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EFFECT OF SEED BIO-PRIMING ON GERMINATION, SEEDLING VIGOR, AND YIELD OF ASHWAGANDHA (*WITHANIA SOMNIFERA* L. DUNAL)

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ABSTRACT

An investigation was conducted at the Plantation Farm, College of Agriculture, Vellanikkara (September 2024-May 2025) to evaluate the efficacy of different seed bio-priming treatments on enhancing the germination, growth, and yield parameters of ashwagandha. The experiment was laid out in a Completely Randomized Design with three replications. Treatments included priming seeds with water, *Trichoderma viride* (10g kg^{-1}), *Pseudomonas fluorescens* (10g kg^{-1}), *Azospirillum* (10g kg^{-1}), panchagavya (5 and 10%), and vermiwash (10 and 20 %). Results revealed significant variations among all measured parameters. Seeds primed with 10 per cent panchagavya recorded the highest germination percentage (86.33), superior root architecture (length 24.86 cm, diameter 1.29 cm), and maximum dry root yield (2.73 g $plant^{-1}$). Panchagavya (5%) and vermiwash (20%) treatments also showed substantial impact, excelling in early vigour, vegetative growth, and biomass accumulation, and were statistically similar to panchagavya (10%). Priming with water and *T. viride* (10g kg^{-1}) consistently showed the least effectiveness. Overall, 10 per cent panchagavya seed priming proved to be a simple and efficient pre-sowing treatment to enhance the growth and productivity of ashwagandha.
***Keywords*:** Ashwagandha, panchagavya, bio-priming, germination, root yield.

Introduction

The use of medicinal plants has been an integral and foundational component of human healthcare systems since the dawn of civilization. Within these exceptional plants are inherent chemical substances used for treating several health issues, which have established the groundwork for both ancient healing traditions and contemporary medical science. From ancient remedies passed down through generations to the sophisticated pharmaceuticals of today, the healing power of plants remains a vital resource for global health. Perhaps no plant better illustrates the bridge between ancient wisdom and contemporary research than the celebrated herb Ashwagandha (*Withania somnifera* (L.) Dunal), which belongs to the Solanaceae family. Native to the arid regions of the Indian subcontinent and beyond, this hardy, erect, evergreen shrub is now a significant crop in India, cultivated on 10,768 hectares, with the majority of production concentrated in Madhya Pradesh and other northwestern states (Kumar *et al*., 2023).

Ashwagandha's benefits are wide-ranging, stemming from its active compounds, most notably withanolides, which are found primarily in the root. Its therapeutic benefits are diverse, encompassing stress-relief, neuroprotection, and potential applications against cancer (Singh *et al*., 2021), Alzheimer's (Choudhary *et al*., 2017), and Parkinson's (Prakash *et al*., 2014) diseases. Consequently, the rising awareness and global demand for these benefits have led to a significant expansion in its cultivation area, active promotion by government bodies, and a thriving international trade for the herb.

Once a niche medicinal crop, ashwagandha is rapidly transitioning into a mainstream agricultural

commodity, presenting both lucrative opportunities and notable challenges for growers across the country. Among these, poor and inconsistent seed germination remains one of the most critical constraints affecting successful crop establishment. Low germination rates not only delay plant stand formation but also disrupt uniform growth, ultimately reducing yield and profitability. Enhancing seed germination and early seedling vigour is therefore essential to ensure sustainable and profitable ashwagandha cultivation. Seed bio-priming has been reported to enhance germination and overall seedling performance in this crop (Narendrakumar, 2019).

In this context, the present study was conducted to evaluate the effect of different seed bio-priming treatments on the germination of ashwagandha.

Materials and Methods

A pot experiment was carried out at the Plantation farm, College of Agriculture, Vellanikkara, from September 2024 to May 2025 to evaluate the effect of different seed bio-priming treatments on germination. Seeds of the ashwagandha variety 'Poshita', procured from the CSIR-CIMAP Research Centre, Bengaluru, were used for the experiment. The experiment was laid out in a Completely Randomized Design (CRD) where seeds were subjected to a range of pre-sowing treatments for a 12-hour period, with each treatment replicated thrice as specified in Table 1. Seeds were sown in 98-cell protrays composed of coirpith compost and FYM mixed in a 1:1 ratio. The progress of seed germination was monitored at regular intervals. After being maintained in the protrays for 45 days, the seedlings were transplanted into 15 kg capacity, UV-stabilized grow bags measuring 40×40 cm with a 600 gauge thickness. Each bag was filled with 13 kg of potting medium prepared in a 3:1:1 ratio of FYM, neem cake, and compost. The crop was cultivated according to the recommended agronomic practices (DMAPR, 2015) and harvested 180 days after planting.

Table 1 : Different seed bio-priming treatments

Treatments	Seed bio-priming
T_1	Soaking in water
T_2	Soaking in *Trichoderma viride* (10g kg^{-1})*
T_3	Soaking in *Pseudomonas fluorescens* (10g kg^{-1})*
T_4	Soaking in *Azospirillum* (10g kg^{-1})*
T_5	Soaking in panchagavya (5%)
T_6	Soaking in panchagavya (10%)
T_7	Soaking in vermiwash (10%)
T_8	Soaking in vermiwash (20%)

* KAU culture

Observations and Measurements

The relevant growth and yield parameters such as number of leaves, plant height, leaf length and width, plant spread (N-S and E-W), number of primary and secondary branches per plant, shoot length, shoot weight (fresh and dry), root length and diameter, number of primary and secondary root branches, root weight (fresh and dry), root yield (fresh and dry) and total biomass production were measured at 60, 120, and 180 days after planting. For each treatment replication, data were collected from five plants that were randomly selected and tagged. For the assessment of leaf characteristics, samples were taken from the fifth mature, fully expanded leaf from the top of the plant. For the assessment of shoot and root parameters, the plants were destructively sampled by uprooting them.

Germination (%)

To calculate the germination percentage, one seed was sown per cell in protrays. A seed was defined as having germinated upon the emergence of its plumule from the seed coat. The number of seeds meeting this criterion was counted daily for 15 days, and the final data was used to determine the germination percentage with the standard equation.

$$Germination\ per\ cent = \frac{Number\ of\ seeds\ germinated}{Total\ number\ of\ seeds\ sown} \times 100$$

Number of days taken for seed germination

A set of 50 seeds were sown in a labelled germination medium for each treatment replication. The number of seeds that sprouted was then counted and recorded at the same time each day. This daily count was used to determine the Mean Germination Time (MGT), which calculates the average time it takes for a seed to germinate.

$$Mean\ Germination\ Time = \frac{\sum(n \times d)}{\sum n}$$

where,

n = the number of seeds that germinated on a specific day

d = the day number

Days to leaf emergence

To determine the Mean Time to Emergence (MTE), seeds were sown in protrays, with the sowing date marked as day zero. Emergence was defined as the appearance of the first true leaf, and the number of seedlings reaching this stage was counted at the same time each day. This daily data was then used to calculate the MTE, which represents the average time it

took for a seedling to emerge across the entire population.

$$Mean\ Time\ to\ Emergence = \frac{\sum(n \times d)}{\sum n}$$

where,

n = the number of seeds that germinated on a specific day

d = the day number

Root-shoot ratio

The plant was separated into its root and shoot portions, and the roots were washed. Both parts were oven-dried at 60-70°C to a constant weight before recording their individual dry weights on a precision scale.

$$Root - shoot\ ratio = \frac{Root\ dry\ weight}{Shoot\ dry\ weight}$$

Root dry recovery

To determine root weights, plants were uprooted, and their roots were washed thoroughly. After blotting dry, the fresh weight was recorded. The roots were then oven-dried at 70°C for 72 hours to a constant weight, at which point the final dry weight was measured. These values were used to calculate the root dry recovery percentage.

$$Root\ Dry\ Recovery\ \% = \frac{Root\ Dry\ Weight}{Root\ Fresh\ Weight} \times 100$$

Harvest index (%)

At maturity, harvested plants were separated into economic (root) and non-economic parts. Both components were oven dried at 70°C for 72 hours to a constant weight, then weighed on a precision scale to determine the economic and biological yields. The harvest index (%) was determined using the established formula (Donald, 1962).

Determination of chlorophyll content (mg g^{-1})

Chlorophyll content was determined using the dimethyl sulfoxide (DMSO) extraction method (Hiscox and Israelstam, 1979). Finely cut leaf bits (0.25 g) were placed in a test tube with five mL of DMSO added and incubated at 60°C for one hour, or until the tissue became colourless. After removing the leaf debris, the resulting extract was transferred to a 25 mL volumetric flask and diluted to the final volume with DMSO. The absorbance of this solution was then measured at wavelengths of 645 nm and 663 nm using a spectrophotometer, and these values were used to calculate the concentrations of chlorophyll a, chlorophyll b, and total chlorophyll with the standard formulas (Arnon, 1949).

All the data recorded from the experiment were subjected to Analysis of Variance (ANOVA) using GRAPES software to determine the statistical significance of the results (Gopinath *et al.*, 2020).

Results and Discussion

The efficacy of various pre-sowing seed treatments on growth, yield, and physiological traits of ashwagandha was clearly demonstrated, with significant variations observed among the eight treatments. Considering germination parameters (Fig. 1-2, Table 2), seed soaking in 10 per cent panchagavya (T_6) showed the highest germination percentage (86.33 ± 1.57), followed by T_8 (81.33 ± 1.57) and T_4 (79.33 ± 1.57), which were on par with each other. The early germination was observed in T_5 (6.89 ± 0.13 days) and T_8 (7.05 ± 0.13 days), while delayed germination noted in T_2. The fastest seedling emergence was also observed in treatments T_5 and T_8, with emergence occurring at 7.64 ± 0.15 days and 7.72 ± 0.15 days, respectively, and were statistically similar.

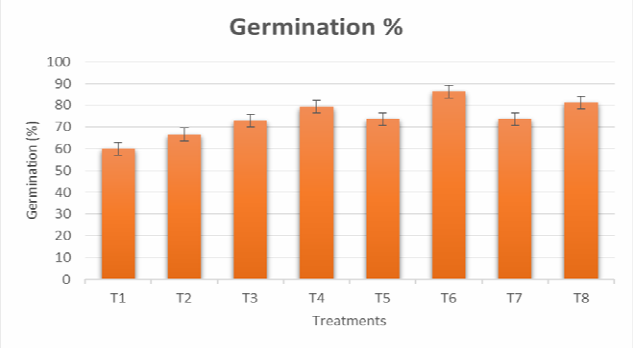

Fig. 1 : Germination percentage under various treatments

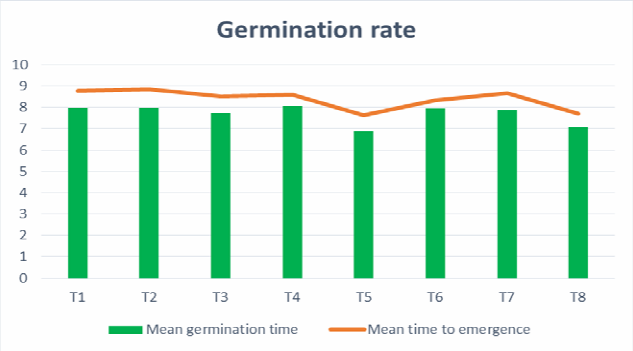

Fig. 2 : Mean Germination Time (MGT) and Mean Time to Emergence (MTE) of ashwagandha as influenced by different treatments

With respect to leaf parameters (Fig. 3, Table 3), treatment T_6 produced the highest number of leaves (211.63 ± 3.54), followed by T_7 (207.93 ± 3.54) and T_5 (206.50 ± 3.54). Treatment T_5 produced the longest leaves, measuring 6.32 ± 0.09 cm, and the widest

leaves, with a statistically significant width of 4.73 ± 0.11 cm. T_5 recorded the maximum leaf area (4341.17 ± 144.45 cm²), which was statistically on par with T_6 (4016.57 ± 144.45 cm²) and T_7 (3950.22 ± 144.45 cm²). While treatments T_5 (2.74 ± 0.13 mg g^{-1}) and T_1 (2.66 ± 0.13 mg g^{-1}) had the highest chlorophyll a content, T_8 was statistically superior for chlorophyll b (0.94 ± 0.08 mg g^{-1}). Consequently, T_5 (3.36 ± 0.15 mg g^{-1}) and T_8 (3.35 ± 0.15 mg g^{-1}) recorded the highest and statistically similar total chlorophyll content.

Table 2 : Effect of seed bio-priming on leaf parameters of ashwagandha

Treatments	No. of leaves per plant	Leaf area (cm^2)	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})
T1	162.81^{d}	2754.03^{f}	2.66^{a}	0.46^{b}	3.12^{ab}
T2	170.33^{d}	2975.83^{ef}	2.23^{b}	0.39^{b}	2.62^{c}
T3	184.77^{c}	3257.15^{de}	2.43^{ab}	0.51^{b}	2.93^{abc}
T4	197.90^{b}	3518.18^{cd}	2.26^{b}	0.40^{b}	2.66^{bc}
T5	206.50^{ab}	4341.17^{a}	2.74^{a}	0.62^{b}	3.36^{a}
T6	211.63^{a}	4016.57^{ab}	2.39^{ab}	0.47^{b}	2.86^{bc}
T7	207.93^{ab}	3950.22^{abc}	2.11^{b}	0.45^{b}	2.56^{c}
T8	201.33^{ab}	3815.98^{bc}	2.41^{ab}	0.94^{a}	3.35^{a}
SE (m)	3.54	144.45	0.13	0.08	0.15
CV (%)	3.18	6.99	9.08	25.93	9.11
CD	10.62	433.06	0.38	0.24	0.46

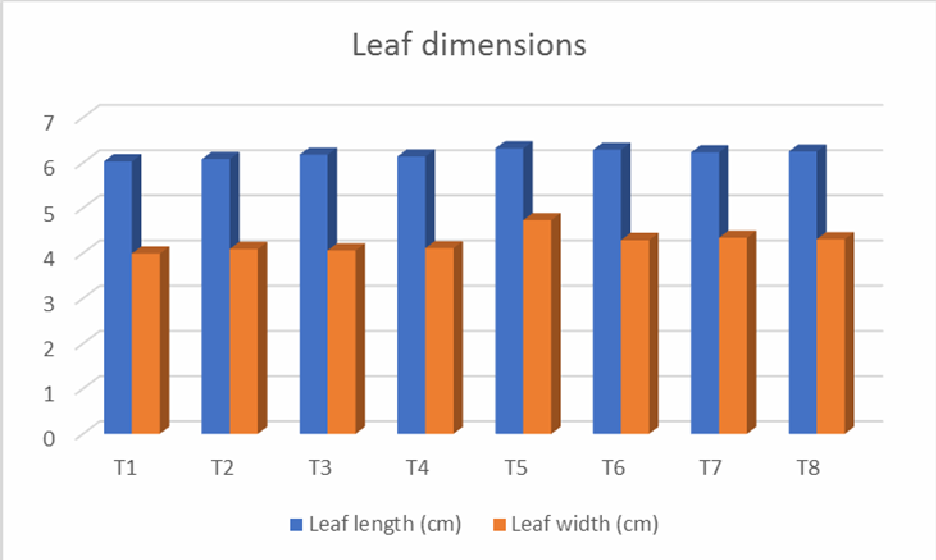

Fig. 3 : Effect of seed bio-priming on the leaf dimensions of ashwagandha

Regarding plant stature (Table 4), T_5 exhibited maximum plant height (80.99 ± 1.44 cm) and a statistically superior plant spread in both North-South (45.20 ± 0.92 cm) and East-West (48.15 ± 0.92 cm) directions. The shortest plants were noted in T_2 (68.82 ± 1.44 cm). As for branches per plant, T_6 showed the highest number of primary branches (5.87 ± 0.24), while T_5 produced the most secondary branches (8.73 ± 0.21); both were statistically on par with several other high-performing treatments.

Table 3 : Effect of seed bio-priming on plant architecture of ashwagandha

Treatments	Plant height (cm)	Plant spread N-S (cm)	Plant spread E-W (cm)	Primary branches	Secondary branches
T_1	69.42	40.11^{bc}	45.92^{abc}	4.00^{c}	7.73^{d}
T_2	68.82	36.39^{d}	43.53^{cd}	5.27^{ab}	7.80^{cd}
T_3	72.59	41.13^{bc}	42.60^{d}	5.33^{ab}	8.40^{abc}
T_4	74.67	40.54^{bc}	44.46^{bcd}	4.73^{b}	8.13^{abcd}

T_5	80.99	45.20[a]	48.15[a]	5.40[ab]	8.73[a]
T_6	80.46	42.12[b]	44.51[bcd]	5.87[a]	8.67[ab]
T_7	78.40	39.07[cd]	43.23[d]	5.53[a]	7.87[cd]
T_8	76.38	41.54[bc]	46.84[ab]	5.47[a]	8.07[bcd]
SE (m)	1.44	0.92	0.87	0.24	0.21
CV (%)	3.31	3.92	3.36	7.81	4.50
CD	NA	2.76	2.61	0.70	0.64

In relation to shoot parameters (Table 5), T_8 (83.37 ± 0.54 cm, 95.22 ± 1.56 g, and 30.09 ± 0.61 g) and T_5 (82.86 ± 0.54 cm, 91.69 ± 1.56 g, and 29.26 ± 0.61 g) produced the longest shoots, fresh shoot weight, and dry shoot weight, and were statistically on par with each other, followed by T_6 (81.12 ± 0.54 cm, 89.71 ± 1.56 g, and 27.57 ± 0.61 g).

Table 4 : Effect of seed bio-priming on shoot growth parameters of ashwagandha

Treatments	Shoot length (cm)	Shoot weight fresh (g)	Shoot weight dry (g)
T_1	71.33[f]	83.23[de]	25.32[de]
T_2	73.88[e]	76.31[f]	22.56[f]
T_3	78.15[c]	78.89[ef]	24.83[e]
T_4	75.57[d]	74.51[f]	22.85[f]
T_5	82.86[a]	91.69[ab]	29.26[ab]
T_6	81.12[b]	89.71[bc]	27.57[bc]
T_7	79.32[c]	85.56[cd]	26.98[cd]
T_8	83.37[a]	95.22[a]	30.09[a]
SE (m)	0.54	1.56	0.61
CV (%)	1.19	3.20	4.06
CD	1.61	4.67	1.84

In terms of yield parameters (Fig. 4, Table 6-8), root length and root diameter were found to be highest in T_6 (24.86 ± 0.41 cm and 1.29 ± 0.01 cm, respectively). The highest number of primary root branches was observed in T_7 (42.67 ± 2.19), which was statistically on par with T_6 (41.33 ± 2.19) and T_8 (40.67 ± 2.19). For secondary root branches, T_6 showed the best results with 434.67 ± 12.86 branches, which was statistically similar to T_8 (420.33 ± 12.86) and T_7 (398.00 ± 12.86). T_6 also recorded the highest fresh root weight (9.52 ± 0.15 g), dry root weight (3.10 ± 0.09 g), fresh root yield (8.78 ± 0.15 g), and dry root yield (2.73 ± 0.08 g), proving statistically superior to most other treatments. For dry root yield, T_6 (2.73 ± 0.08 g) was on par with T_8 (2.71 ± 0.08 g), T_7 (2.65 ± 0.08 g), and T_5 (2.57 ± 0.08 g). The lowest fresh (7.65 ± 0.15 g) and dry root weights (2.42 ± 0.09 g) were observed in T_2. The maximum root dry recovery was achieved in T_7 (32.87 ± 0.69 %). No significant variations were observed for the root-shoot ratio and total biomass production. Although not statistically significant, T_8 produced the highest total biomass (33.02 g), followed by T_5 (32.08 g) and T_6 (30.67 g), while the lowest biomass was recorded in T_2 (24.97 ± 0.64 g). The highest harvest index was observed in T_4 (9.15 ± 0.31 %), which was statistically on par with T_6 (8.92 ± 0.31 %), whereas the lowest values were observed in treatments T_2 (7.81 ± 0.31 %) and T_3 (7.68 ± 0.31 %).

Table 5 : Effect of seed bio-priming on root growth parameters of ashwagandha

Treatments	Root length (cm)	Root diameter (cm)	Primary root branches	Secondary root branches
T_1	17.47[f]	0.90[g]	32.00[d]	305.00[c]
T_2	19.48[e]	1.08[de]	34.33[cd]	252.33[d]
T_3	20.63[de]	1.05[ef]	33.00[d]	356.00[b]
T_4	23.36[b]	1.03[f]	35.67[bcd]	272.33[cd]
T_5	22.18[bc]	1.11[cd]	37.67[abcd]	290.67[cd]
T_6	24.86[a]	1.29[a]	41.33[ab]	434.67[a]

T_7	20.01^{de}	1.21^{b}	42.67^{a}	398.00^{a}
T_8	21.10^{cd}	1.14^{c}	40.67^{abc}	420.33^{a}
SE (m)	0.41	0.01	2.19	12.86
CV (%)	3.36	2.25	10.20	6.53
CD	1.23	0.04	6.56	38.57

Table 6 : Effect of seed bio-priming on root weight per plant

Treatments	Root weight fresh (g)	Root weight dry (g)	Root dry recovery (%)
T_1	8.43^{de}	2.67^{cde}	31.65
T_2	7.65^{g}	2.42^{e}	31.57
T_3	8.23^{ef}	2.66^{de}	32.30
T_4	7.91^{fg}	2.55^{de}	32.24
T_5	8.71^{cd}	2.82^{bcd}	32.37
T_6	9.52^{a}	3.10^{a}	32.54
T_7	9.27^{ab}	3.05^{ab}	32.87
T_8	9.06^{bc}	2.93^{abc}	32.39
SE (m)	0.15	0.09	0.69
CV (%)	3.08	5.67	3.73
CD	0.46	0.27	NA

Table 7 : Effect of seed bio-priming on biomass and harvest index of ashwagandha

Treatments	Root-shoot ratio	Total biomass production (g)	Harvest index
T_1	0.11	27.98	8.21^{bcd}
T_2	0.11	24.97	7.81^{d}
T_3	0.11	27.49	7.68^{d}
T_4	0.11	25.40	9.15^{a}
T_5	0.10	32.08	8.00^{cd}
T_6	0.12	30.67	8.92^{ab}
T_7	0.12	30.03	8.82^{abc}
T_8	0.10	33.02	8.20^{bcd}
SE (m)	0.01	0.64	0.31
CV (%)	6.13	3.80	6.35
CD	NA	NA	0.92

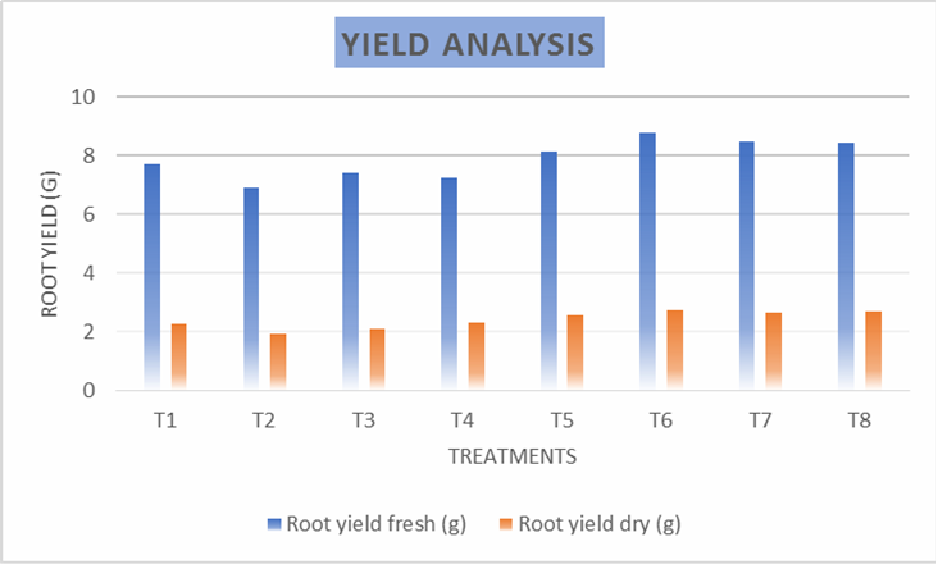

Fig. 4 : Fresh and dry root yield of ashwagandha under various treatments

The findings of various researchers revealed that panchagavya showed a positive effect on the growth and yield characters of ashwagandha. The superior performance of bio-priming seeds with panchagavya (T_6) across multiple key metrics can be attributed to its growth-promoting hormones and beneficial microbes, which provided a sustained advantage throughout the crop's life cycle. The adequate amount of nutrients found in panchagavya aligns with the study conducted by Geetha *et al.* (2013). Similar results were reported by Marmat *et al*. (2021) in their research on tomato. The early onset of germination on the fourth day in ashwagandha seeds treated with panchagavya suggests a rapid overcoming of seed dormancy (Ankad *et al*., 2018). This might be due to improved water imbibition facilitated by panchagavya priming, because seeds have a thick outer coat and take more time to initiate germination if sown unprimed. Water imbibition is the initial step of germination, and inadequate moisture level hampers the germination process. Additionally, this makes the seed's exposure time lesser to soil-borne pathogens and allows it to establish at a faster rate.

The increase in root and shoot length was due to the presence of easily absorbable organic C, N, P, and K in panchagavya (Jain *et al*., 2013). Following the successful shoot establishment promoted by the panchagavya seed treatment, the subsequent growth in plant height was both rapid and substantial. In addition to providing an immediate source of energy for creating new plant tissues through its amino acids, sugars, and organic carbon, panchagavya also contains crucial growth promoting hormones like cytokinins. This finding is consistent with the work of Basavaraj *et al*. (2015) who also observed that the application of panchagavya resulted in a higher number of branches, along with increased plant height and leaf area in French beans. It is also evident from the results by Vasumathi (2001) in *Phyllanthus amarus*, Sanjutha *et al*. (2008) in *Andrographis paniculata* and Singh *et al*. (2015) in cashew. Similarly, the treatment T_8, which involved soaking in 20 per cent vermiwash, showed increased shoot growth that can be attributed to vermiwash's properties as a biofertilizer, particularly its ability to mimic the action of Gibberellic acid (Fathima and Sekar, 2014). The soluble humic and fulvic acids found in vermiwash are known to promote the growth of lateral roots and the development of more root hairs. This is in accordance with Siddiqui *et al*. (2008).

The application of FYM at 90 days after planting provided a critical mid-season nutritional boost that maintained the vigorous growth initiated by the panchagavya seed treatment, with these combined effects lasting until the final yield. This approach is supported by Bhattacharyya *et al*. (2007), who reported that long term FYM application significantly increases soil organic carbon and the availability of essential nutrients like nitrogen. Since the application of FYM helped the crop to synthesize more amount of photosynthates, it resulted in the translocation of stored reserves to the roots and increased its biomass, ultimately providing higher root yield. This is in accordance with Vijaya *et al*. (2013). Furthermore, Vajantha *et al*. (2012) found that nutrient management influenced soil enzyme activity, noting that increased dehydrogenase and phosphatase activity helped the ashwagandha crop to gain more roots. The nutrients and hormones present in panchagavya itself contributed to increased root formation, enhanced resistance to drought, and increased the overall vigour of the plant (Sarma and Talukdar, 2024). This combination of a strong start from priming and sustained nutrition likely contributed to the high harvest index observed in the panchagavya treatment.

Conclusion

Based on its consistently superior and balanced performance, seed treatment with 10 per cent panchagavya (T_6) can be recommended as an effective, economical, and sustainable bio-priming strategy for farmers aiming to maximize the yield and profitability of their ashwagandha crop. This technique, when integrated with other good agricultural practices like using high-quality seeds and ensuring FYM supplementation, provides growers with a powerful method to improve germination and establish a vigorous, uniform crop stand.

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