also be looked at through the same lens.

*Cymbopogon citratus*, commonly known as Lemongrass, is a perennial crop. *C. citratus* contain phytochemicals comprising geranial (43%) and neral (31%) that together form citral (Oliveira, *et al.* 2011). Lemongrass oil, extracted from Lemongrass, *Cymbopogon citratus* comprises of chemicals like geranial and neral, myrcene, linalool, and geranyl acetate (Mukarram, *et al.* 2021) . These secondary metabolites have been reported to inhibit seed germination and seedling growth of wheat, black mustard and *Amaranthus palmeri* (Dudai N., *et al.* 1999). Reports claim that the essential oil of *C. citratus* decreases root length in lettuce (Alves, *et al.* 2004), and the germination of weed seeds, such as *Amaranthus blitoids, Amaranthus palmeri, Euphorbia hirta, Sinaps nigra, Trifolium capestri, Lycopersicum esculentum* and *Triticum aesto* (Dudai N., *et al.* 1999).

The majority of research on the plant's allelopathic potential studies was conducted on essential oil, which isn't an economically efficient option for farmers. So, to establish its role as a green, cost-effective and easy-to-use plant-based weedicide, we explored the aqueous extract of the leaves. The plant being easily available in India, can be grown per our needs.

## MATERIAL & METHODS

### Preparation of leaf extract

Fresh leaves were taken for preparation of aqueous leaf extract by the maceration (MAC) method. 25g of fresh leaves of *C. citratus* were harvested, cleansed, mid vein of leaves was removed and cut into small pieces and then ground in distilled water. The obtained crude extract concentration was considered as 100%. To make further concentrations of the crude extract (25% and 50%) was prepared by adding distilled water.

**Seeds selection and their viability test**

All the experiments were performed as petri-plate experiment in the laboratory of Department of Botany, Patna University. The seeds used as representatives included *Cicer arietinum* (Bengal gram), *Zea mays* (maize) and *Amaranthus retroflexus* (redroot pigweed).

The seed germination and viability test were used, counting daily the number of germinated seeds before the test installation. Seeds were surface sterilized in Mercuric chloride solution and then rinsed with distilled water thoroughly. 10 seeds each of *Zea mays, Cicer arietinum* and 100 seeds of *Amaranthus retroflexus* were kept separately in each petriplate setup. Germination was observed after 6 days.

**Petri - dish experiment and observation on seed germination related parameters**

Surface sterilized seeds of *Zea mays, Cicer arietinum* and *Amaranthus retroflexus* respectively, were taken in well sterilized petri- dishes and treated with 10 ml of *C. citratus* leaves extract at different concentrations (0%, 25%, 50% and 100%) independently. Distilled water was used as control. 10 ml of distilled water was added every 24 hours to maintain the moisture of the filter paper in the petri dishes. The filter papers were changed, and seeds were washed after 36 h of treatment to avoid pathogen contamination. Termination of the experiment was done after three consecutive recordings showed no further variation.

**Analysis of morphological parameters -**

**Germination test:** The germination percentage of the treated plants was calculated by the given formula:

$$\text{Germination percentage} = \frac{\text{Total number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

(Khan & Ungar, 1997)

**The length of shoot and root and fresh weight estimation of treated plants**

Three plants from each petri-plates of different concentrations were taken out very carefully from the petri-plate and blotted dry to avoid the surface water droplets. Three plants from each petri-plates weigh on electric weighing machine one by one separately and finally the average value calculated. The shoot and root length were measured using a thread and a scale.

**Analysis of physiological parameters -**

**Pigment estimation**

The pigment estimation of chlorophyll a, chlorophyll b (total chlorophyll) and carotenoid was performed by Arnon's (1949) method. 0.1g of leaves of treated plants at different concentration were taken separately and ground in 10 ml of 80% acetone using mortar and pestle. The mixture was then filtered through filter paper and the extract was transferred to cuvette to analyze on spectro photometer at 663 nm, 645 nm for chlorophyll a and chlorophyll b, and 480 nm & 510 nm for carotenoid (Arnon, 1949).

**Protein estimation**

Estimation of Protein content in leaf tissues were done using Lowry method (Lowry, *et al.* 1951). 0.5g of fresh leaves of treated plant at different concentration were taken separately and ground with mortar & pestle in 10 ml distilled water. The mixture was then filtered using filter paper. The sample were then analysed on a spectrophotometer at 640 nm. The protein concentration in each sample was determined using the bovine serum albumin standard curve, and protein was calculated in µg/mg DW (S.R.H. Amiri, *et al.* 2017).

**RESULTS & DISCUSSION**

**Morphological effects**

Aqueous extract of *C. citratus* leaves showed significant effect in reducing the germination percentage of *Zea mays* and *Cicer arietinum* and total inhibitory effect on the germination of *Amaranthus retroflexus* (Figure I). In the case of *Zea mays,* the germination percentage remained quite good at all concentrations (25%, 50%, and

100%) of the extract, while for *Cicer arietinum* the germination percentage was reduced markedly with the increasing concentrations of the leaf extract. The extract caused complete inhibition of germination of *Amaranthus retroflexus* at all concentrations. Germination percentage of a 100% was achieved for *Zea mays* at 25% and 50% of concentration in alignment with that of the control while the germination percentage was reduced to 80% at 100% concentration of *Cymbopogon citratus* leaf extract. The germination percentage for *Cicer arietinum* was seen to be 80% at 0% concentration of the leaf extract (control setup) which significantly reduced to 12.5 %, 25% and 0% at 25%, 50% and 100% of the concentration of the treatment given respectively. The inhibitory effect was prominently seen in *Amaranthus retroflexus* seeds where the germination was completely inhibited (0% germination) at all concentrations of aqueous leaf extract.


**a. Germination in *Zea mays* after 8 days of treatment.**


**b. Germination in *Cicer arietinum* after 8 days of treatment.**


**c. Germination in *Amaranthus retroflexus* after 8 days of treatment.**

**Figure I : Effect of aqueous leaf extract of Cymbopogon citratus on the germination of *Zea mays* (a), *Cicer arietinum* (b) and *Amaranthus retroflexus* (c) after 8 days of treatment at different**

Among the three test seeds only *Zea mays* was selected for further studies, rest two discarded due to no proper germination.

The analysis of the data obtained for the fresh weight of *Zea mays* showed a decreasing trend as the extract concentration increased from 0% to 100%. The average fresh weight of the plant at control was found to be 1.27±0.03 g while at 25%, 50 % and 100 % concentrations of the leaf extract it was found to be 1.14± 0.06 g, 1.08± 0.02 g and 0.74±0.06 g respectively. The shoot and the root lengths of *Zea mays* at control was found to be 17.2 cm and 19.2 cm respectively which decreased with the increasing concentration of the given treatment. At 25 % of the treatment concentration the shoot and the root lengths were observed to be 16.8 cm and 14.6 cm respectively gradually declining to 14.1 cm and 15.2 cm at 50% concentration while were found to be 14.3 cm and 15 cm at 100% treatment concentration.

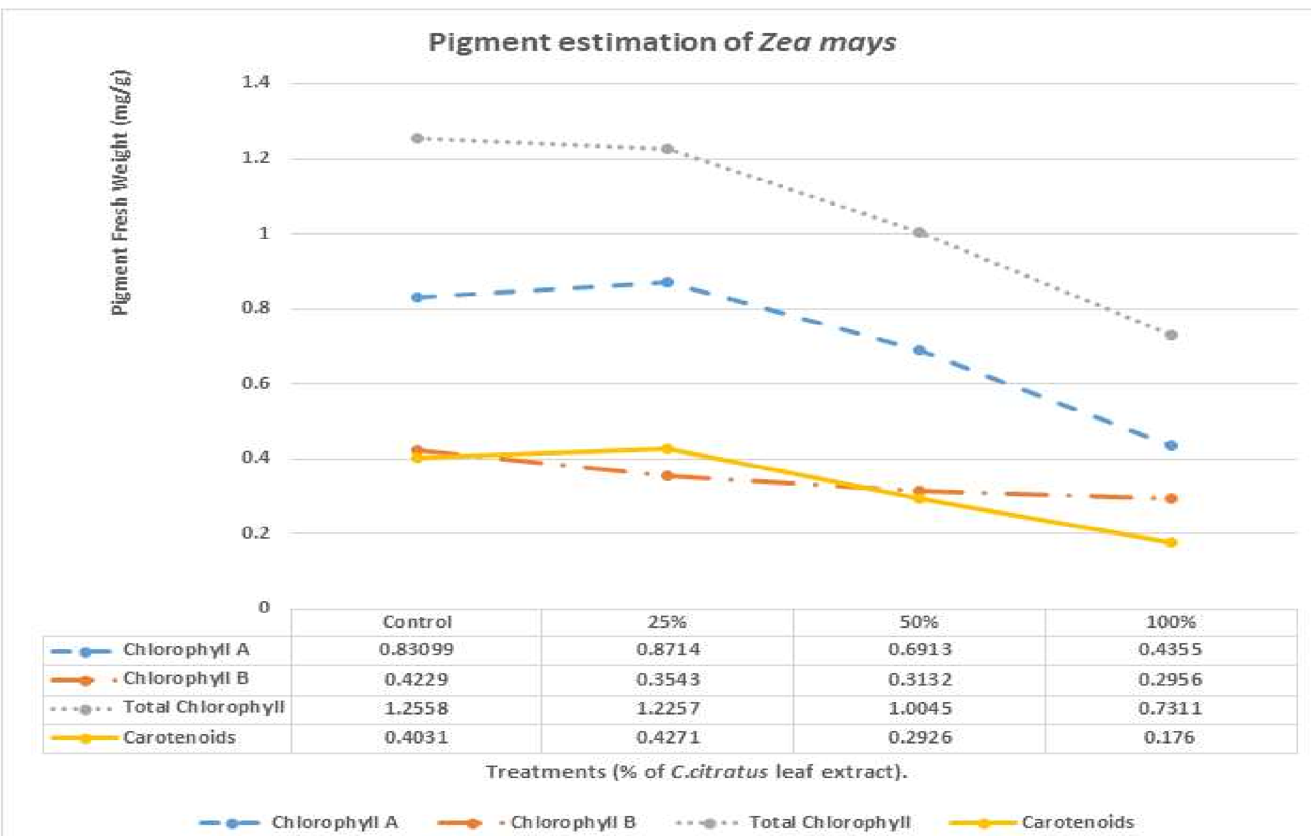
These results stand true to depicting the allelopathic nature of *Cymbopogon citratus*. The observations settle well with the claims made by various authors. Several authors marked that the essential oil of *Cymbopogon citratus* decreased the germination of seeds like *Amaranthus blitoides, Euphorbia hirta, Trifolium campestris, Amaranthus palmeri* and *Triticum aesto* (Dudai, *et al.* 1999). It is also known to cause adverse effect on the germination and growth of *B. pilosa* (Fortes, *et al.*, 2009). Reduced the root length in lettuce, as an after effect of treatment with lemongrass leaf extract, has also been observed (Alves, *et al.* 2004). The aqueous leaf extract of lemongrass at significantly increased root cell death of ruzi grass at 10 % (w/v) concentration (Sukkhaeng, *et al.*, 2023). However, *C. citratus* extract did not reduce the germination percentage in soyabean (Fortes 2009). *C. citratus* extracts inhibited growth and development in *Portulaca oleracea* L. and *B. pilosa* L. (Souza, *et al.*, 1998). The negative effect on the germination of seeds can be alluded to the decreased activity of α - amylase due to herbicidal effect of lemongrass extracts (Poonpaiboonpipat, *et al.,* 2013).

**Result of physiology parameters:**

**1. Pigment content:**

On observing the chlorophyll and carotenoid content estimation results, it was found that a decrease in chlorophyll values occurred with the concurrent increase in treatment concentration. The total chlorophyll content decreased from 1.2558 mg/g at control to 1.2257 mg/g at 25%, 1.0045 mg/g at 50% and 0.7311 mg/g at 100% treatment concentration respectively while the carotenoid content showed considerable decrease from 0.4031 mg/g at control to 0.4271 mg/g at 25%, 0.2926 mg/g at 50% and 0.1760 mg/g at 100% of leaf extract concentration (Figure 2).

A drop in chlorophyll and carotenoids contents in response to the *C. citratus* aqueous leaf extract suggests its negative impact on its photosynthesis. The observed effects were similar to the effects of essential oils of *E. citriodora* and *A. scoparia* that reduced chlorophyll content and thus affected photosynthetic activity (Batish *et al.,* 2004). Results predict that a decline in carotenoids content may also lead to a carotenoid deficiency - induced photooxidation of chlorophyll (Dankov *et al.,* 2009).




| | Control | 25% | 50% | 100% |
|---|---|---|---|---|
| Chlorophyll A | 0.83099 | 0.8714 | 0.6913 | 0.4355 |
| Chlorophyll B | 0.4229 | 0.3543 | 0.3132 | 0.2956 |
| Total Chlorophyll | 1.2558 | 1.2257 | 1.0045 | 0.7311 |
| Carotenoids | 0.4031 | 0.4271 | 0.2926 | 0.176 |

**Fig. 2- Reduction in pigment contents in *Zea mays* after the treatment of *C. citratus* aqueous leaf extract.**

## 2. Protein content

Total protein present in the leaves of *Zea mays* treated at different concentrations of the aqueous leaves extract of *C. citratus* along with control condition showed an adverse impact on the protein content of the plant. The values of protein content (in µg/g) declined successively as compared to that of control (Figure 3).

Some studies have reported that the volatile oil from several allelopathic plants and their monoterpenes caused accumulation of $H_2O_2$ in some plants (Mutlu, *et al.,* 2010). Accumulation of $H_2O_2$ in plants further enhances lipid peroxidation, thus increased oxidative stress and leading to disruption of cell metabolic activities including protein synthesis and degradation. Probably the decrease in protein content was due to increase in lipid peroxidation caused by lemongrass leaf extract, the latter holding true evidently in a few studies (Sukkhaeng, *et al.* 2023).

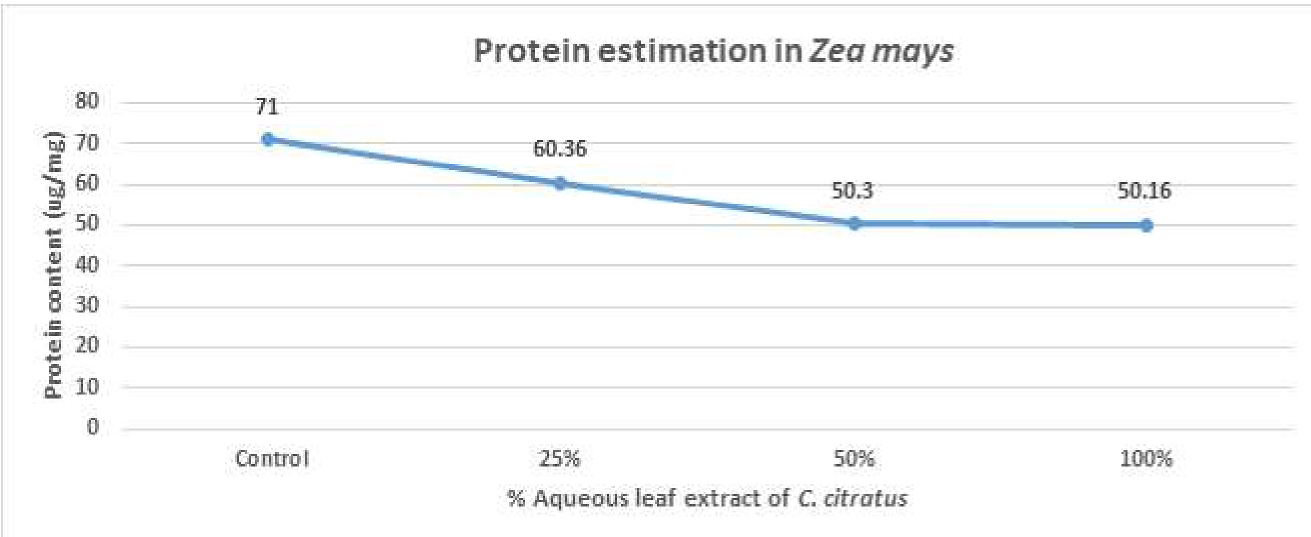



**Fig. 3- Reduction in protein content in *Zea mays* after the treatment of *Cymbopogon citratus* aqueous leaf extract.**

## CONCLUSION

The uncontrolled use of chemical herbicides to manage weeds has resulted in the development of herbicide-resistant weed biotypes, environmental contamination, and damage to non-target plants and species. All of the aforementioned risks prompted scientists to look for alternative approaches, such as using natural plant compounds as herbicides. Thus, the study concluded that the plant *C. citratus* had promising allelopathic properties. The aqueous extract of *C. citratus* can be utilized as a powerful natural herbicide and can serve as models for the development of new herbicides that are both safe for the environment and human health.

In our study, the allelopathic effects of *C. citratus* aqueous leaf extract on seed germination of three plant species including two dicot plants (i.e. *Amaranthus retroflexes* and *Cicer arietinum*) and one monocot plant (i.e. *Zea mays*) were studied. Allelopathic activity of *C. citratus* aqueous leaf extract was more noticeable for the two plant species which belongs to dicot (i.e. *A. retroflexus* and *C. arietinum*) than the plant belongs to monocot (i.e. *Zea mays*). Hence, on the basis of result we can conclude that the dicots are more sensitive to the aqueous leaf extract of the *C. citratus* and monocots are less sensitive. It can be concluded that the monocot plants have more stress tolerant ability than dicot plants or dicots are more sensitive to the *C. citratus* leaf extract. Weed i.e. *Amaranthus retroflexus* is highly sensitive to the *C. citratus* leaf extract.

Hence, *C. citratus* can be used as a bioherbicide for managing weeds. This natural herbicide offers a sustainable and environmentally friendly approaches to weed management. Further research and field trials will be essential to fully understand its efficacy and practical application in weed control practices.

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