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Screening of natural biostimulants and optimization of seed priming concentrations for enhancing germination, seedling vigour and reserve mobilization in mungbean (*Vigna radiata* L. Wilczek)

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Abstract

Rapid and uniform seed germination is essential for achieving optimum crop establishment and productivity in mungbean (*Vigna radiata* L. Wilczek). Seed priming with natural biostimulants has emerged as an environmentally sustainable strategy to enhance seed performance by improving reserve mobilization and early seedling growth. The present study was conducted to screen different natural biostimulants and determine their optimum concentrations for seed priming in mungbean under laboratory conditions.

The experiment was carried out in a completely randomized design with a factorial arrangement consisting of seventeen natural biostimulants, including seaweed extract, cow urine and aqueous extracts of medicinal plants, along with hydroprimed control. Each biostimulant was evaluated at four concentrations (2, 3, 4 and 5%) with three replications. Seeds were primed for 12 h before incubation under laboratory conditions. Germination percentage, vigour indices, and α -amylase activity were recorded after seven days.

Biostimulant treatments significantly influenced all measured parameters. Among the concentration levels, 3% exhibited the highest overall performance with respect to germination, seedling growth, vigour indices and reserve mobilization, whereas 5% resulted in a decline in most traits, indicating possible phytotoxic effects at higher concentrations. Among the tested biostimulants, *Leucas aspera* (2%) produced the highest germination percentage and vigour index, seaweed extract (3%) recorded maximum seedling biomass and vigour index II, while cow urine (3%) showed the highest α -amylase activity and mobilization efficiency. These responses demonstrate that moderate concentrations of natural biostimulants effectively enhance metabolic activity during germination by promoting enzyme activation and efficient utilization of seed reserves.

The study concludes that natural biostimulants can serve as sustainable alternatives to synthetic seed treatments for improving early seedling establishment in mungbean. Based on the overall performance, a priming concentration of approximately 3% was found to be optimum for most biostimulants, although the ideal concentration varied among individual extracts. The identified superior treatments provide promising candidates for subsequent pot and field evaluation.

Keywords: α -amylase, biostimulants, germination, mungbean, reserve mobilization, seed priming, seedling vigour

Introduction

Mungbean (*Vigna radiata* L. Wilczek) is one of the most important pulse crops cultivated in tropical and subtropical regions because of its high protein content, short duration and ability to improve soil fertility through biological nitrogen fixation. It serves as an inexpensive source of dietary protein, vitamins and minerals and plays a vital role in sustainable cropping systems. However, poor seedling establishment caused by slow or uneven germination frequently limits productivity, particularly under adverse environmental conditions such as high temperature, moisture deficit and soil salinity. Improving early seedling vigour therefore remains a major objective in mungbean production.

Seed priming is a simple and effective pre-sowing treatment in which seeds are partially hydrated to initiate metabolic activities associated with germination without allowing radicle emergence. Following drying, primed seeds exhibit faster and more synchronized germination, improved enzyme activity, greater stress tolerance and enhanced seedling

establishment. Seed priming has become an important agronomic technique because it is inexpensive, environmentally safe and easily adopted by farmers.

Recently, natural biostimulants have attracted considerable attention as alternatives to synthetic growth regulators due to increasing concerns regarding environmental sustainability and chemical inputs. Plant-derived extracts, seaweed products and animal-derived organic formulations contain diverse bioactive compounds, including phytohormones, amino acids, vitamins, phenolics, osmoprotectants and essential mineral nutrients, which stimulate physiological and biochemical processes in plants. These compounds improve nutrient uptake, activate antioxidant defence systems, enhance enzyme activities and promote plant growth under both normal and stressful conditions. Consequently, natural biostimulants are increasingly being incorporated into sustainable crop production systems.

Seaweed extracts are among the most widely studied biostimulants because they contain naturally occurring auxins, cytokinins, gibberellins, betaines and polysaccharides that stimulate germination and seedling growth. Similarly, cow urine has long been recognized in traditional agriculture as a source of macro- and micronutrients, growth-promoting substances and beneficial microorganisms. Several medicinal plants such as *Leucas aspera*, *Ocimum tenuiflorum*, *Acalypha indica*, *Euphorbia hirta* and *Heliotropium indicum* possess diverse secondary metabolites with antioxidant and antimicrobial properties that may influence seed metabolism during germination. Nevertheless, comparative information regarding their suitability as seed priming agents and the optimization of their application concentrations remains limited.

The beneficial effects of seed priming are largely mediated through enhanced reserve mobilization during germination. Activation of hydrolytic enzymes, particularly α -amylase, facilitates the degradation of starch into soluble sugars that supply energy for embryo growth and seedling development. Enhanced reserve mobilization is therefore closely associated with improved germination, seedling vigour and biomass accumulation. Measuring α -amylase activity together with mobilization efficiency provides valuable insights into the physiological mechanisms underlying the action of different biostimulants.

Although numerous studies have demonstrated the beneficial effects of individual biostimulants, systematic screening of a broad range of natural biostimulants under identical experimental conditions is scarce. Furthermore, information on the optimum concentrations required to maximize seedling performance while avoiding inhibitory effects is limited. Determining the most effective priming concentration is essential because excessive concentrations may induce osmotic stress or phytotoxicity, reducing germination and seedling growth.

Therefore, the present investigation was undertaken to (i) compare the efficacy of different natural biostimulants as seed priming agents for mungbean, (ii) determine the optimum concentration for enhancing germination and seedling vigour, and (iii) investigate their influence on α -amylase activity and reserve mobilization during early seedling development. The findings are expected to identify promising eco-friendly seed priming agents that can improve crop establishment and provide a scientific basis for their subsequent evaluation under pot and field conditions.

Materials and Methods

The present investigation was conducted during 2024 at Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India, to evaluate the effect of ethnobotanical plant extracts, seaweed extract and cow urine on Mungbean seedling traits. The study comprised an *in vitro* screening and dose optimization experiment followed by a pot experiment under controlled conditions. Healthy seeds of mungbean local variety 'HUM-16' sourced from TCA Dholi, RPCAU were surface sterilized with 0.1% HgCl_2 solution for 2-3 min followed by washing with distilled water. Seeds were primed with 16 different treatments at four concentrations viz., 2%, 3%, 4% and 5%. The treatments were extracts made from the ethnobotanical plants i.e., *Lantana Camara*, Moringa, Catnip, *Leucas aspera*, *E. hirta*, *Achyranthes*, *Calotropis*, *Oxalis*, *Datura*, *T. procumbens*, *A. indica*, *Ocimum* and *I. heliotrope*. The ethnobotanicals materials for extract preparation are easily available locally. Fresh, fully expanded young and tender leaves of ethnobotanical plants were collected, washed and air-dried briefly to remove surface moisture. For the stock extract, 250 g of leaves were blended with 1000 mL Cow Urine (1:4 w/v), and the slurry was left for 12 hrs and filtered first through muslin cloth and then through Whatman No. 1 paper to produce a clear solution. Working solutions of 2%, 3%, 4% and 5% were prepared by diluting the 2 mL, 3 mL, 4 mL, 5 mL stock solution with 100 mL of distilled water respectively. These mixtures were prepared fresh on the day of priming.

Seeds were primed with each priming agent at respective concentrations for 12 hours at room temperature. The experiment was set up in petri plates with each replication having 20 seeds of Mungbean followed by incubation. Control seeds were soaked only in distilled water, and all other aspects of their handling were the same as for treated seeds. Data were collected from 7 days old seedling. The parameters recorded from the experiment along with their formulae are as follows: Germination percentage (G%) = $[\text{Number of germinated seeds} / \text{Total number of seeds}] \times 100$; Vigour index I (VI-I) = Germination percentage $\times$ Seedling length [cm] (Abdul-Baki and Anderson, 1973); Vigour index II (VI-II) = Germination percentage $\times$ Seedling dry weight [mg] *et al.* (Abdul-Baki and Anderson, 1973), and α -amylase (Bernfeld, 1955) ^[15]. Length was measured with the aid of scale ruler, while dry weight was measured on electronic weighing balance (with precision of 0.1 mg) after drying the seedlings in hot air oven at $65 \pm 2^\circ\text{C}$ for one day.

Statistical analysis

The experiment was conducted in a Completely Randomized Design (CRD) with three replications to evaluate the effects of different seed priming treatments. All statistical analyses were conducted in R software using the *GRAPESAgri1* package developed for agricultural data analysis.

Results and Discussion

Effect of biostimulants and priming concentration on germination percentage

Seed priming with different natural biostimulants significantly ($p \leq 0.05$) influenced germination percentage of mungbean (Table 1). Significant effects were observed for treatment (T), concentration (C) and their interaction (T $\times$ C), indicating that the response of mungbean seeds depended on both the type of biostimulant and the concentration applied.

Among the tested biostimulants, Indian heliotrope recorded the highest mean germination (89.25%), followed by cow urine (90.50%), *Leucas aspera* (86.67%), catnip (85.42%) and seaweed extract (84.17%). Hydroprimed control maintained a consistently high germination (91.67%) across all concentrations. The lowest mean germination was observed with Aloe (67.50%), followed by *Achyranthes* (71.67%) and *Calotropis* (72.92%).

Considering concentration means, 3% priming produced the highest overall germination (85.25%), which was statistically comparable to 2% (84.76%) but significantly

higher than 4% (80.61%) and 5% (73.51%). Germination progressively declined with increasing concentration beyond 3%, indicating that excessive concentrations inhibited seed germination.

Among individual treatment × concentration combinations, the maximum germination (95.33%) was recorded with Indian heliotrope (3%), closely followed by seaweed extract (2%) and *Leucas aspera* (2%) (95.00%). Conversely, the lowest germination (50.00%) was observed with Aloe (5%), demonstrating a pronounced inhibitory effect of higher extract concentration. Figure 1

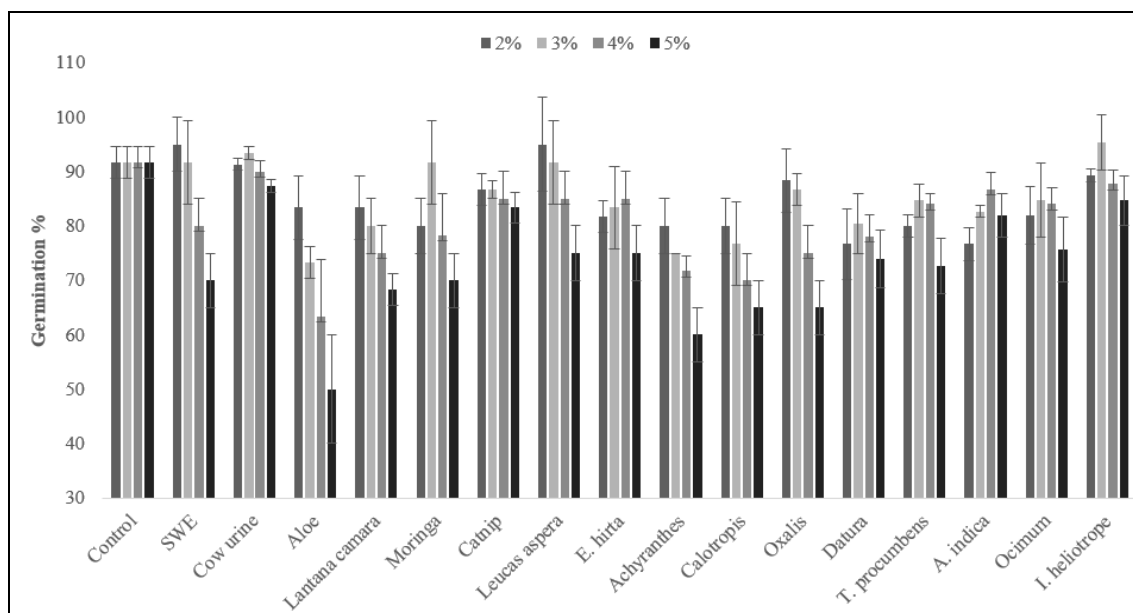


Fig 1: Effect of seed priming treatments on Germination percentage in Mungbean seedlings

Table 1: Germination percentage (%)

Treatment (T)	Concentration (C)				Mean (T)
	2%	3%	4%	5%	
Control	91.67 ± 2.89	91.67 ± 2.89	91.67 ± 2.89	91.67 ± 2.89	91.67 ± 2.46
SWE	95.00 ± 5.00	91.67 ± 7.64	80.00 ± 5.00	70.00 ± 5.00	84.17 ± 11.45
Cow urine	91.33 ± 1.15	93.33 ± 1.15	90.00 ± 2.00	87.33 ± 1.15	90.50 ± 2.58
Aloe	83.33 ± 5.77	73.33 ± 2.89	63.33 ± 10.41	50.00 ± 10.00	67.50 ± 14.54
LC	83.33 ± 5.77	80.00 ± 5.00	75.00 ± 5.00	68.33 ± 2.89	76.67 ± 7.18
Moringa	80.00 ± 5.00	91.67 ± 7.64	78.33 ± 7.64	70.00 ± 5.00	80.00 ± 9.77
Catnip	86.67 ± 2.89	86.67 ± 1.53	85.00 ± 5.00	83.33 ± 2.89	85.42 ± 3.18
<i>Leucas aspera</i>	95.00 ± 8.66	91.67 ± 7.64	85.00 ± 5.00	75.00 ± 5.00	86.67 ± 9.85
<i>E. hirta</i>	81.67 ± 2.89	83.33 ± 7.64	85.00 ± 5.00	75.00 ± 5.00	81.25 ± 6.08
<i>Achyranthes</i>	80.00 ± 5.00	75.00 ± 0.00	71.67 ± 2.89	60.00 ± 5.00	71.67 ± 8.35
<i>Calotropis</i>	80.00 ± 5.00	76.67 ± 7.64	70.00 ± 5.00	65.00 ± 5.00	72.92 ± 7.82
<i>Oxalis</i>	88.33 ± 5.77	86.67 ± 2.89	75.00 ± 5.00	65.00 ± 5.00	78.75 ± 10.69
<i>Datura</i>	76.67 ± 6.43	80.33 ± 5.51	78.00 ± 4.00	74.00 ± 5.29	77.25 ± 5.17
<i>T. procumbens</i>	80.00 ± 2.00	84.67 ± 3.06	84.00 ± 2.00	72.67 ± 5.03	80.33 ± 5.71
<i>A. indica</i>	76.67 ± 3.06	82.67 ± 1.15	86.67 ± 3.06	82.00 ± 4.00	82.00 ± 4.51
<i>Ocimum</i>	82.00 ± 5.29	84.67 ± 6.81	84.00 ± 3.00	75.67 ± 6.03	81.58 ± 5.96
<i>I. heliotrope</i>	89.33 ± 1.15	95.33 ± 5.03	87.67 ± 2.52	84.67 ± 4.51	89.25 ± 5.12
Mean (C)	84.76 ± 7.09	85.25 ± 7.84	80.61 ± 8.54	73.51 ± 11.06	
Interaction	T	C	T × C		
SE(m)	1.43	0.7	2.87		
CD	4.01	1.95	8.02		

The stimulatory effect of moderate concentrations of biostimulants can be attributed to improved water uptake, activation of metabolic pathways and enhanced enzyme activity during germination. Plant-derived biostimulants contain naturally occurring phytohormones, amino acids, vitamins and phenolic compounds that promote embryo growth and accelerate the repair of cellular components

during imbibition. Similar improvements in germination following seed priming with natural biostimulants have been reported in several crops, where enhanced metabolic activation resulted in faster and more uniform seedling emergence (Paparella *et al.*, 2015; Roupael and Colla, 2020; Yakhin *et al.*, 2017) [6, 2, 5].

The decline in germination at 5% concentration suggests that higher concentrations may create osmotic stress or introduce phytotoxic levels of secondary metabolites, thereby restricting water uptake and delaying metabolic activation. Similar concentration-dependent inhibitory responses have been documented for several botanical extracts, where excessive phenolics, alkaloids and other allelochemicals interfered with germination and early seedling growth (du Jardin, 2015; Roupheal and Colla, 2020) [1, 2].

The superior performance of Indian heliotrope, cow urine and Leucas aspera indicates that these biostimulants possess favorable bioactive compounds capable of stimulating germination without causing toxicity at optimum concentrations. The overall findings demonstrate that 3% represents the optimum concentration for enhancing germination in mungbean under laboratory conditions, whereas concentrations above this threshold become progressively inhibitory.

3.2 Effect of biostimulants on Vigour Index I

Vigour Index I differed significantly among treatments, concentrations and their interaction (Table 2), indicating that seedling vigour was markedly influenced by both the nature of the biostimulant and priming concentration.

Among the treatments, *Leucas aspera* recorded the highest mean Vigour Index I (2017.91), followed closely by Indian heliotrope (1999.16), cow urine (1915.78) and seaweed extract (1913.93). The control exhibited a mean value of 1874.33. The lowest vigour was observed in *Achyranthes* (962.88), *Datura* (954.85) and *Calotropis* (1011.92). Figure 2.

Across concentrations, the highest mean Vigour Index I was obtained at 3% (1602.08), followed by 2% (1529.47), whereas 4% (1435.03) and particularly 5% (1167.64) significantly reduced seedling vigour. The highest individual value (2435.33) was recorded in Indian heliotrope at 3%, followed by cow urine (3%) (2261.93) and *Leucas aspera* (2%) (2299.40).

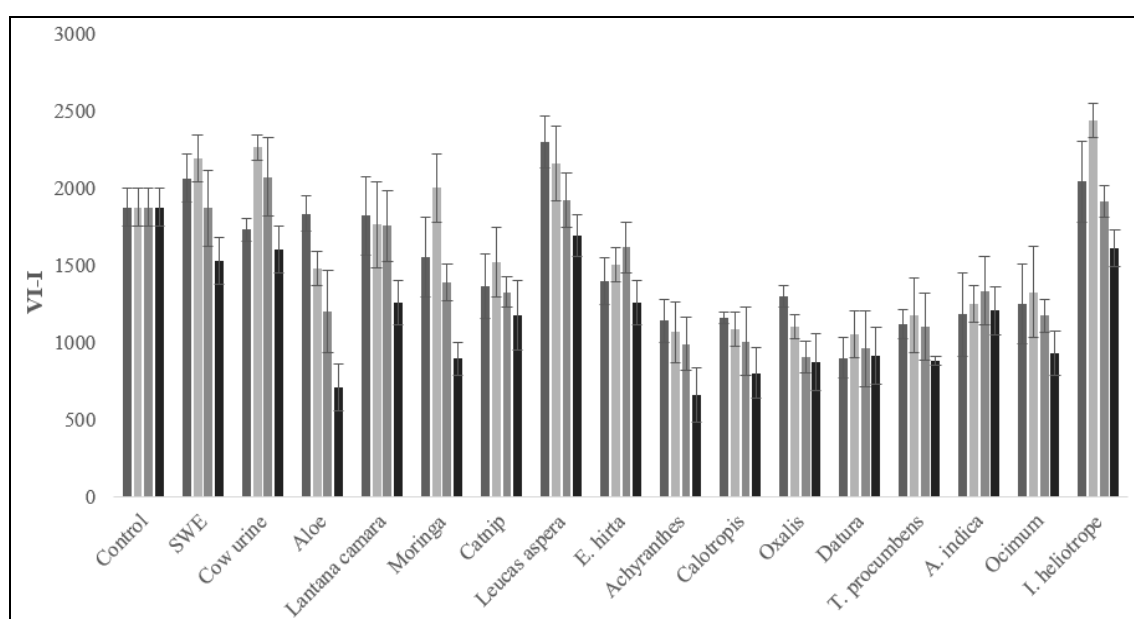


Fig 2: Effect of seed priming treatments on Seed Vigour Index I in Mungbean seedlings

Table 2: Vigour Index I

Treatment (T)	2%	3%	4%	5%	Mean (A)
Control	1874.33 ± 121.04	1874.33 ± 121.04	1874.33 ± 121.04	1874.33 ± 121.04	1874.33 ± 103.22
SWE	2062.75 ± 350.21	2192.92 ± 154.23	1870.31 ± 245.16	1529.73 ± 351.38	1913.93 ± 357.83
Cow urine	1728.53 ± 82.93	2261.93 ± 76.91	2070.67 ± 252.55	1602.00 ± 149.81	1915.78 ± 306.00
Aloe	1834.33 ± 108.15	1478.42 ± 115.83	1201.50 ± 266.23	707.67 ± 172.93	1305.48 ± 455.79
LC	1818.47 ± 277.56	1761.81 ± 251.74	1754.67 ± 228.61	1256.29 ± 243.63	1647.81 ± 319.73
Moringa	1551.67 ± 219.22	1998.83 ± 257.60	1387.67 ± 119.36	892.50 ± 103.44	1457.67 ± 442.80
Catnip	1361.67 ± 227.72	1520.43 ± 210.23	1325.00 ± 99.87	1175.83 ± 227.10	1345.73 ± 212.22
Leucas aspera	2299.40 ± 244.06	2159.52 ± 167.44	1920.79 ± 174.63	1691.92 ± 137.97	2017.91 ± 289.08
E. hirta	1396.23 ± 111.85	1502.21 ± 150.50	1615.29 ± 164.04	1255.50 ± 145.80	1442.31 ± 185.44
Achyranthes	1138.17 ± 196.19	1065.00 ± 138.90	989.67 ± 173.41	658.67 ± 179.58	962.88 ± 241.86
Calotropis	1158.83 ± 109.51	1082.00 ± 38.69	1005.67 ± 219.88	801.17 ± 162.74	1011.92 ± 188.17
Oxalis	1301.00 ± 74.96	1101.83 ± 69.58	903.67 ± 104.17	870.33 ± 182.32	1044.21 ± 206.01
Datura	896.67 ± 154.72	1051.33 ± 131.37	958.07 ± 244.35	913.33 ± 186.95	954.85 ± 169.22
Tridax procumbens	1114.00 ± 240.81	1176.27 ± 94.81	1100.27 ± 218.55	877.73 ± 29.14	1067.07 ± 186.94
Acalypha indica	1178.93 ± 120.70	1247.80 ± 266.73	1332.67 ± 222.19	1204.00 ± 157.77	1240.85 ± 181.15
Ocimum	1246.13 ± 294.66	1325.33 ± 258.10	1173.00 ± 108.29	929.67 ± 245.85	1168.53 ± 254.82
Indian heliotrope	2039.93 ± 112.58	2435.33 ± 262.73	1912.23 ± 103.10	1609.13 ± 117.27	1999.16 ± 339.37
Mean (B)	1529.47 ± 429.61	1602.08 ± 485.91	1435.03 ± 420.38	1167.64 ± 399.99	1433.55
Interaction	T	C	T x C		
SE(m)	54.11	26.25	108.22		
CD	151.33	73.4	302.65		

Vigour Index I integrates germination percentage with seedling length and therefore reflects the combined effect of rapid germination and subsequent seedling growth. The enhanced vigour observed in Indian heliotrope, *Leucas aspera* and seaweed extract treatments indicates improved mobilization of seed reserves and more efficient utilization of stored carbohydrates for seedling elongation. Previous studies have shown that natural biostimulants enhance cell division, cell elongation and nutrient mobilization through increased synthesis of endogenous phytohormones and activation of metabolic enzymes (Calvo *et al.*, 2014; Rouphael and Colla, 2020; Bulgari *et al.*, 2019) ^[3, 2, 4].

The marked decline in Vigour Index I at 5% concentration suggests that excessive extract concentrations negatively affected seedling growth, possibly because of osmotic imbalance or inhibitory secondary metabolites that restricted root and shoot elongation.

Effect on Vigour Index II

Vigour Index II also exhibited significant variation among treatments and concentrations (Table 3). Mean values ranged from 1.40 in *Achyranthes* to 2.44 in Indian heliotrope.

Among all treatments, Indian heliotrope recorded the highest mean Vigour Index II (2.44), followed by *Leucas aspera* (2.21), seaweed extract (2.12), cow urine (2.09) and the control (2.08). The maximum individual value (3.02) was obtained with Indian heliotrope at 3% concentration.

The concentration effect followed a trend similar to that observed for germination and Vigour Index I. Mean Vigour Index II was highest at 3% (2.00), followed by 2% (1.97), while 4% (1.78) and 5% (1.57) showed a gradual decline. Figure 3.

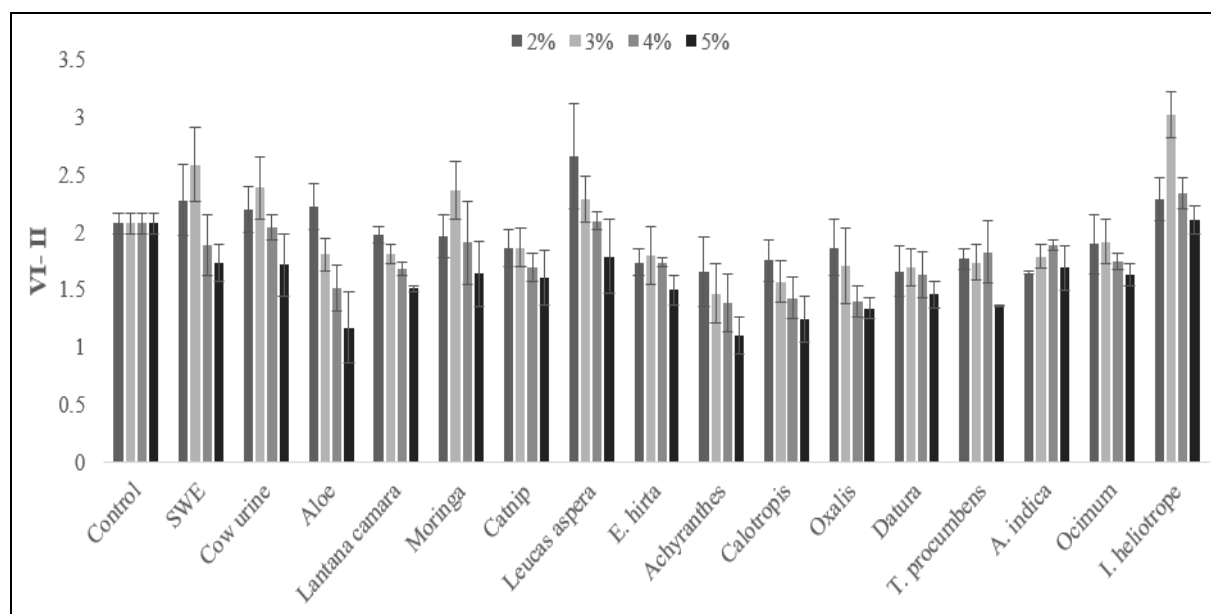


Figure 3. Effect of seed priming treatments on Seed Vigour Index II in Mungbean seedlings

Table 3: Vigour Index II

Treatment (T)	2%	3%	4%	5%	Mean (A)
Control	2.08 ± 0.09	2.08 ± 0.09	2.08 ± 0.09	2.08 ± 0.09	2.08 ± 0.08
SWE	2.28 ± 0.31	2.59 ± 0.32	1.89 ± 0.26	1.73 ± 0.16	2.12 ± 0.42
Cow urine	2.20 ± 0.20	2.39 ± 0.27	2.04 ± 0.11	1.72 ± 0.27	2.09 ± 0.32
Aloe	2.22 ± 0.20	1.81 ± 0.14	1.52 ± 0.20	1.17 ± 0.31	1.68 ± 0.45
LC	1.98 ± 0.07	1.81 ± 0.08	1.68 ± 0.06	1.51 ± 0.03	1.74 ± 0.19
Moringa	1.97 ± 0.19	2.37 ± 0.25	1.91 ± 0.36	1.64 ± 0.28	1.97 ± 0.36
Catnip	1.86 ± 0.16	1.87 ± 0.17	1.70 ± 0.12	1.61 ± 0.24	1.76 ± 0.19
Leucas aspera	2.66 ± 0.46	2.29 ± 0.20	2.10 ± 0.08	1.79 ± 0.32	2.21 ± 0.42
E. hirta	1.74 ± 0.12	1.80 ± 0.25	1.74 ± 0.04	1.50 ± 0.13	1.69 ± 0.18
Achyranthes	1.66 ± 0.30	1.47 ± 0.26	1.39 ± 0.25	1.10 ± 0.16	1.40 ± 0.30
Calotropis	1.76 ± 0.18	1.57 ± 0.18	1.43 ± 0.18	1.24 ± 0.20	1.50 ± 0.26
Oxalis	1.87 ± 0.25	1.71 ± 0.33	1.40 ± 0.13	1.34 ± 0.09	1.58 ± 0.30
Datura	1.66 ± 0.22	1.70 ± 0.16	1.63 ± 0.20	1.46 ± 0.12	1.61 ± 0.18
Tridax procumbens	1.77 ± 0.09	1.74 ± 0.15	1.83 ± 0.27	1.36 ± 0.01	1.68 ± 0.24
Acalypha indica	1.65 ± 0.01	1.79 ± 0.10	1.89 ± 0.04	1.69 ± 0.19	1.76 ± 0.13
Ocimum	1.90 ± 0.26	1.92 ± 0.19	1.75 ± 0.07	1.63 ± 0.10	1.80 ± 0.19
Indian heliotrope	2.29 ± 0.19	3.02 ± 0.20	2.34 ± 0.14	2.11 ± 0.12	2.44 ± 0.39
Mean (B)	1.97 ± 0.33	2.00 ± 0.43	1.78 ± 0.30	1.57 ± 0.32	1.83
Interaction	T	C	T x C		
SE(m)	0.06	0.03	0.12		
CD	0.16	0.08	0.32		

Since Vigour Index II incorporates seed germination and seedling dry matter accumulation, the higher values obtained with Indian heliotrope, *Leucas aspera* and seaweed extract indicate superior biomass production during early seedling establishment. Enhanced dry matter accumulation is generally associated with efficient reserve mobilization and increased photosynthetic capacity during early growth (Paparella *et al.*, 2015; Rouphael and Colla, 2020) [6, 2].

Effect on α -amylase activity

α -Amylase activity was significantly affected by treatment, concentration and their interaction (Table 4). The enzyme activity ranged from 0.18 to 0.81 mg maltose g⁻¹ FW min⁻¹,

demonstrating considerable variation among the evaluated biostimulants.

Among treatments, Indian heliotrope and cow urine recorded the highest mean α -amylase activity (0.72), followed closely by *Leucas aspera* (0.69) and seaweed extract (0.64). The lowest activity was observed in catnip (0.18).

Across concentrations, the highest mean enzyme activity was observed at 3% (0.44), followed by 2% (0.42), whereas activity declined to 0.40 and 0.35 at 4% and 5%, respectively. The maximum α -amylase activity (0.81 mg maltose g⁻¹ FW min⁻¹) was recorded in Indian heliotrope at 3% concentration. Figure 4

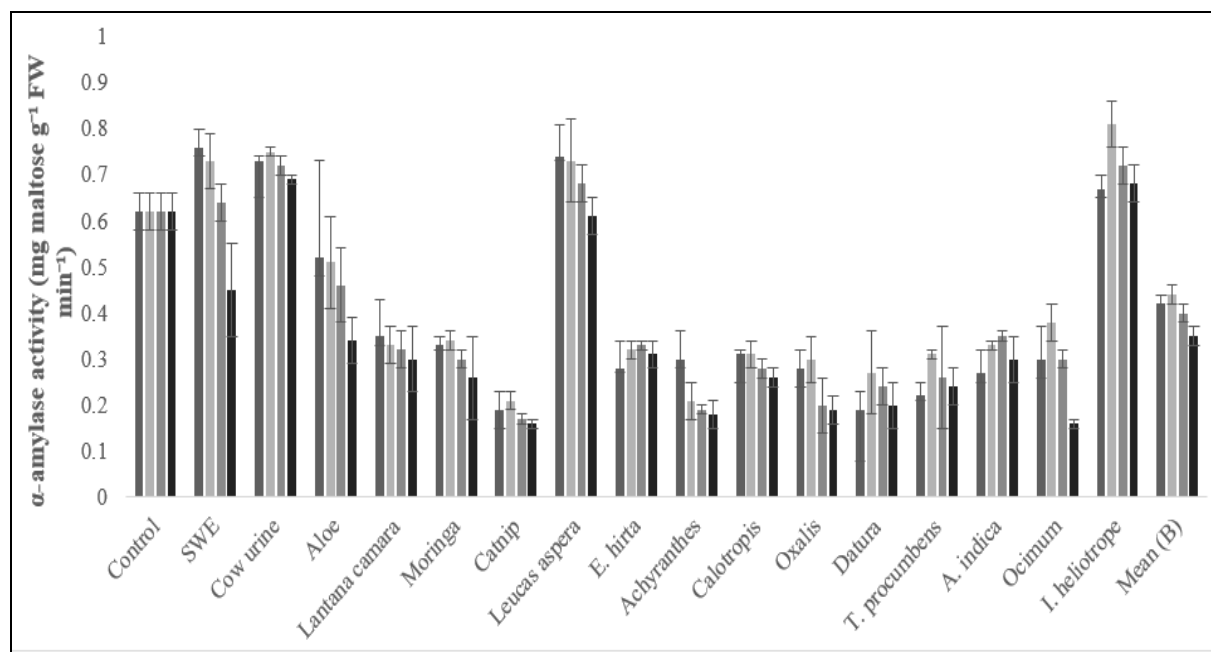


Figure 4. Effect of seed priming treatments on α -Amylase Activity (mg maltose g⁻¹ FW min⁻¹) in Mungbean seedlings

Table 4: Alpha Amylase

Treatment (T)	2%	3%	4%	5%	Mean (A)
Control	0.62 ± 0.04	0.62 ± 0.04	0.62 ± 0.04	0.62 ± 0.04	0.62 ± 0.04
SWE	0.76 ± 0.04	0.73 ± 0.06	0.64 ± 0.04	0.45 ± 0.10	0.64 ± 0.14
Cow urine	0.73 ± 0.01	0.75 ± 0.01	0.72 ± 0.02	0.69 ± 0.01	0.72 ± 0.02
Aloe	0.52 ± 0.21	0.51 ± 0.10	0.46 ± 0.08	0.34 ± 0.05	0.46 ± 0.13
LC	0.35 ± 0.08	0.33 ± 0.04	0.32 ± 0.04	0.30 ± 0.07	0.32 ± 0.05
Moringa	0.33 ± 0.02	0.34 ± 0.02	0.30 ± 0.02	0.26 ± 0.09	0.31 ± 0.05
Catnip	0.19 ± 0.04	0.21 ± 0.02	0.17 ± 0.01	0.16 ± 0.01	0.18 ± 0.03
Leucas aspera	0.74 ± 0.07	0.73 ± 0.09	0.68 ± 0.04	0.61 ± 0.04	0.69 ± 0.08
E. hirta	0.28 ± 0.06	0.32 ± 0.02	0.33 ± 0.01	0.31 ± 0.03	0.31 ± 0.04
Achyranthes	0.30 ± 0.06	0.21 ± 0.04	0.19 ± 0.01	0.18 ± 0.03	0.22 ± 0.06
Calotropis	0.31 ± 0.01	0.31 ± 0.03	0.28 ± 0.02	0.26 ± 0.02	0.29 ± 0.03
Oxalis	0.28 ± 0.04	0.30 ± 0.05	0.20 ± 0.06	0.19 ± 0.03	0.24 ± 0.07
Datura	0.19 ± 0.04	0.27 ± 0.09	0.24 ± 0.04	0.20 ± 0.05	0.23 ± 0.06
Tridax procumbens	0.22 ± 0.03	0.31 ± 0.01	0.26 ± 0.11	0.24 ± 0.04	0.26 ± 0.06
Acalypha indica	0.27 ± 0.05	0.33 ± 0.01	0.35 ± 0.01	0.30 ± 0.05	0.31 ± 0.04
Ocimum	0.30 ± 0.07	0.38 ± 0.04	0.30 ± 0.02	0.16 ± 0.01	0.29 ± 0.09
Indian heliotrope	0.67 ± 0.03	0.81 ± 0.05	0.72 ± 0.04	0.68 ± 0.04	0.72 ± 0.07
Mean (B)	0.42 ± 0.21	0.44 ± 0.21	0.40 ± 0.20	0.35 ± 0.19	0.4
Interaction	T	C	T x C		
SE(m)	0.02	0.01	0.03		
CD	0.04	0.02	0.09		

α -Amylase plays a crucial role during germination by hydrolyzing starch reserves into soluble sugars that supply energy for embryo growth. The increased α -amylase activity observed in the superior treatments indicates enhanced

metabolic activation and reserve mobilization during germination. Similar increases in α -amylase activity following seed priming have been reported in legumes and cereals, where priming accelerated starch degradation,

respiration and seedling establishment (Paparella *et al.*, 2015; Farooq *et al.*, 2019; Roupheal and Colla, 2020) [6, 7, 2]. The decline in enzyme activity at higher concentrations may be attributed to inhibitory effects of concentrated phytochemicals on enzyme synthesis or activity, resulting in reduced reserve mobilization and consequently poorer seedling performance.

Conclusion

Across all four parameters evaluated, 3% concentration consistently produced the best overall response, indicating that this concentration provided an optimal balance between stimulation of metabolic activity and avoidance of phytotoxic effects. Among the biostimulants, Indian heliotrope, *Leucas aspera*, seaweed extract and cow urine were consistently superior, exhibiting higher germination, greater seedling vigour and enhanced α -amylase activity. These findings identify these treatments as promising candidates for further evaluation under pot and field conditions.

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