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Research Paper

In-Vitro Evaluation of Aqueous Leaf Extract of *Eucalyptus globulus* L. on *Vigna aconitifolia* L.

Shinde K. L., Sonawane T.P., Donde R.H

Assistant Professor, Department of Botany, Jijamata College of Science and Arts Dnyaneshwar Nagar, Bhende. Tal. Newasa, Dist. Ahilyanagar, Maharashtra. 414605 affiliated by Savitribai Phule Pune University, Pune

Email: ranidonde96@gmail.com

Abstract: This study evaluated the allelopathic potential of *Eucalyptus globulus* L. aqueous leaf extract on *Vigna aconitifolia* L. (moth bean) under in-vitro conditions. Different concentrations (5%, 10%, 15% & 20%) of leaf extract were tested on seed germination and seedling growth. Germination percentage was noted on the 4th and 6th day of treatment. Root length and shoot length were measured on the 10th day of germination. Result showed a concentration-dependent inhibition of germination percentage, root length, shoot length and total seedling length compared to control. The highest inhibition was observed at 20% extract concentration. The study suggests that *Eucalyptus globulus* L. leaf extract contains phytochemicals with allelopathic effects, potentially impacting *Vigna aconitifolia* L. growth and productivity.

Keywords: *Eucalyptus globulus* L., *Vigna aconitifolia* L., seedling length.

Introduction: Allelopathy is a biological phenomenon in which plants release bioactive compounds into the environment that influence the growth and development of neighboring plants. These biochemical substances, commonly known as allelochemicals, may either stimulate or inhibit seed germination and seedling growth (Herro and Callaway, 2003; Gurmu 2015; Regu 2018; Yakubu et al., 2018). *Eucalyptus globulus* is fast growing tree widely cultivated for timber, pulp, fuelwood and essential oil production. It is known to contain various secondary metabolites such as phenolics compounds are reported to possess strong allelopathic properties that may affect the germination and growth of nearby crop plants (Regu. 2018). Leaf litter and aqueous extracts of *Eucalyptus globulus* have been shown to influence soil chemistry

and plant physiology (Gurmu 2015). *Vigna aconitifolia* L. (moth bean) is an important drought-resistant legume cultivated mainly in arid and semi-arid regions. It serves as a valuable source of protein and plays a significant role in improving soil fertility through nitrogen fixation. Since moth bean is often grown in areas where eucalyptus plantations are present, it is necessary to evaluate any potential inhibitory or stimulatory effects of eucalyptus leaf extracts on its growth (Regu. 2018). In-vitro studies provide controlled environmental conditions to assess the direct effects of plant extracts on seed germination and early seedling development.

Aims and Objective:

To evaluate the in-vitro effects of aqueous leaf extract of *Eucalyptus globulus* on the seed germination and seedling growth of *Vigna aconitifolia* L. To study the effects of these extracts on the seed germination percentage of *Vigna aconitifolia* L. seeds under controlled laboratory conditions.

Material and Method:

Collection of plant material:

Fresh, healthy leaves of *Eucalyptus globulus* L. were collected from mature plants growing in a pesticide-free area. The leaves were washed thoroughly with tap water followed by distilled water to remove dust and contaminants.

Healthy, uniform seeds of wheat were purchased from the local market for present investigation

Preparation of aqueous leaf extract:

The collected leaves were shade-dried at room temperature for 7-10 days. The dried leaves were ground into fine powder using a sterile electric grinder. About 10 grams of leaf powder was soaked in 100 ml distilled water and kept on a shaker for 24 hrs. at room temperature. The mixture was filtered through muslin cloth followed by Whatman No.1 filter paper. The filtrate obtained was considered as 100 % stock solution. Different concentrations (5%,10%,15%&20%) were prepared by diluting the stock solution with distilled water.

Seed viability test: A seed viability test is performed to determine whether seeds are alive and capable of germinating under suitable environmental conditions. It helps evaluate the quality and planting value of seeds before sowing or experimental use.

Water Floatation Test: A total 300 seeds of moth bean were placed in a container filled with distilled water and left for 24 hrs. Seeds that settled at the bottom were considered viable. Seeds that remain floating were rejected as they were likely non-viable.

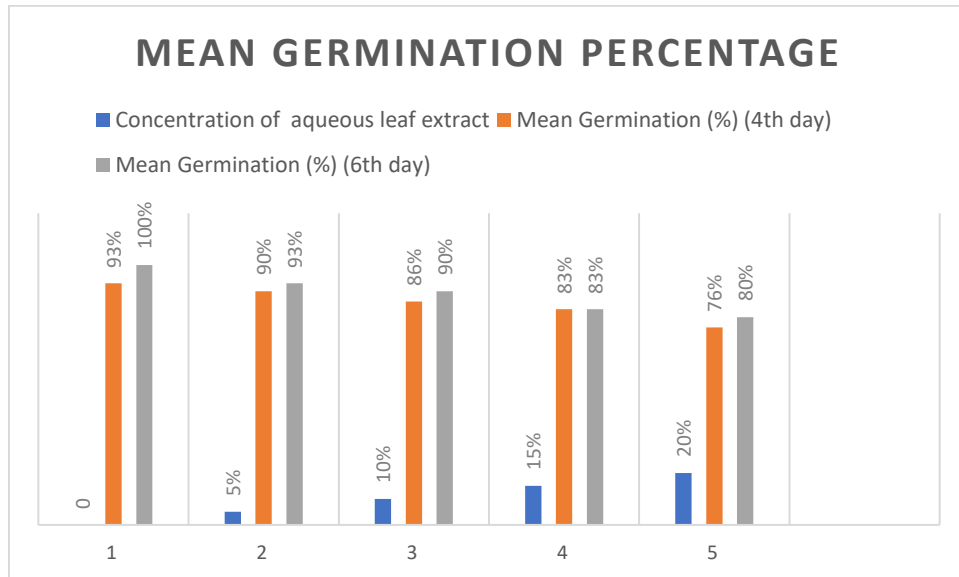
Sterilization of glassware: All glassware used in the experiment was thoroughly cleaned and sterilized prior to use to prevent microbial contamination. Initially, the glassware such as petri dishes, conical flasks, beakers, measuring cylinders was washed with tap water followed by distilled water to remove any residues. After drying the glassware was sterilized in an autoclave at 121 c under 15 psi pressure for 15-20 minutes when moist heat sterilization was required.

Application of treatment: After surface sterilization healthy seeds of moth bean were placed in sterilized petri dishes lined with Whatman No.1 filter paper. Different concentrations of aqueous leaf extract of *Eucalyptus globulus L.* were used as treatment solution. about 5 ml of the respective extract concentrations was added to each petri dish to moisten the filter paper uniformly the control group received an equal quantity (5ml) of distilled water instead of plant extract. Each treatment was replicated 3 times, with 10 seeds per petri dish. The petri dishes were incubated under laboratory conditions at room temperature. The moisture level was maintained by adding the respective treatment solution whenever required. observations on germination and seedling growth were recorded regular intervals.

Result & Discussion

Table no.1 Effect of aqueous leaf extract of *Eucalyptus globulus L.* on seed germination of *Vigna aconitifolia L.*

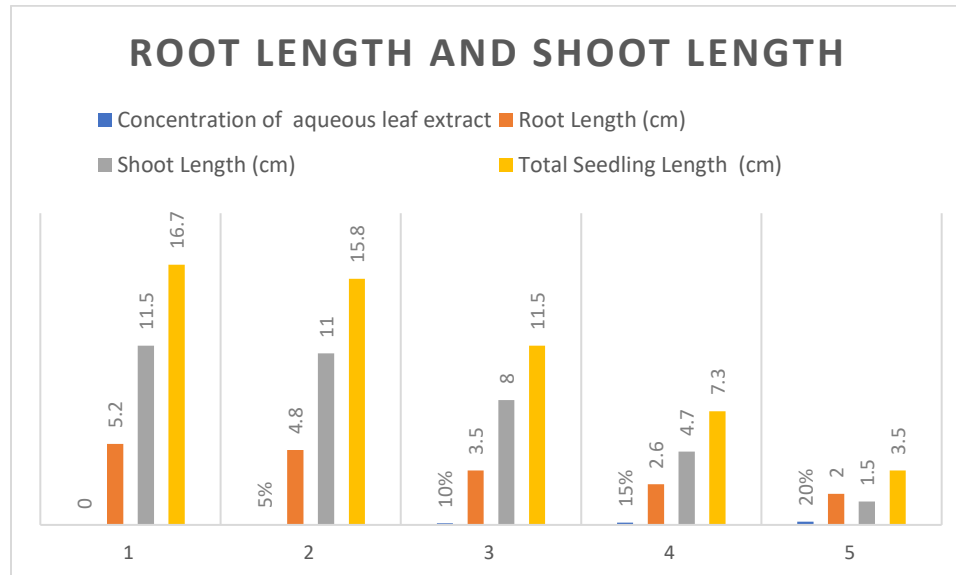
Sr.No	Concentration of aqueous leaf extract (V/V)	Mean Germination (%) (4 th day)	Mean Germination (%) (6 th day)
1	Control	93%	100%
2	5 %	90%	93%
3	10 %	86%	90%
4	15 %	83%	83%
5	20 %	76%	80%



Graph No.1: Effect of aqueous leaf extract of *Eucalyptus globulus L.* on seed germination of *Vigna aconitifolia L.*

Table no.2 Effect of aqueous leaf extract of *Eucalyptus globulus L.* on root and shoot growth of *Vigna aconitifolia L.* (After 10 days of germination)

Sr.No	Concentration of aqueous leaf extract (V/V)	Root Length (cm)	Shoot Length (cm)	Total Length (cm)	Seedling
1	Control	5.2	11.5	16.7	
2	5 %	4.8	11	15.8	
3	10 %	3.5	8	11.5	
4	15 %	2.6	4.7	7.3	
5	20 %	2	1.5	3.5	



Graph No.2: Effect of aqueous leaf extract of *Eucalyptus globulus L.* on root and shoot growth of *Vigna aconitifolia L.* (After 10 days of germination)

The present study revealed that the allelopathic influence of *Eucalyptus globulus L.* on seed germination and seedling growth of *Vigna aconitifolia L.*

Seed Germination:

The aqueous leaf extract of *Eucalyptus globulus L.* showed a significant effect on the germination of *Vigna aconitifolia L.* seeds under in vitro conditions. Seed germination percentage decreased with increasing concentration of the extract. The control treatment (distilled water) showed the highest germination percentage, whereas treatments containing higher concentrations of extract showed reduced germination. Seed germination in the control was observed to be around 90-100%, while treatments with (5%,10%,15% & 20%) extract concentrations showed progressively lower germination rates. This reduction suggests the presence of allelopathic compounds in *Eucalyptus globulus L.* leaves that may inhibit seed germination.

Seedling Growth:

The root and shoot length of *Vigna aconitifolia L.* seedlings were also significantly affected by the *Eucalyptus globulus* leaf extract. Seedlings grown in control conditions exhibited the highest root and shoot length indicating normal growth. However, as the concentrations of *Eucalyptus* extract increased both root and shoot growth were inhibited.

Discussion:

The result confirms that *Eucalyptus globulus* L. possesses allelopathic potential. The inhibitory effects observed in this study may be due to bioactive compounds such as eucalyptol, phenolic acids and flavonoids which are known to suppress germination and seedling growth of neighboring plants. The findings of the present study are consistent with earlier research reports that demonstrated that inhibitory effects of eucalyptus species on crop plants. Several studies have shown that leaf extracts and leaf litter of *Eucalyptus* release allelochemicals into the soil, which suppress the germination and growth nearby vegetation. Therefore, the presence of *Eucalyptus globulus* plantations near agricultural fields may negatively influence the growth of certain crops including legumes like *Vigna aconitifolia* L.

Conclusion:

The in vitro evaluation indicates that *Eucalyptus globulus* L. leaf extract has a concentration-dependent inhibitory effect on germination and seedling growth of *Vigna aconitifolia* L. Higher concentrations significantly reduced germination percentage, root length and shoot length. These results highlight the allelopathic potential of *Eucalyptus globulus* L. and suggest that its residues or extracts may affect crop growth in agricultural ecosystems.

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