



Effect of *Piper betle* L. leaves Extract on Seed Germination and Early Seedling Growth of Some Oil Seeds

Ayodhya Dattatray Kshirsagar* and Riyaj Raju Inamdar

Department of Botany, Chandmal Tarachand Bora College, Shirur

*Email: drayodhya11@gmail.com

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Abstract

This study assessed the impact of *Piper betle* L. leaf extract on seed germination and early seedling development of two oilseed crops, sunflower (*Helianthus annuus* L.) and safflower (*Carthamus tinctorius* L.). Aqueous leaf extracts were prepared at different concentrations and applied under controlled laboratory conditions. Germination percentage, root length, shoot length, and seedling vigor index (SVI) were measured to evaluate early growth responses. The results demonstrated a concentration-dependent effect of the extract on both species. Lower concentrations promoted better germination performance, with sunflower showing maximum germination at 2.5% and safflower at 10% concentration. However, increasing extract concentration progressively reduced germination, root and shoot elongation, and SVI. Root growth was more severely inhibited than shoot growth, indicating greater sensitivity of below-ground tissues. Comparatively, safflower exhibited higher tolerance than sunflower at moderate extract levels, maintaining relatively better growth parameters. Overall, the findings suggest that *P. betle* leaf extract possesses biologically active compounds capable of influencing early crop growth. While higher concentrations exhibit inhibitory effects, lower concentrations may serve as a potential eco-friendly seed priming agent for improving early-stage performance in selected oilseed crops.

INTRODUCTION

Seed germination and early seedling development are critical stages in a plant's life cycle, determining the establishment, vigor and productivity of crops. These phases are highly sensitive to biochemical and environmental stimuli, making them useful indicators for assessing the influence of natural compounds on plant growth processes. In recent years, interest has grown in exploring plant-derived extracts for their effects on seed physiology, both as potential growth stimulants and as natural regulators of germination.

Piper betle L. commonly known as betel vine, is a perennial climber valued in traditional medicine for its rich profile of bioactive compounds, including phenolics, alkaloids, and essential oils. These constituents can influence metabolic activities in living systems not only in humans and animals but also in plants. The

allelopathic interactions of plant extracts, where one plant's biochemical secretions affect the growth of another, have been examined across several species with mixed results, ranging from growth promotion to inhibition depending on concentration and plant type.

Plant leaves extracts are known to contain treasures of bioactive compounds which can influence germination and early seedling growth. These phytochemicals may act as natural growth stimulators or inhibitor. Phenolic compounds in betel leaf extracts may have allelopathic effects on seed germination and seedling growth of crops and weeds (Choopayak *et al.*, 2022). Despite its well-known medicinal applications, limited studies have explored the use of betel leaf extract as a natural biostimulant for seed germination and seedling growth.

Carthamus tinctorius L. and *Helianthus annuus* L. are commercially important cultivated oilseed crops belonging from Asteraceae family. These plants are usually cultivated in arid and semi-arid zone where rain fed conditions, low rainfall and high evapotranspiration during vegetation periods restricts the crops growth (Kaya *et al.*, 2003). Safflower is a drought-tolerant crop, while sunflower is well known for its high oil content and different climatic tolerance (Singh and Nimbkar 2006). These treatments enhancing the germination percentage and early growth of selected crops could contribute considerably to their yield potential and overall productivity. Improving their germination rate and seedling vigor can significantly contribute to higher yield potential and better crop establishing under field conditions. Still recent studies have explored the use of plant extracts, particularly betel leaf extract, on the germination and early growth of crops (Thonsoongnern, and Phraprasert, 2019). Using leaf extracts, such as betel leaf, can offer a sustainable and eco-friendly approach to increase germination and seedling vigor, reducing the reliance on chemical growth regulators.

This study investigates the effects of different concentrations of betel leaf extract on germination percentage and early seedling growth of safflower and sunflower. It aims to determine whether the extract acts as a growth promoter or inhibitor during early developmental stages. The results may provide useful insights into the potential use of betel leaf extract as a sustainable, plant-based input for improving seed performance, particularly in organic and low-chemical farming systems.

MATERIALS AND METHODS

Collection of Plant Material:

Fresh mature leaves of *P. betle* L. were collected from healthy plants in the Shirur (Pune). While Seeds of safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.) were collected from local seed supplier from Shirur (Pune).

Preparation of *P. betle* L. leaves aqueous extract:

For preparing plant leaves extract, fresh, healthy betel leaves were collected, rinsed with tap water followed by distilled water, and blotted dry. 50 gm fresh leaves were chopped and boiled in 100 mL distilled water for 10 min. Then let it be cooled, filtered through muslin and Whatman No. 1 filter paper. The stock was stored at 4 °C and used for further experiment.

Qualitative phytochemical screening:

For the confirmation of phytochemical presence in Distilled water Betel leaf extract, qualitative

analysis was done with the help of Sanathi and Sengottuvel *et al.*, 2016.

Seed surface sterilization:

Healthy, disease free, uniform seeds of Safflower and Sunflower were selected for further study. Surface-sterilization of selected seeds were done by immersion in 70% ethanol for 30 seconds followed by 1% sodium hypochlorite for 2 minutes, then rinsed thoroughly with 2-3 times sterile distilled water (ISTA, 2019). Sterilized seeds were blotted dry on sterile filter paper.

Seed Treatment and Germination Setup:

Different concentration of plant extract (0%, 2.5 %, 5%, 10%, 15%, 20%) were prepared from the stock by dilution with distilled water. By using Seed priming (soak) method, sterilized seeds Safflower and Sunflower were soaked in different concentration for 08 hours at room temperature (25 ± 2 °C), then surface dried and inoculated to sterile Petri dishes. Experiments were arranged in a completely randomized design with three replicates per treatment. Each replicate consisted of 10 seeds placed in a sterile Petri dish lined with two layers of germination paper. The dishes were incubated at 25 ± 2 °C under 12-hour light and dark cycles for 14 days. Moistened regularly with distilled water. Germination was monitored daily. Seeds were considered germinated when the radicle emerged to at least 2 mm (ISTA, 2021).

The number of seeds germinated was observed daily and the final data was recorded at 7 days after treatment. The results obtained were converted into percentage germination. The germination percentage was computed using the following formula:

Germination (%) = $\frac{\text{Number of seed germinated}}{\text{total number of seed for test}} \times 100$

For Safflower and Sunflower seeds, shoot and root length was recorded at 7 -14 days after treatment. All germinated seedlings root length and shoot length was measured from the root-shoot zone to tip. In the same way, root length was measured from the base to the top of the radicles of all germinated seedlings. Vigor Index (VI) of the seedlings was calculated using following formula
Vigor index = Germination (%) \times (Root length + Shoot length)

RESULTS AND DISCUSSION:

Qualitative Phytochemical Screening

Various aspects of *P. betle* L. leaves were extensively investigated due to its phytochemical constituents present in its leaves, and the previous studies revealed that the plant contains a wide range of medicinal and nutritional importance.

During present study aqueous extract also showed the presence of phytochemicals such as alkaloids, terpenoids, phenols, tannins, protein, glycosides and saponins. The qualitative results indicate that was aqueous extracted a wider range of phytochemicals compared. Similar trends have been reported by Biswas *et al.*, 2022.

Effect of *Piper betle* L. leaves Extract on Seed Germination of safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.)

The results of this study revealed that the Betel leaf extract significantly influenced the germination rate and early seedling growth of the Safflower and Sunflower. The results are summarized in Table 1 and Table 2

During present study, Table 1 and 2 shows the mean germination percentage, mean root length, mean of shoot length and vigor index of safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.) seeds treated with various concentrations of *P. betle* L. leaves extract.

During present study, *C. tinctorius* L seed showed the highest germination ($85.33 \pm 1.53\%$) in 10% concentration of leaves extract, while the lowest germination ($16.05 \pm 2.0\%$) was recorded in the 20% concentration of leaves extract treatment (Table 1). Seeds treated with concentrations (10%) of the leaf extract showed a significant increase in germination percentage compared to the control. While in *H. annuus* L seeds recorded highest germination ($69.00 \pm 0.82\%$) at 2.5% concentration of leaves extract. A gradual decrease in seed germination percentage were observed with increasing concentration (5%, 10%, 15% 20%) of *P. betle* L. leaves extract. This indicates a dose-dependent inhibitory effect on *H. annuus* seed germination. *P. betle* L. leaves extract contains allelochemicals such as eugenol and flavonoids that can influence seed germination and seedling growth by inhibiting enzymatic activity (Srichand and Supaporn, 2019).

The results indicate that *P. betle* L. leaves extract can be phytotoxic at higher concentrations, it might have potential as a natural biostimulant, depending on how it is applied. Similar observations were made by Kumar *et al.* (2015), who reported concentration-dependent effects of *P. betle* L. extracts on *Phaseolus mungo* germination. Thus, understanding the appropriate concentration range is essential if *P. betle* L. leaves extract is to be utilized for agricultural purposes.

The germination of *C. tinctorius* L. and *H. annuus* L. seeds was influenced by the concentration of *P. betle* L. leaves extract. In *C. tinctorius* L. seed germination in lower

concentrations of *P. betle* L. leaves extract (5 % and 10 %) caused stimulation, while higher concentrations (15 % and 20%) resulted in strong inhibition. In *H. annuus* L. seed germination in lower concentrations of *P. betle* L. leaves extract (2.5%) caused stimulation, while higher concentrations (15% and 20%) resulted in strong inhibition. Hormetic effect (stimulation at low dose, inhibition at high dose) is well documented in seed bioassays. Root and shoot lengths were significantly reduced at higher concentrations (15% and 20%) of betel leaf extract, indicating a strong inhibitory effect.

In present study, *C. tinctorius* L. and *H. annuus* L. recorded maximum vigor index at 10% and 2.5% concentration of *P. betle* L. leaves extract respectively. Low concentrations *P. betle* L. leaves extract respectively (2.5%) promoted elongation, while higher concentrations reduced growth in sunflower seed. Root and shoot elongation were optimal at 2.5 % extract for *H. annuus* and for *C. tinctorius* 10 % and 5%, indicating stimulatory effects of bioactive compounds in betel leaf.

Seedling vigor index combines germination and growth parameters, providing measure of early seedling performance Maximum seedling vigor occurred at 2.5% extract for *H. annuus* and 10% for *C. tinctorius*. SVI declined sharply at 5–20%, indicating inhibitory effects at higher concentrations for sunflower seed germination.

Conclusion:

The leaves extract of *Piper betle* L. had a significant influence on seed germination and early seedling development in both *Carthamus tinctorius* L. and *Helianthus annuus* L. Germination percentage, root length, shoot length, and seedling vigor index declined progressively as the concentration of the extract increased. In *Helianthus annuus*, lower concentrations (2.5%) showed slight inhibition with nearly normal growth, indicating mild tolerance or possible biostimulatory effects. However, higher concentrations acted as growth inhibitors. In contrast, *Carthamus tinctorius* demonstrated comparatively greater tolerance, maintaining higher germination rates, longer root and shoot lengths, and a better vigor index at 10% extract concentration than *H. annuus*. The consistent reduction in Seedling Vigor Index (SVI) with increasing extract levels confirms the concentration-dependent phytotoxic effects of betel leaf extract. Nevertheless, at lower concentrations, betel leaf extract shows potential as a natural and environmentally friendly seed priming agent for both crops

Table 1. Effect of *Piper betle* L leaves extract concentration on Safflower (*Carthamus tinctorius* L.) seed germination and growth parameters

Concentration of Extract (%)	Germination (%)	Root Length (cm)	Shoot Length (cm)	Seedling Vigor Index
Control (0%)	72± 2.65	4.7 ± 0.7	7.0± 1.11	835.2
2.5%	60±2.0	3.3 ± 1.23	5.08± 1.24	501.6
5%	62.65 ± 1.53	5.4 ± 1.19	7.3 ± 0.41	777
10%	85.33 ± 1.53	7.5± 1.78	8.1 ± 1.37	1365.3
15%	40.65 ± 1.57	4.3± 1.16	5.7 ± 1.28	378.2
20%	16.05± 2.0	2.3 ± 0.25	3.53 ± 0.15	92.8

Values are mean ± SD of three replicates (n=3).

Table 2. Effect of *Piper betle* L leaves extract concentration on sunflower (*Helianthus annuus* L.) seed germination and growth parameters

Concentration of Extract (%)	Germination (%)	Root Length (cm)	Shoot Length (cm)	Seedling Vigor Index
Control (0%)	55.33 ± 1.40	4.9± 1.11	6.63±0.78	778.78
2.5%	69.00± 0.82	6.9±0.99	8.00±1.23	1030.4
5%	10.65± 0.94	5.1±1.11	5.77±0.56	116.27
10%	6.33±1.24	3.2±0.72	4.75±0.73	50.35
15%	3.65± 0.47	2.03±0.19	3.43±0.6	20.04
20%	2.33± 1.69	1.7±0.15	2.35±0.16	9.64

Values are mean ± SD of three replicates (n=3).

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