

ALLELOPATHIC IMPACT OF AQUEOUS LEACHATE OF *EUCALYPTUS GLOBULUS L.* LEAVES ON SEED GERMINATION, GROWTH, AND BIOCHEMICAL CONTENTS OF SEEDLING OF *ELEUSINE CORACANA GAERTN*

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ABSTRACT

The background to the study: Allelopathy can be distinguished from other plant interference strategies because the donor species produces inhibitory compounds into the environment to cause harm. This work aimed to evaluate the allelopathic impact of aqueous leachate of *Eucalyptus globulus* leaf litter on seed germination, biochemical, and growth parameters of finger millet (*Eleusine coracana*) seedlings.

Methods: This experiment was conducted using aqueous leaf litter leachate at a concentration of 100%, 80%, 60%, 40%, and 20% using water as a control.

Results: The experiment showed that all the test concentrations of leachate had a deleterious effect on seed germination, seedling growth-like length, fresh and dry weight, and biochemical parameters such as total chlorophyll and total carbohydrate of the test crop, *Eleusine coracana* seedlings. Further, it was also observed that the inhibitory effects were gradually increased with a rise in the leaf leachate concentration.

Conclusion: It is recommended that crops not be associated with different species of *Eucalyptus* due to the allelopathic influence on germination and early vegetative developmental stages of crops.

Keywords: *Eucalyptus* leaf litter leachate, Finger millet, Allelochemicals, Seed germination, Seedling growth, Total chlorophyll, Total carbohydrates, Ragi.

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INTRODUCTION

Molisch, in 1937, introduced the term Allelopathy which alludes to all biochemical interactions (inhibitory and Stimulatory) among plants. Allelopathy can be distinguished from other plant interference strategies, because the donor species produces inhibitory compounds into the environment to cause harm. Muller, in 1969, suggested that allelopathy alludes to the overall effect (Stimulatory and inhibitory) of one plant on the other. In both natural and agricultural ecosystems, allelopathy is just as significant as competition in influencing plant growth. Most parts of the plant, such as the root, rhizome, stem, flower, and leaves, produce allelochemicals (Joshi and Joshi, 2021). The chemicals released by one plant interfere with other plants' seed germination and seedling growth (Alam and Islam, 2002, Aragão, 2017). The allelochemicals harm crop plants' seed germination, development, and yield (Herro and Callaway, 2003; Gurmu 2015; Regu 2018; Yakubu *et al.*, 2018).

Eucalyptus is mainly planted in agroforestry schemes. According to Luna (2016), agro forestry is meant primarily to obtain multiple benefits through interactive and international land use. *Eucalyptus*-based agroforestry also has a prominent role in the economic expansion of developed countries in the tropics. *Eucalyptus* species, append to the family *Myrtaceae*, is native to Australia. *Eucalyptus* species can be luxuriously grown in tropical regions (Dawar *et al.*, 2007).

Agronomical features of test crop (*Eleusine coracana*)

Finger millet is a vital millet cultivated in Odisha, Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Uttarakhand, Karnataka, few hilly regions of the northeastern areas of India for its high-yielding potentiality, nutritional value, and usefulness in diabetes. Finger millets (Mandia in Odia) are primarily grown in Odisha in an area of 1.66 lakh hectares with

a production of 1.44 lakh tons (Agrawal, 2019). Of all the millets and cereals, finger millet has the highest amount of calcium and potassium. Also rich in essential amino acids such as methionine, tryptophan, threonine, isoleucine, and valine. Finger millets are safe for people who have a Gluten allergy and Celiac disease Kochhar 2011.

Millets are often grown on soil with less fertility. Millets are superior to wheat and rice in terms of vitamins, proteins, minerals, etc. Finger millet is a nutraceutical and is nutritious, non-glutinous, and non-acid-forming food. It has many nutraceutical and health-promoting properties. The most abundant source of calcium (300-350 mg/100 g), finger millet also has high fiber content and more sulfur-rich amino acids with antioxidant activities (Chandra, 2019).

METHODS

Dry fallen leaves of *Eucalyptus* were collected from Mathuranath, Balasore. The leaf litter leachates were made by soaking dry leaves in distilled water in a 1:5 ratio for 72 h, following the method adopted by Padhy *et al.* in 1992. The leachate is now used as a stock solution. From this, different leachate concentrations were prepared after dilution with distilled water. Experiments were conducted using aqueous leaf extract at 100%, 80%, 60%, 40%, and 20% and distilled water as control. Freshly prepared leachates were used to hydrate the finger millet seeds in the laboratory. The finger millet seeds were sterilized with 0.1% Mercuric chloride for one minute, followed by a thorough rinse of the seeds many times to remove the Mercuric chloride (Rawat *et al.*, 2016).

The germination experiment was conducted in five replicas for each concentration and control set in large sterilized Petri dishes, circular-cut blotting paper was placed in the Petri dish, and 20 finger millets

seeds were kept at an equal distance on the blotting paper of each Petri dish. To hydrate the test seeds required, leachates and distilled water were poured into the respective Petri dishes.

In this investigation, pure line seeds of finger millet were used to find the germination and seedling growth in response to allelochemicals present in the aqueous extract of *Eucalyptus* leaf.

RESULTS AND DISCUSSION

The allelopathic effects of *Eucalyptus* leaf litter leachate clearly showed inhibition in a concentration-dependent manner on the percentage of seed germination, length, fresh and dry weight, and biochemical content of root and shoot of *E. coracana*. The study exhibited maximum phytotoxicity at 100% extract treatment compared to the control. The result showed a considerable decrease in seed germination, seedling root, shoot length, fresh and dry weight, total chlorophyll, and carbohydrate content of the test crop with increasing leachate concentration.

Table 1: Effects of aqueous leachete of *Eucalyptus* leaves on seed germination of Ragi (*Eleusine coracana* Geartn) (Each datum is mean of five replicates±SE)

Concentration of leachate (%)	Days after soaking (DAS)	Percentage of germination
1 Control	2	3
	4	96.4±2.2
	6	97.3±2.3
20	2	99.2±2.4
	4	62.7±1.9
	6	66.1±2.0
40	2	87.2±2.1
	4	42.0±1.1
	6	46.0±1.4
60	2	50.6±1.7
	4	33.0±1.1
	6	36.0±1.2
80	2	45.0±1.5
	4	22.0±0.6
	6	25.2±0.9
100	2	30.0±1.0
	4	9.2±0.2
	6	12.9±0.4
		15.0±0.6

The data on the allelopathic effect on seed germination is presented in Table 1. Data on the allelopathic effect on some morphological characters at a very early seedling stage, like length, dry and fresh weight of shoot, and root, are presented in Tables 2 and 3 which show the biochemical contents, that is, total chlorophyll and total carbohydrates of test seedlings.

Data given in the Table 1 showed that all the concentrations of *Eucalyptus* leaf leachates eloquently reduce the seed germination of finger millet. The highest seed germination percentage was observed in 6 DAS in the control set (99.2%), while the minimum observed in the leaf leachate treatment of 100% concentration was 15%. It was noticed that all the leachate concentrations tested considerably inhibited the germination rate.

Germination is an essential stage of plant development. Any biochemical changes during germination provide the basic framework for further growth and development. A decline in germination might be caused by different allelochemicals and the phytotoxin in the leachate, which checks the amylase, peroxidase, acid phosphatase activity, or activation of the hydrolyzing enzyme (Alam and Islam, 2002).

Table 2 shows that all concentrations of *Eucalyptus* leaf leachate significantly decrease the development of test seedlings. The maximum shoot and root length was observed in 12 DAS in the control set, that is, 4.3±0.03 cm and 8.4±0.027 cm, respectively, while minimum shoot and root length was observed in 100% leachate treatment set, that is, 0.4±0.003 and 0.5±0.01 cm. Maximum shoot, root fresh and dry weight was observed in 12 DAS in control set 7.1±0.14 mg and 1.8±0.08 and 1.5±0.06 and 0.46±0.05 mg, respectively, while minimum shoot, root fresh, and dry weight found in 100% leachate treatment set, that is, 0.92±0.01, 0.45±0.003, 0.14±0.002, and 0.04±0.001 mg respectively.

Table 3 showed that the highest total chlorophyll was observed in 12 DAS in the control set, that is, 0.703±0.02 mg/g fresh weight. While at 12 DAS, the minimum total chlorophyll was estimated in 100% leachate concentration set, 0.123±0.004 mg/g fresh wt. Table 3 also showed total carbohydrate content, maximum observed in 12DAS in the control set of both shoot and root, that is, 0.399±0.003 and 0.396±0.002 mg/g fresh weight, respectively, while lowest total carbohydrate in shoot and root found in 100% leachate treatment set, that is, 0.126±0.003 and 0.092±0.005 mg/g fresh weight, respectively.

Eucalyptus species produce inhibitory substances (Patel et al., 2002). These allelopathic substances or allelochemicals are exuded by the

Table 2: Effects of aqueous leachete of *Eucalyptus* leave on seedling growth of Finger millet (*Eleusine coracana* Geartn) (Each datum is mean of 15 seedlings±SE)

Conc. of leachate (%)	Days after soaking (DAS)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (mg)	Root fresh weight (mg)	Shoot dry weight (mg)	Root dry weight (mg)
1 Control	2	3	4	5	6	7	8
	8	2.8±0.07	7.7±0.18	5.9±0.13	1.3±0.07	0.97±0.03	0.17±0.02
	10	3.4±0.11	7.9±0.22	6.5±0.13	1.6±0.08	1.2±0.03	0.34±0.03
20	12	4.3±0.03	8.4±0.27	7.1±0.14	1.8±0.08	1.5±0.06	0.46±0.05
	8	2.1±0.08	5.0±0.12	4.8±0.08	0.86±0.02	0.73±0.02	0.13±0.01
	10	2.5±0.09	5.5±0.16	5.3±0.09	1.2±0.02	0.81±0.02	0.21±0.01
40	12	3.1±0.14	6.2±0.17	5.4±0.10	1.3±0.05	0.94±0.03	0.26±0.01
	8	1.2±0.04	1.7±0.09	2.4±0.05	0.73±0.01	0.41±0.004	0.12±0.006
	10	1.6±0.04	2.2±0.10	2.8±0.06	0.82±0.01	0.53±0.006	0.14±0.007
60	12	2.1±0.06	2.5±0.11	3.1±0.06	1.1±0.02	0.62±0.007	0.18±0.008
	8	0.9±0.02	1.2±0.06	1.5±0.02	0.51±0.008	0.26±0.002	0.06±0.002
	10	1.1±0.03	1.4±0.05	1.8±0.02	0.65±0.008	0.41±0.003	0.08±0.003
80	12	1.4±0.05	1.7±0.07	2.2±0.03	0.86±0.01	0.47±0.004	0.12±0.003
	8	0.5±0.008	0.7±0.02	0.88±0.01	0.34±0.003	0.14±0.001	0.03±0.002
	10	0.6±0.007	0.9±0.03	1.1±0.01	0.46±0.004	0.27±0.002	0.07±0.003
100	12	0.7±0.007	1.2±0.04	1.3±0.02	0.61±0.005	0.32±0.003	0.09±0.004
	8	0.1±0.002	0.2±0.01	0.57±0.01	0.16±0.001	0.09±0.001	0.02±0.001
	10	0.2±0.002	0.3±0.01	0.71±0.01	0.22±0.002	0.11±0.001	0.03±0.001
	12	0.4±0.003	0.5±0.01	0.92±0.01	0.45±0.003	0.14±0.002	0.04±0.001

Table 3: Effects of aqueous leachate of *Eucalyptus* leaves on total chlorophyll and total carbohydrate content of Ragi (*Elusine coracana*) seedlings (Each datum is mean of 15 seedlings, \pm SE from mean)

Conc. of leachate (%)	Days after soaking (DAS)	Total chlorophyll content (mg/g fresh wt.)	Total carbohydrate content (mg/g fresh wt.)	
			Shoot	Root
Control	8	0.347 \pm 0.002	0.215 \pm 0.002	0.214 \pm 0.003
	10	0.551 \pm 0.001	0.302 \pm 0.001	0.298 \pm 0.002
	12	0.703 \pm 0.002	0.399 \pm 0.003	0.396 \pm 0.002
20	8	0.287 \pm 0.003	0.26 \pm 0.003	0.223 \pm 0.003
	10	0.410 \pm 0.002	0.318 \pm 0.005	0.366 \pm 0.002
	12	0.548 \pm 0.001	0.396 \pm 0.003	0.400 \pm 0.003
40	8	0.253 \pm 0.002	0.217 \pm 0.002	0.180 \pm 0.003
	10	0.306 \pm 0.001	0.284 \pm 0.002	0.216 \pm 0.003
	12	0.378 \pm 0.001	0.360 \pm 0.003	0.259 \pm 0.001
60	8	0.089 \pm 0.006	0.147 \pm 0.003	0.098 \pm 0.002
	10	0.168 \pm 0.004	0.174 \pm 0.005	0.127 \pm 0.005
	12	0.271 \pm 0.003	0.218 \pm 0.003	0.163 \pm 0.003
80	8	0.069 \pm 0.003	0.077 \pm 0.003	0.044 \pm 0.002
	10	0.141 \pm 0.002	0.109 \pm 0.005	0.068 \pm 0.004
	12	0.167 \pm 0.002	0.144 \pm 0.003	0.101 \pm 0.001
100	8	0.051 \pm 0.006	0.046 \pm 0.003	0.031 \pm 0.002
	10	0.109 \pm 0.003	0.099 \pm 0.005	0.047 \pm 0.004
	12	0.123 \pm 0.004	0.126 \pm 0.003	0.092 \pm 0.005

Eucalyptus plant and can benefit or damage the growth of another plant. The reduction in length and weight of Ragi seedlings might have been caused by the harmful effect of allelochemicals in water uptake by seedlings and a diminution in other physiological processes (Patel *et al.*, 2002; Scavo *et al.*, 2019).

The result of the present research was in accord with Padhy *et al.* (2000), Herro and Callaway, 2003, Rassaeifar *et al.* 2013, Manoj, (2014), Ghanuni *et al.*, (2015), Joshi and Joshi (2016 and 2017), Li *et al.*, in 2021, Abdalla *et al.*, in 2021, Mushtaq *et al.*, in 2020, Sikolia and Ayuma, in 2018, Nega and Gudeta, in 2019, Mandal *et al.*, in 2018, Mishra, in 2018, Dafaallah *et al.*, in 2019, Yakubu *et al.*, in 2018, and Epee Missé, in 2019. They had reported a significant decrease in seed germination and seedling growth (root and shoot length and weight). An analogous phytotoxic effect on germination and seedling growth of the medicinal and aromatic plant studied by Zubay *et al.*, 2021, allelopathic effects of sesame extracts on seed germination of bamboo observed by Zhao *et al.* (2022), *Senna garrettiana* leaves extract to inhibit the growth of *Lepidium sativum* L. and *Echinochloa crus-Galli* (L.) studied by Krumsri *et al.* 2022., and Aragão *et al.*, (2017) found that the sesquiterpene in *Lepidaploa rufogrisea* had a phytotoxic effect on weeds. In their investigation, Nega and Gudeta (2019) reported that allelochemicals in the *Eucalyptus globulus* plant components severely affect teff and barely seed germination and early seedling growth.

The reduction in seed germination and seedling growth might be due to an imbalance of metabolism and metabolic treatment, regulated by various emergence activities from seed (Padhy *et al.*, 2000). It has been observed that the influence of allelochemicals on seed sprouts and the development of roots may be due to a reduction in cell division. Nishida *et al.* (2005) reported that in plant root apical meristem, monoterpenoids such as 1,8-cineole, beta-pinene, Camphor, α pinene, and camphene influenced DNA synthesis and cell proliferation.

Allelochemicals can hinder the action of Na⁺/K⁺ATPase involved in the absorption and ion transport through the cell membrane. Many previous studies explained the increase of free radical concentrations resulting in damage to the membrane system of plants (Lin, 2010, Al Harun *et al.*, 2014, Sunmonu and Van student 2014, Aragão *et al.*, 2017).

CONCLUSION

The present research showed that aqueous extract of *E. globulus* leaf different at different concentration gradients inhibited seed germination,

weight (fresh and dry), length, and biochemical parameters of Ragi seedlings. More study is required to explore the possible biochemical and physiological mechanisms related to the allelopathic impact of plants. It is recommended that crops not be associated with different species of *Eucalyptus* due to the allelopathic influence on germination and early vegetative developmental stages of crops.

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