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Short communications

Growth, yield and alkaloid content in ashwagandha (*Withania somnifera* (L.) Dunal) as influenced by organic amendments and phytostimulants

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Abstract
A study was conducted at Kerala Agricultural University to examine the effect of organic amendments and phytostimulants on maximizing the yield and quality of ashwagandha (*Withania somnifera* (L.) Dunal). The experiment employed a randomized block design (factorial) with 16 treatment combinations of farmyard manure, vermicompost, and foliar phytostimulants (seaweed fertilizer, humic and fulvic acids, and chitosan) using the JawaharAshwagandha 20 (JA-20) variety. The results showed that vermicompost at 5 t/ha significantly improved plant height, number of branches per plant, fresh biomass, and dry biomass. Seaweed fertilizer enhanced growth metrics, and its combination with vermicompost at 5 t/ha improved root characteristics, achieving a fresh root yield of 614 kg/ha. Farmyard manure at 10 t/ha with seaweed fertilizer produced comparable results, with a fresh yield of 590 kg/ha and higher total alkaloid content of 2.43%. This combination also yielded the highest benefit-cost ratio of 2.25. The study suggests that integrating organic amendments and seaweed fertilizer can enhance ashwagandha's root growth and alkaloid content, making farmyard manure at 10 t/ha with seaweed fertilizer a promising approach for ashwagandha cultivation.

Keywords: Ashwagandha, Farmyard manure, Phytostimulants, Seaweed fertilizer, Vermicompost, *Withania somnifera*,

Withania somnifera (L.) Dunal, known as 'Ashwagandha' in Malayalam or Indian Ginseng in English, is a drought-tolerant medicinal plant native to arid regions across Africa, Asia, and the Mediterranean region (Kumar et al., 2023). Renowned for its restorative properties and therapeutic benefits, ashwagandha has been an important ingredient of Ayurvedic and Unani medicines, offering relief from cancer, anxiety, inflammation, and neurological disorders (Singh and Sharma, 2018). Ashwagandha roots, which are rich in fiber, starch, and alkaloids, are the most economically important part of the plant (Gupta et al., 2021).

Organic amendments play a critical role in safeguarding the therapeutic qualities of ashwagandha and other medicinal plants, yielding high-quality, chemical-free biomass with optimal efficacy (Jat et al., 2021). Phytostimulants are substances or microorganisms that enhance plant growth and quality, promoting physiological responses in plants to improve growth, nutrient efficiency, and stress tolerance. Common phytostimulantsin agriculture include humic and fulvic acids, which are produced when plant, animal, and microbial matter breaks down in the soil; seaweed fertilizer, an organic biostimulant that improves nutrient absorption and increases resistance to environmental stress; and chitosan, a biopolymer made from crustacean shells. This study explored the synergistic effects of organic amendments and phytostimulants on ashwagandha's root growth and alkaloid content, contributing to the advancement of sustainable and eco-friendly agricultural systems.

The study was carried out at the AgronomyDepartment, College of Agriculture, Vellanikkara, Kerala Agricultural University. The variety Jawahar Ashwagandha-20 (JA-20) was used for the experiment.The seedlings were raised in protrays containing a mixture of vermicompost and coir pith in a 1:10 ratio. The 45-day-old seedlings were transplanted to mulched beds.

A randomized block design(Factorial) with 16 treatment combinations was adopted, with each combination replicated thrice. The individual plots measured 3.15 m x 3.15 m, with plants spaced 45 cm apart. The treatments comprised two organic amendments, each at two levels - farmyard manure at 5 t/ha and 10 t/ha, vermicompost at 2.5 t/ha and 5 t/ha - combined with four phytostimulant levels: water spray, 0.4% seaweed fertilizer, 0.4% humic and fulvic acid, and 0.1% chitosan. The phytostimulants were applied as foliar sprays one month and three months after planting.

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Biometric parameters were observed 90 days after planting (DAP), and root characteristics and alkaloid content were measured at harvest (120 DAP). The data was statistically analyzed using KAU GRAPES online software(Gopinath et al., 2020).

The combined use of organic amendments and phytostimulantsprofoundly impacted the growth and development of ashwagandha, yielding notable improvements.Farmyard manureat 10 t/ha and vermicompostat 5 t/ha resulted in taller plants (49.06 cm and 49.22 cm, respectively). The number of branches per plant was also significantly higher in farmyard manureat 10 t/ha and vermicompostat 5 t/ha, with an average value of 9.30 and 9.53, respectively. The application of vermicompost @ 5 t/ha resulted in a higher fresh biomass yield of 93.88 g/ plant and was on par with farmyard manure @ 10 t/ha (93.16 g/plant). The lowest fresh biomass yield was observed in vermicompost @ 2.5 t/ha (70.25 g/plant). The dry biomass yield was higher invermicompost @ 5 t/ha (26.30 g/plant) and was on par with farmyard manure @ 10 t/ha (25.28 g/ plant) and farmyard manure @ 5 t/ha (23.58 g/plant).

Among phytostimulants, the application of seaweed fertilizer resulted in the highest fresh biomass yield (131.85 g/plant), followed by humic and fulvic acid (90.53 g/plant), chitosan (78.77 g/plant), and water spray (71.05 g/plant). Similarly, seaweed fertilizer recorded the highest dry biomass yield (33.95 g/plant).

The interaction between phytostimulants and organic amendments significantly impacted fresh and dry biomass yield. The combination of farmyard manure at 5 t/ha with seaweed fertilizer showed the highest fresh biomass of 168.90 g. The same trend was observed for dry biomass yield, with the highest dry biomass in farmyard manure at 5 t/ha with seaweed fertilizer (47.60 g),which was on par withvermicompost at 5 t/ha with seaweed fertilizer (42.00 g), followed by farmyard manure at 10 t/ha with seaweed fertilizer (41.30 g).These results are consistent with the findings of Jat et al. (2021), who observed a notable increase in ashwagandha plant biomass after applyingorganic amendments and phytostimulants.

Applying organic amendments and phytostimulants significantly influenced ashwagandha's root characteristics and total alkaloid content. Specifically, farmyard manure at 10 t/ha enhanced root proliferation (4.52 roots/plant), while vermicompost at 5 t/ha increased root length (27.75 cm) and diameter (7.24 mm), resulting in higher fresh and dry root

Table 1. Effect of organic amendments and phytostimulantson plant height, number of branches per plant, fresh biomass and dry biomass per plant of aswagandha

Treatments	Plant height at 90 DAP	No. of Branches at 90 DAP	Plant fresh biomass/plant (g)	Plant dry biomass/plant (g)
A1: FYM @ 5 t/ha	42.76	8.26	84.90	23.58
A2: FYM @ 10 t/ha	49.06	9.30	93.16	25.28
A3: Vermicompost @ 2.5 t/ha	41.33	8.47	70.25	21.33
A4: Vermicompost @ 5 t/ha	49.22	9.53	93.88	26.30
CD (0.05)	5.174	0.86	7.37	2.91
B1: Water spray	44.66	8.42	71.05	19.63
B2: Seaweed fertilizer	50.70	10.09	131.85	33.95
B3: Humic&fulvic acid	42.23	8.61	90.53	22.05
B4: Chitosan	44.78	8.39	78.77	20.85
CD (0.05)	5.17	0.86	7.37	2.91
A1 X B1	36.46	8.30	38.40	19.20
A1 X B2	46.16	9.36	168.90	47.60
A1 X B3	39.93	7.60	70.00	22.40
A1 X B4	48.46	7.76	62.30	25.10
A2 X B1	55.50	7.96	80.80	15.20
A2 X B2	51.82	10.66	115.20	41.30
A2 X B3	45.73	9.90	97.20	21.00
A2 X B4	43.20	8.66	79.50	23.60
A3 X B1	39.75	8.46	20.00	16.50
A3 X B2	47.86	9.86	97.30	24.90
A3 X B3	38.90	7.73	89.60	27.30
A3 X B4	38.80	7.80	74.10	16.60
A4 X B1	46.93	9.13	25.00	27.60
A4 X B2	56.96	10.46	146.00	42.00
A4 X B3	44.33	9.20	105.30	17.50
A4 X B4	48.67	9.33	99.20	18.10
CD (0.05)	NS	NS	14.75	5.81

yields (440 kg/ha and 161 kg/ha, respectively). Vermicompost at 5 t/ha also led to a higher total alkaloid content (2.07%) in the roots.

Phytostimulant application significantly influenced ashwagandha's root development and alkaloid production. Seaweed fertilizer proved remarkably effective, resulting in a notable increase in the number of roots per plant (4.91), as well as enhanced root length (28.10 cm) and diameter (7.61 mm). Applyingseaweed fertilizer also improved the fresh and dry root yields (584 kg/ha and 180 kg/ha, respectively).

The phytostimulant treatments significantly influenced the total alkaloid content of roots. The results revealed that seaweed fertilizer recorded the highest alkaloid content (2.17%), significantly superior to the other treatments. The treatment with seaweed fertilizer was superior to water spray, humic and fulvic acid, and chitosan by 26.74%, 27.65%, and 21.22%, respectively.

The interaction effect of organic amendments and phytostimulants was significant for all the parameters. The treatment combination of vermicompost @ 5 t/ha + seaweed fertilizer recorded a higher fresh root yield (614 kg/ha) and was on par with farmyard manure @ 10 t/ha + seaweed fertilizer (590 kg/ha). The same trend was observed for dry root yield, with the treatment combination of vermicompost @ 5 t/ha + seaweed fertilizer showing a higher dry root yield (239 kg/ha).The fresh and dry root yields of ashwagandha obtained in this study were lower than those reported in traditional ashwagandha-growing areas, where yields typically range from 1500-2000 kg/ha (Khabiya*et al.,*2024), likely due to non-optimal agro-climatic conditions. Future studies should focus on identifying region-specific cultivars and developing location-specific agronomic packages for non-traditional areas to improve yields.

The combined application of organic manures and phytostimulants increased the total alkaloid content in ashwagandha roots. A higher total alkaloid content of 2.43% was recorded in farmyard manure 10 t/ha + seaweed fertilizer and was on par with farmyard manure 5 t/ha + seaweed fertilizer (2.30 %), farmyard manure 5 t/ha + chitosan (2.30%) and vermicompost5 t/ha + seaweed fertilizer (2.06 %). The lowest alkaloid contents were observed in treatments of farmyard manure@ 5t/ha +water spray (1.63%), farmyard manure @ 10 t/ha + water spray (1.90%), and vermicompost @ 5 t/ha + humic and fulvic acid (1.76%).

Table 2. Effect of organic amendments and phytostimulantson root characteristicsand total alkaloid content of ashwagandha

Treatments	No. of roots/ plant	Length of roots (cm)	Diameter of roots (mm)	Fresh root yield/ha (kg/ha)	Dry root yield/ha (kg/ha)	Total alkaloid (%)	B:C ratio
Organic manures							
A1: FYM @ 5 t/ha	3.93	22.43	5.59	332	124	1.62	
A2: FYM @ 10 t/ha	4.52	26.40	7.30	420.	154	1.73	
A3: Vermicompost @ 2.5 t/ha	4.02	23.58	5.64	412	137	1.96	
A4: Vermicompost @ 5 t/ha	4.62	27.75	7.24	440	161	2.07	
CD (0.05)	0.27	2.60	0.69	35.82	6.72	0.19	
Biostimulants							
B1: Water spray	4.01	22.80	5.55	343	131	1.72	
B2: Seaweed fertilizer	4.91	28.10	7.61	584	180	2.17	
B3: Humic&fulvic acid	4.05	27.13	5.44	353	135	1.70	
B4: Chitosan	4.11	22.13	7.17	323	130	1.79	
CD (0.05)	0.27	2.60	0.69	35.82	6.72	0.19	
Interaction							
A1 X B1	3.93	23.60	4.73	372	145	1.63	1.92
A1 X B2	4.40	28.40	6.68	364	120	2.30	1.57
A1 X B3	3.86	14.80	5.30	404	165	1.60	2.16
A1 X B4	3.50	22.90	5.64	188	66	2.30	0.87
A2 X B1	4.26	26.80	5.07	337	140	1.90	1.44
A2 X B2	5.13	22.60	9.92	590	220	2.43	2.25
A2 X B3	4.56	34.60	6.73	293	95	1.90	0.97
A2 X B4	4.10	21.60	7.48	320	160	2.03	1.64
A3 X B1	3.76	22.40	6.24	369	120	1.76	0.74
A3 X B2	4.63	32.60	5.76	527	140	1.86	0.86
A3 X B3	3.56	21.70	4.77	239	175	1.53	1.08
A3 X B4	4.10	17.60	5.78	411	115	1.30	0.71
A4 X B1	4.06	18.40	6.15	294	120	1.56	0.44
A4 X B2	5.46	28.80	8.06	614	239	2.06	0.89
A4 X B3	4.20	37.40	4.95	477	105	1.76	0.39
A4 X B4	4.73	26.40	9.78	374	180	1.53	0.66
CD (0.05)	0.54	5.20	1.38	71.65	13.46	0.39	

Manohar et al. (2012) reported that vermicompost positively influenced ashwagandha by providing essential nutrients, boosting metabolic rates, and increasing plant auxin activity. Similarly, research by Raja and Veerakumari (2013) demonstrated that vermicompost application significantly impacted ashwagandha's root characteristics, including length, diameter, and fresh and dry weight. The improvements were likely due to enhanced plant growth regulator availability and increased microbial activity.

Plants treated with seaweed fertilizer displayed a higher root count, with roots that were 19% longer than the control and thicker roots with a diameter of 7.61 mm. Also, these plants exhibited exceptional performance in terms of fresh and dry root yield per hectare. Research by Pacheco et al. (2019) revealed that seaweed fertilizer application increased root biomass by 28.5% in yarrow plants (*Achillea millefolium*) compared to control plots. The stimulatory effect of seaweed fertilizer on root development in ashwagandha can be attributed to the presence of plant growth hormones such as auxin and cytokinin. Additionally, seaweed fertilizer provides essential macro- and micronutrients that support shoot and root growth (Mafakheri and Asghari, 2018). Similar findings were reported by Mazeed et al. (2022), who found that foliar application of phytostimulants enhanced ashwagandha's quality parameters. Furthermore, Elansary et al. (2016) observed that seaweed fertilizer application resulted in the highest essential oil content in mint plants.

The integration of farmyard manure and seaweed fertilizer yielded a favorable benefit-cost (B:C) ratio of 2.25, making it the most economically viable treatment(Table 2). While combinations based on vermicompost exhibited superior root development, their higher cost reduced profitability. However, adopting on-farm vermicompost production can offset this drawback, minimizing expenses.

The use of organic manures, such as vermicompost or farmyard manure, in conjunction with seaweed fertilizer, was synergistic and augmented root production and alkaloid content in ashwagandha. Among the organic amendments and phytostimulants combinations tested in the present investigation for ashwagandha cultivation, the combination of farmyard manure @ 10 t/ha and seaweed fertilizer emerged as the most promising, as revealed by better root yield, enhanced alkaloid content, and a better benefit-cost (BC) ratio.

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