RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume 59 Issue 4: 543-549 (April 2025)

Phytopriming of *Vigna mungo* (L.) Hepper (Blackgram) Seeds using Aqueous Extracts of *Moringa oleifera* Lam, *Gliricidia sepium* (Jacq.) Walp and *Pongamia pinnata* (L.) Pierre Improves Seedling Emergence, Growth and Yield

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10.18805/IJARe.A-6334

ABSTRACT

Background: Phytopriming can enhance seed germination and seedling emergence. Seeds when primed with plant leaf extracts result in better establishment and growth of field crops.

Methods: This study examines the potential of leaf extracts of *Moringa oleifera* Lam, *Gliricidia sepium* (Jacq.) Walp and *Pongamia pinnata* (L.) Pierre on the growth and yield of black gram through seed hydropriming.

Result: Pot studies revealed a 10% increase in shoot length and 3% increase in fresh weight upon priming with *P. pinnata* (L.) Pierre leaf extract in comparison to liquid fertilizer treatment. Also, 6% increase in root nodule formation was observed in *G. sepium* (Jacq.) Walp and *P. pinnata* (L.) Pierre treated plants. Testing the biochemical parameters revealed a 20% increase in total phenolics, 12% increase in total carbohydrates and 64% increase in chlorophyll pigment in the case of *P. pinnata* (L.) Pierre leaf extract treated group. The data presented shows that a 4% *M. oleifera* Lam leaf extract, 10% *G. sepium* (Jacq.) Walp, or 4% *P. pinnata* (L.) Pierre leaf extract can be used for improving seedling emergence, plant growth and yield. Among the three, the best overall growth and yield can be obtained by using a 4% *P. pinnata* (L.) Pierre leaf extract as a seed priming agent.

Key words: Black gram, Growth enhancement, Phytopriming, Plant extracts.

INTRODUCTION

In recent decades, seed priming has been demonstrated to effectively improve seed germination and support seedling growth. Various methods of seed priming such as biopriming, halopriming, hormonal priming, hydropriming and osmopriming have been used to improve germination (Lutts *et al.,* 2016). Among these techniques, hydropriming is the most commonly followed and effective method (Pandita *et al.,* 2007). Hydropriming involves soaking seeds in an aqueous solvent for 24 hours or overnight prior to sowing in the field. This process facilitates the rapid absorption of water and oxygen by the seeds, triggering a range of biochemical activities. Utilization of storage compounds namely carbohydrates, lipids, proteins and the production of ROS, the activation of enzymes like POX, APX and GH, are among these activities (Hussain *et al.,* 2018). Crucial to successful seed germination, antioxidant enzymes can act as molecular markers for seed vigor (Pagano *et al.,* 2019). A study conducted on *V. mungo* (L.) Hepper (black gram) demonstrated that the application of *Spirulina platensis* extract positively influenced the germination of seedlings and their subsequent development (Quang Thinh and Sundareswaran, 2018). *Ocimum sanctum* leaf extracts increased the number of nodules in *V. mungo* (L.) Hepper. The presence of nodules indicates the plant's ability to form a symbiotic relationship with rhizobia, which enhances nitrogen fixation and promotes plant growth (Dugesar *et al.,* 2017).

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How to cite this article: Krishnasamy, R. and Obbineni, J.M. (2025). Phytopriming of *Vigna mungo* (L.) Hepper (Blackgram) Seeds using Aqueous Extracts of *Moringa oleifera* Lam, *Gliricidia sepium* (Jacq.) Walp and *Pongamia pinnata* (L.) Pierre Improves Seedling Emergence, Growth and Yield. Indian Journal of Agricultural Research. 59(4): 543-549. doi: 10.18805/IJARe.A-6334.

Submitted: 29-10-2024 **Accepted:** 16-01-2025 **Online:** 06-03-2025

M. oleifera Lam is a widely used plant source, with its leaves and slender fruit also known as drumstick commonly used in food across South Asia. The diverse culinary and medicinal applications of Moringa reflect its significance as a valuable plant resource (Baldisserotto *et al.,* 2018). Its leaf extract, when applied to wheat plants, has shown positive effects on nutrient availability, photosynthesis, root development and overall plant growth and yield (Khan *et al.,* 2017).

G. sepium (Jacq.) Walp is also a fast-growing tree species and has the ability to fix nitrogen in the soil (Kaba *et al.,* 2019). It has been mostly used as fodder and for firewood, live fencing, intercropping and shade crops in coffee plantations (Alamu *et al.,* 2023; Beedy *et al.,* 2010). *P. pinnata* (L.) Pierre is a nitrogen-fixing tropical tree that thrives in nitrogen-deficient and contaminated soils, demonstrating its ability to enrich the soil with essential nutrients (Unnikannan *et al.,* 2013). It has diverse applications in agriculture and biology, with its residual seed oil cake utilized as a soil amendment to improve soil quality and nutrient content and its leaves used as green leaf manure and for pest control (Jathanna *et al.,* 2020).

V. mungo (L.) Hepper, known as black gram is a nitrogen-fixing tropical pulse crop, has suffered declining yields due to seed-borne infections and poor seed quality (Prakash *et al.,* 2020). One potential solution to this issue involves utilizing plant extracts as hydropriming agents to enhance germination, seedling vigor and growth. To overcome the poor seed quality and declining yields in black gram, the study conducted to examine the use of *Pongamia pinnata* leaf extract (*PLE*), *Gliricidia sepium* leaf extract (*GLE*) and *Moringa oleifera* leaf extract (*MLE*) as priming agents to improve the seed viability as well as plant growth.

Materials and Methods

The experiment was conducted from October 2022 to December 2022 at the research cum instructional farm of Vellore Institute of Technology, Vellore, Tamil Nadu, India.

Materials

Leaves of *M. oleifera* Lam, *G. sepium* (Jacq.) Walp and *P. pinnata* (L.) Pierre were collected from various locations in Vellore, India. All the reagents and chemicals were obtained from HiMedia Laboratories Pvt. Ltd and Sisco Research Laboratories (India).

Extraction process

The leaves were washed, dried and ground before being extracted with distilled water. The leaf samples were combined with distilled water (1:10 *w/v)*. The solution was then placed in a water bath for 20 mins with a set temperature of 60°C (Krishnaraj *et al.,* 2012). The solution was allowed to cool down to room temperature and filtered. The filtered extract was stored in an airtight container at 4°C. For priming, different concentrations of the extract in distilled water were prepared by dilution (2%, 4%, 6%, 8% and 10%) (Radwan *et al.,* 2019).

Seed priming test

Surface sterilization of *V. mungo* (L.) Hepper seeds was done by gentle agitation in 4% sodium hypochlorite solution for 10 mins. Then, the seeds were soaked for 3 hrs in the aforementioned concentrations of the different leaf extracts. A control condition was also setup by soaking seeds in distilled water. To carry out the seed germination test, a sterile Petri dish was taken and two sterile filter papers were placed on it. Distilled water (~10-15 mL) was added to the dish to keep the filter papers moist. Twelve seeds were then placed per plate and kept at room temperature. After 10 days, seedling growth parameters were measured. The experiment was conducted in triplicate and repeated twice to ensure reliable results and account for any experimental variations (Ismail *et al.,* 2016).

Pot studies

The pot studies were conducted under net house in Vellore, India. Polyvinyl chloride pots (height - 25 cms and outer diameter - 30 cms) were used. A total of 15 pots were filled with 10 kg of dehydrated and sieved red soil (pH 7.1 and EC 237 mSm^{-1}) and split into 5 groups with 3 pots in each group. Pots in each group were labeled as group A to E. The labels referred to the different treatments given *viz.*, group A for distilled water only, group B for liquid fertilizer (N: P: K 19:19:19), group C for undiluted *MLE*, group D for undiluted *GLE* and group E for undiluted *PLE*.

Surface sterilized *V. mungo* (L.) Hepper (blackgram) seeds were soaked for 1 hr in the respective solutions. Eight seeds that were soaked in the respective solutions were sown in each pot. Thinning was done after ten days and only two plants (showing uniform growth) were retained per pot. 1 lit of tap water was applied to each pot three times a week to maintain the soil moisture. 20 mL of the respective solution *i.e.*, distilled water for group A, 4% liquid fertilizer (N: P: K 19:19:19) for group B, undiluted *MLE* for group C, undiluted *GLE* for group D and undiluted *PLE* for group E was given per plant by foliar application on the 15th, 30th and 45th day after sowing (Karthik *et al.,* 2020).

Measurement of physiological parameters

On the 70th day after sowing, two plants from each group were harvested, washed and analyzed for root nodules, root and shoot length, fresh and dry weight, grain weight and number of pods per plant.

Measurement of biochemical parameters

On the 47th day leaf samples were collected and stored at -80°C. For the measurement of biochemical parameters samples were prepared for each assay as per previously reported protocol (Karthik *et al.,* 2020). The total carbohydrate in the sample was estimated using phenol-sulphuric acid method (Chow and Landhäusser, 2004). Using Lowry's method, total protein was estimated (Lowry *et al.,* 1951). For the estimation of total amino acid, ninhydrin method was used (Yemm *et al.,* 1955). Folin Ciocalteau reagent was used to estimate the total phenol (Rekha *et al.,* 2012). Photosynthetic pigments were estimated using a previously published method (Sarkar *et al.,* 2018).

Results and Discussion

Effect of hydropriming using leaf extracts on seedling growth

Leaf extracts at different concentrations were used for hydropriming the *Vigna mungo* (L.) Hepper (blackgram)

seeds. Seeds hydroprimed with 4% *MLE* displayed the longest root length of 7.91±0.23 cm and the longest shoot length of 16.47±0.36 cm was observed in the 10% extract group (Fig 1A). Seeds hydroprimed with 10% *GLE* exhibited the longest root length of 7.99±0.25 cm, whereas seeds primed with a 6% extract had the longest shoot length of 13.74±0.24 cm (Fig 1B). Seeds hydroprimed with 2% *PLE* showed the longest root length (7.26±0.38 cm) whilst the longest shoot length was observed in the case of 4% extract (14.03±0.31 cm) (Fig 1C).

From Fig 1 it is evident that hydropriming with *MLE*, *GLE and PLE* has led to better seedling growth and it can be concluded that 4% *MLE*, 10% *GLE* and 4% *PLE* are effective in promoting seedling growth.

Effect of hydropriming using leaf extracts on plant growth and yield

Pot experiments were conducted to examine the effect of the different leaf extracts on the growth characteristics and yield of the blackgram. Experiments showed that plants treated with commercial fertilizer (19:19:19) showed the highest root length (39.67±1.05 cm) (Fig 2A). On the other hand, *PLE* treated plants showed the highest shoot length (42.35±1.53 cm) (Fig 2B).

Plant biomass analysis revealed that the fresh weight of *PLE* treated group was the highest (50.09±0.71 g) and that of the untreated plant was lowest (30.31±1.33 g) (Fig 3A). The dry weight of plants treated with liquid fertilizer was highest (23.14±0.89 g) and that in the untreated group was lowest (10.50±0.31 g) (Fig 3B). The morphological differences between the plants of different treatment groups, *viz.*, control, liquid fertilizer, *MLE*, *GLE* and *PLE* can be seen in Fig 4.

Other growth characteristics such as root nodules per plant and yield characteristics such as pods per plant as well as grain weight were measured. Treatment with *PLE* resulted in the highest number of well-developed root nodules per plant followed by *GLE* treated group, liquid fertilizer treated group and *MLE* treated group (Fig 5A). The grain weight and number of pods per plant were highest in liquid fertilizer treated group. Plants treated with *PLE* also showed a significantly high grain weight and number of pods per plant (Fig 5B and Fig 5C).

Assessment of plant metabolite levels upon hydropriming

The total protein, carbohydrate, phenol, chlorophyll and carotenoid were measured using biochemical assays. Total protein concentration was found to be highest in *MLE*

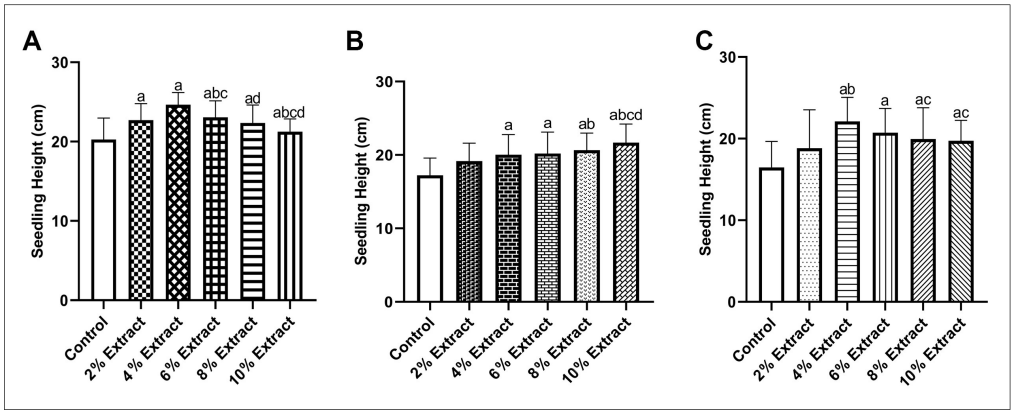


Fig 1: Effect of hydropriming using varying concentrations of A. *MLE* B. *GLE* and C. *PLE* on growth of *V. mungo* (L.) Hepper (blackgram) seedlings as estimated by measuring the seedling height.

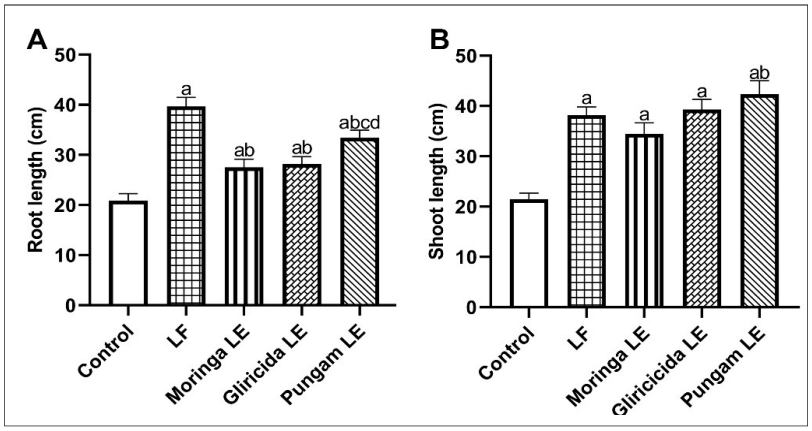


Fig 2: Effect of hydropriming using liquid fertilizer (N: P: K 19:19:19), *MLE*, *GLE and PLE* on A. Root Length and B. Shoot Length of *V. mungo* (L.) Hepper (blackgram) plants.

treated group (0.536±0.0153 mg/ml) (Fig 6A). Carbohydrate content was highest in the plants treated with *PLE* (0.5915±0.043 mg/ml) (Fig 6B). Phenol content was highest in the plants treated with *PLE* (0.3668±0.021 mg/ml) (Fig 6C). The photosynthetic pigment chlorophyll a was highest in the plants treated with *PLE* (0.6536±0.018 mg/ml) (Fig 7A). Chlorophyll b content was highest in plants treated with liquid fertilizer (0.4923±0.051 mg/ml) (Fig 7B). Carotenoid content in the liquid fertilizer group was highest at 0.0537±0.003 mg/ml (Fig 7C).

Seedling and plant growth can be enhanced by using several plant leaf extracts and organic compounds as priming agents (Duary *et al.,* 2022). Poor seed quality and lower germination rates can be overcome by hydropriming with plant leaf extracts. In the current study, we have assessed the effect of hydropriming using aqueous leaf extracts of *M. oleifera* Lam, *G. sepium* (Jacq.) Walp and *P. pinnata* (L.) Pierre on the growth and yield of *V. mungo* (L.) Hepper (blackgram). Biomass analysis revealed significant growth enhancement in the case of seeds hydroprimed with different plant extracts. Seeds hydroprimed with 4% *MLE* showed a 21% increase, 10% *GLE* showed a 20% and 4% *PLE* showed the highest increase of 27% in seedling height. In pot trials, growth parameters such as dry weight and fresh weight as well as root and shoot length showed a significant increase. Notably, *GLE* treated plants

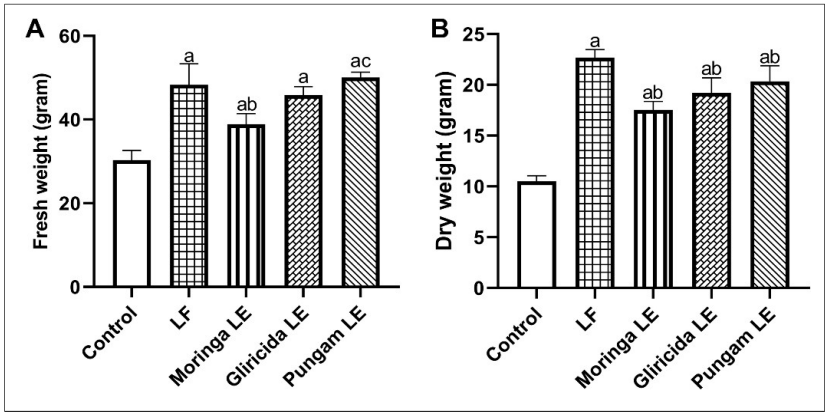


Fig 3: Increase in A. fresh weight B. dry weight of *V. mungo* (L.) Hepper (blackgram) plants upon hydropriming using liquid fertilizer (N: P: K 19:19:19), *MLE*, *GLE* and *PLE*.

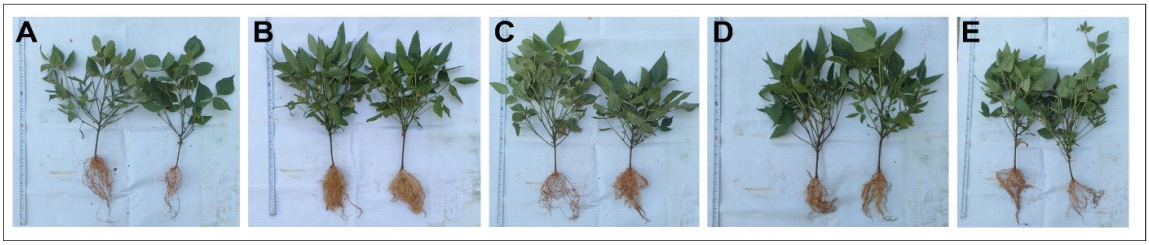


Fig 4: Morphological differences in *V. mungo* (L.) Hepper (blackgram) plants hydroprimed using A. water, B. liquid fertilizer (N: P: K 19:19:19), C. *MLE*, D. *GLE* and E. *PLE*.

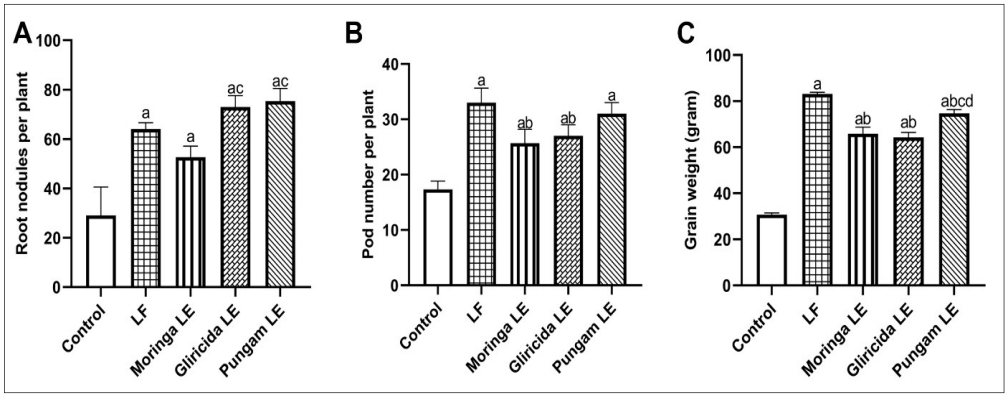


Fig 5: Growth and yield characteristics of *V. mungo* (L.) Hepper (blackgram) plants on hydropriming using liquid fertilizer (N: P: K 19:19:19), MLE, GLE and PLE. A. Root nodules per plant. B. Pods per plant. C. Grain weight.

exhibited a 3% increase in shoot length and *PLE* treated plants exhibited a 10% increase in shoot length as well as 3% increase in fresh weight in comparison to liquid fertilizer-treated group. The improved emergence of the seedlings as well as increase in plant growth can be attributed to the several plant nutrients, *i.e.*, N, P, K and Ca present in *MLE*, *GLE* and *PLE* (Alamu *et al.,* 2023; Foidl *et al.,* 2001; Islam *et al.,* 2021). The stimulatory effect may also be due to the presence of several bioactive compounds in the extracts (ElSayed *et al.,* 2022). Another notable observation is a 6% increase in root nodules per plant in *GLE* and *PLE* treated plants. This may be attributed to flavones which are vital in mediating the molecular dialogue between rhizobia and legume plants. This symbiosis allows legume plants to access a significant source of nitrogen through biological nitrogen fixation, contributing to their growth, development and ecological adaptation (Zhang *et al.,* 2009). *GLE* and *PLE* have been reported to contain many flavones thus playing a role in a multitude of applications (Jurd and Manners,1977; Marzouk *et al.,* 2007). Biochemical attributes such as total protein, phenol and carbohydrates also showed a significant increase upon hydropriming. Treatment with *MLE* showed a 25% increase in total protein and treatment with *PLE* resulted in a 12% increase in total carbohydrates as well as 20% increase in total phenol. *MLE*, *GLE* and *PLE* treatments resulted in a significant increase in chlorophyll a by 30%, 49% and 64% respectively. An increase in chlorophyll pigments would facilitate an improvement in the water use efficiency as well as gas exchange attributes. There is also an increase in photosynthetic and respiration activities. Previous studies have reported that seeds pelleted with pungam leaf powder showed increased chlorophyll (Prakash *et al.,* 2021) and exogenous application of *MLE,* improved chlorophyll pigments (Rashid *et al.,* 2021).

Overall, the *PLE* treated group exhibited higher shoot length, fresh weight and other physiological parameters. The extract is known to have an active compound myo-inositol which acts as a signaling molecule involved in various physiological processes (Sharma *et al.,* 2020). *GLE* and *MLE* have an active compound called quercetin which plays a pivotal role in seed germination, photosynthesis and pollen development. Another flavonoid called rutin is also present in these extracts. It regulates the biosynthesis of growth hormones such as cytokinin, auxin and gibberellins (Gorni *et al.,* 2022). *MLE* is also rich in vitamins, alkaloids and phytohormones which regulate the plant growth (Karthiga *et al.,* 2022). Hydropriming of seeds with plant leaf extracts holds great promise and can be particularly beneficial for farming systems using low

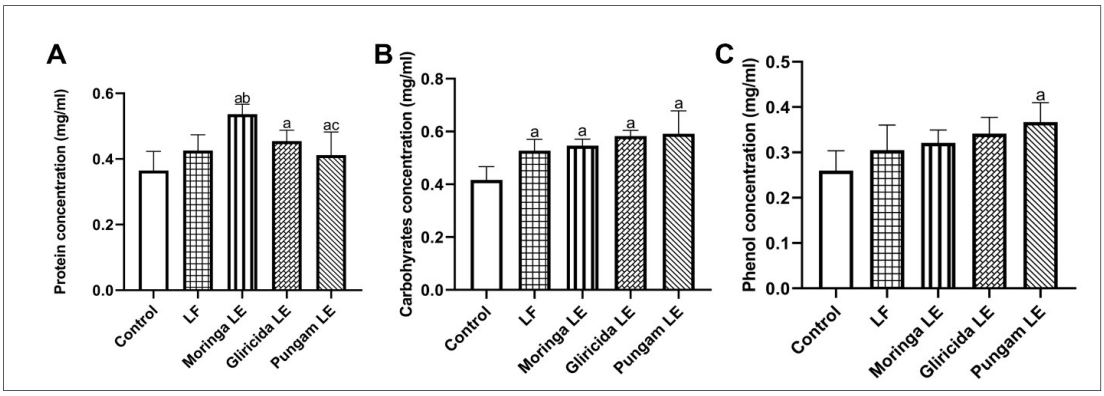


Fig 6: Concentration of A. Protein B. Carbohydrate and C. Phenol in *Vigna mungo* (L.) Hepper (blackgram) plants on hydropriming using liquid fertilizer (N: P: K 19:19:19), *MLE*, *GLE* and *PLE*.

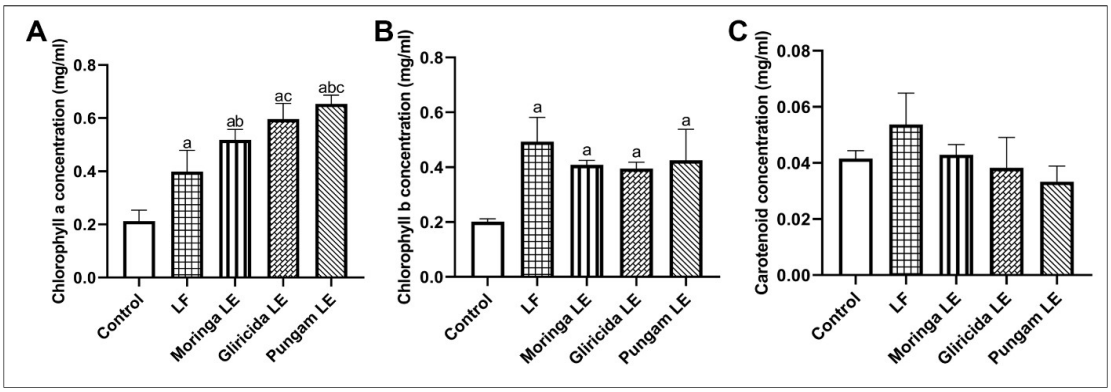


Fig 7: Estimation of photosynthetic pigments A. chlorophyll a B. chlorophyll b and C. carotenoid in *V. mungo* (L.) Hepper (blackgram) plants on hydropriming using liquid fertilizer (N: P: K 19:19:19), *MLE*, *GLE* and *PLE*.

external agricultural inputs and to a large extent depending on on-farm resources (Carrillo-Reche *et al.,* 2018). *MLE* at 4%, *GLE* at 10% and *PLE* at 4% can be used by the farmer as a priming agent for *V. mungo* (L.) Hepper (blackgram) seeds to enhance growth and productivity with the best results obtained using *PLE*.

Conclusion

Vigna mungo (L.) Hepper (blackgram) seeds hydroprimed with 4% *M. oleifera* Lam extract, 10% *G. sepium* (Jacq.) Walp extract and 4% *P. pinnata* (L.) Pierre leaf extract showed significant improvement in seedling growth and development. Subsequent pot trials revealed that all tested leaf extracts exhibited notable benefits in terms of yield attributes and plant growth parameters. Among the different extracts, *P. pinnata* (L.) Pierre leaf extract displayed the most pronounced effects, resulting in superior plant growth and increased grain weight. Based on these findings, it can be concluded that the application of these leaf extracts as an alternative to commercial fertilizers holds promise for sustainable agricultural practices. By harnessing the natural growth-promoting properties of these leaf extracts, farmers can potentially reduce their reliance on synthetic chemical fertilizers, mitigating their negative environmental impact. Further investigations are warranted to gain an in-depth understanding of the underlying mechanisms behind the beneficial effects of these leaf extracts. Field trials encompassing a broader range of crop species and environmental conditions would provide valuable insights.

Acknowledgement

This work was funded by VIT under the rGEMS grant and the Science and Engineering Research Board (SERB), Govt. of India, vide grants TAR/2022/000312 and SRG/2020/001690.

Disclaimers

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Conflict of interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. The authors declare no conflict of interest.

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