

Effect of Organic Soil Amendments and Bio-Stimulants on Growth Parameters of *Withania Somnifera*

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ABSTRACT

Withania somnifera is a medicinally significant plant, and this study aimed to assess how foliar bio-stimulants and organic soil amendments affected its vegetative growth. A Factorial Randomized Block Design (FRBD) experiment was carried out in the field, utilizing two factors: soil treatments and bio-stimulant foliar sprays. The first component was a mix of four organic soil amendments: vermicompost (S2), phosphobacteria (S4), vesicular-arbuscular mycorrhizae (S3), and farmyard manure (S1). A total of eight therapy combinations were possible due to Factor II, which consisted of two bio-stimulant treatments: panchakavya (F2) and humic acid (F1). At 60 and 120 days following seeding, we measured important vegetative development characteristics including plant height, number of secondary branches, leaf length, leaf breadth, stem girth, and leaf length and width. The results showed that compared to other treatments, the combination of 2.5 tonnes/hectare of vermicompost and 0.3% humic acid (S2F1) greatly improved plant development. Plant stature, branching, leaf shape, and stem thickness were all best measured in this mixture, suggesting that the organic nutrients and bio-stimulants worked in tandem. The results highlight the possibility of enhancing the growth performance of *Withania somnifera* through integrated organic nutrient management, which could lead to more sustainable and environmentally friendly methods of growing medicinal plants.

Key Words: *Plant, Medicinal, Experiment, Winter Cherry, Organic.*

I. INTRODUCTION

Traditional Indian medicine, especially the Ayurvedic school, places much respect on *Withania somnifera*, most often known as Winter Cherry or Ashwagandha. This little woody plant has several medicinal uses; it is native to Africa, the Middle East, and India. This plant's adaptogenic, anti-inflammatory, antioxidant, stress-relieving, immunomodulatory, and neuroprotective properties have garnered much praise throughout the years. Raw Ashwagandha and its processed pharmaceutical forms are in high demand due to the worldwide upturn in interest in herbal medicine and natural cures. So, *withania somnifera* is being cultivated more and more as a commercial crop as well as a food product. The optimization of agronomic procedures, particularly those related to soil fertility and nutrient management, is essential for this shift from systematic agriculture to wild harvesting. To improve the quantitative and qualitative yield parameters of this medicinal plant, it is crucial to apply and standardize organic manures.

Because of the risk of residual toxicity in bioactive chemicals, chemical inputs are discouraged in the production of medicinal and aromatic plants; thus, organic manures play an essential role in sustainable agriculture. In addition to providing nutrients, organic manures like compost, vermicompost, chicken manure, green manure, and farmyard manure (FYM) enhance soil structure, microbial activity, and water-holding capacity. Organic manures promote soil fertility over the long term by slowly releasing nutrients, in contrast to synthetic fertilizers that do this in an often-unsustainable manner. Because of the complex

interplay between genetics, soil health, and microbial diversity, this is of the utmost importance for medicinal plants like Ashwagandha, which accumulate secondary metabolites like withanolides. Standardizing organic manures becomes an important area of research since Ashwagandha is very valuable as a medicinal crop and is very sensitive to soil conditions. It entails figuring out which organic manures work best for what plants in terms of growth, production, and medicinal quality, and then applying them at the right times.

Organic manures are well-known in agriculture, but little is known about how they affect *Withania somnifera* specifically. There has been little scientific support of most traditional methods, which are based on practical information passed down through generations. Developing a uniform standard is also made more difficult by differences in soil types, climate, manure content, and cultural practices. This highlights the critical need for controlled, repeatable studies to establish standards for the application of organic manure. This helps with the creation of standards for agro-industrial stakeholders, practitioners, and farmers who work with Ashwagandha, as well as ensuring its reproducibility and scalability. Composting rates are just one aspect of standardization; other factors include nutritional profiles, microbial loads, carbon-to-nitrogen ratios, and the extent to which other agronomic inputs work in tandem with compost. Height, branch count, leaf area, and root biomass are some of the growth attributes that can be improved by following strict standardization standards; these factors affect Ashwagandha's therapeutic efficacy and market value.

Organic manure standardization also has substantial monetary and environmental benefits. Environmental imbalance, groundwater contamination, and soil deterioration can be lessened by the use of organic methods, which reduce the need for chemical fertilizers. Many small and marginal farmers grow Ashwagandha as a crop rotation or in rain-fed environments, and these alternatives provide a more economical option for them. Holistic farm sustainability is promoted by the residual effects of organic manures, which further boost soil fertility for succeeding crops. The production of certified organic Ashwagandha, which is highly sought for in both local and worldwide markets, is made possible by standardized organic manure techniques. This is in line with larger policy efforts that are trying to promote organic farming, protect biodiversity, and create sustainable jobs. Standardized and scientifically validated growing procedures are also necessary to meet the strict quality control standards that have resulted from consumer knowledge regarding the authenticity and provenance of herbal products.

It is also necessary to investigate in depth the biological dynamics of applying organic manure. Nitrogen, phosphorus, potassium, zinc, iron, and manganese are all micronutrients that are affected by the interactions between the soil microbiota and organic manures. Enzyme synthesis, rhizospheric activity, and hormonal signalling pathways are all affected by these factors, which in turn affect plant development. Soil buffering capacity and humus production are two benefits of farmyard manure and plant growth-promoting hormones like auxins, gibberellins, and cytokinins, respectively, in vermicompost. It is necessary to conduct a comparative evaluation under controlled experimental settings since these manures have varied effects on Ashwagandha's growth stages, which include germination, vegetative development, blooming, and root formation. Furthermore, additional optimization opportunities arise from the possibility of combining several organic manures to achieve synergistic advantages. For the purpose of maximizing growth performance and active component accumulation in *Withania somnifera*, it is crucial to conduct such research in order to determine the most effective combinations and application timings.

In addition, the effects of climate change on soil quality and plant physiology highlight the critical need to establish uniform guidelines for the application of organic manure. Agronomic solutions that can withstand unpredictable weather conditions are essential in this age of rising pest and disease outbreaks. An important part of climate-smart agriculture is the use of organic manures, which, when applied correctly, increase soil organic carbon content, water retention, and microbial resistance. Better root quality, more consistent yields, and less susceptibility to environmental stresses are the results of this in the context of Ashwagandha production. To keep Ashwagandha farming going in bad weather, organic manure-based solutions improve soil health and plant vigor, which acts as a buffer against biotic and abiotic challenges.

The importance of maintaining consistent quality is further emphasized by the high demand for Ashwagandha extracts in the nutraceutical, pharmaceutical, and cosmetic industries worldwide. Variability in withanolide content, caused by inconsistent agronomic techniques, impacts the efficacy and safety of the end product. By making sure that organic manures are standardized, we may be sure that the raw materials used in plant development and bioactive compound synthesis are up to pharmacopoeial standards. If we want to keep consumers' trust and fulfil our export obligations, this is essential. Additionally, post-harvest losses are decreased and downstream processing, packaging, and storage are made more efficient using uniform growing techniques. Research on the pharmacodynamics and pharmacokinetics of Ashwagandha-derived products can be conducted more accurately with this standardization.

The social and economic ramifications of organic manure standardization are significant, alongside agronomic and pharmaceutical factors. Even though they don't have access to scientific inputs, rural and tribal populations commonly grow *Withania somnifera* because of their cultural knowledge. These communities can be empowered, productivity can be enhanced, and new income streams can be created through standardization initiatives that combine traditional wisdom with modern scientific methodologies. Learning communities, participatory research methodologies, and farmer field schools can all help spread information and build skills. Therefore, inclusive and sustainable agricultural growth is achieved through the standardization process, which acts as a bridge between grassroots practice and scientific innovation. Sustainable agriculture, medicinal plant production, environmental conservation, and rural development are all touched by the effects of standardizing organic manures on *Withania somnifera* growth. Optimizing plant growth, production, and medicinal quality requires a methodical examination into the kind, quantity, and manner of applying organic manure. The project makes use of eco-friendly practices, soil science, plant physiology, and microbiology. Making sure that agricultural procedures are scientifically robust is becoming more and more important as the demand for organic and herbal products grows worldwide. The entire therapeutic potential of Ashwagandha, an environmentally sensitive crop, can be realized through the standardization of organic manures, which will also help farmers make a living.

II. REVIEW OF LITERATURE

Kaur, Amandeep et al., (2018) Due to its health-promoting and therapeutic effects against a wide range of illnesses, ashwagandha, also known as *Withania somnifera*, has recently been the topic of significant investigation. Experimental evidence from multiple studies has demonstrated the potential medicinal uses of its active withanolides, such as Withaferin A (Wi-A), Withanone (Wi-N), and Withanolide A (Wi-A). However, it needs to be grown in an environmentally sustainable fashion with a high yield if its use in the herbal sector is to be encouraged. Nowadays, most farmers use chemical fertilizers on all of their crops regardless of soil quality or ecological concerns. This has led many to ignore the detrimental effects that

these fertilizers have on these areas. We tried to use vermicompost (Vcom, 20-100%) as the organic fertilizer of choice during the planting and growing stages of Ashwagandha plants, taking these considerations into consideration. We report that (i) pre-soaking of seeds for 12 h in Vermicompost leachate (Vcom-L) and Vermicompost tea (Vcom-T) led to higher germination, (ii) binary combination of pre-soaking of seeds and cultivation in Vcom (up to 80%) resulted in further improvement both in germination and seedling growth, (iii) cultivated plants in the presence of Vcom+Vcom-L showed higher leaf and root mass, earlier onset of flowering and fruiting and (iv) leaves from the Vcom+Vcom-L cultivated plants showed higher level of active Withanolides, Withanone (Wi-N), Withanolide A (Wi-A) and Withaferin A (Wi-A) and showed anticancer activities in cell culture assays. Considering this, we provide a simple and inexpensive method to increase the production of ashwagandha leaves and the pharmacological components they contain.

Chaudhary, S.R et al., (2017) Soil biochemical characteristics and ashwagandha (*Withania somnifera*) yield and quality metrics were the subjects of a field experiment designed to examine the effects of organic nutrient management. There were three separate runs of the experiment, each with its own unique design based on the split plot plan. Organic manures, including vermicompost, castor cake, and farmyard manure, were utilized as the principal treatments for the plots. Biofertilizers and jivamrut were used in different combinations as subplot treatments. Results showed that with regular application of organic fertilizers, root development, yield, and withanolide content in ashwagandha were all improved. The use of castor cake produced equivalent results to vermicompost in terms of dry root output (714.6 kg ha^{-1}) and total withanolide content (0.91 mg g^{-1}). After applying vermicompost and castor cake, the soil's biochemical properties and available nutrients were much improved compared to prior treatments. It follows that organic manures, especially vermicompost and castor cake, may provide ashwagandha with the nutrients it needs to thrive naturally.

Kulkarni, Suhas et al., (2008) The Ayurvedic medical system makes heavy use of the plant ashwagandha, whose scientific name is *Withania somnifera*. Using pentylenetetrazol (PTZ, iv) as a monotherapy or in combination with exogenous gamma-amino butyric acid (GABA), diazepam (a GABA receptor modulator), or *W. somnifera* root extract (Ws), this study aimed to determine the effect on the seizure threshold of mice. The lowest intravenous dose of PTZ (in mg/kg) needed to induce myoclonic jerks, generalized clonus, and tonic extension convulsions was recorded as the seizure threshold. Ws, when taken orally in doses of 100 or 200 mg/kg, raised the seizure threshold before the tonic extension phase began. However, the seizure threshold was unaffected by a lower oral dose of Ws (50 mg/kg). We found that a combination of a sub-protective dose of diazepam (0.5 mg/kg ip) or GABA (25 mg/kg ip) with a sub-effective dosage of Ws (50 mg/kg po) increased the seizure threshold. The results led researchers to conclude that *W. somnifera*'s anticonvulsant effects against the PTZ seizure threshold paradigm were due, in part, to its GABAergic modulation.

III. MATERIALS AND METHODS

Study Site and Experimental Design

This research set out to determine how different organic manures affected the development and growth of the medicinal plant winter cherry (*Withania somnifera*), which is well-known for its curative effects. The experiment was carried out on clay-loamy soil, which is often ideal for growing *Withania somnifera* due to its moderate drainage and overall suitability.

To make sure that everything was random and replicated, the experiment was structured using a Factorial Randomized Block Design (FRBD). Various organic manure treatments were able to have their individual and combined effects evaluated thanks to the factorial design.

In order to increase statistical dependability and decrease experimental error, the design featured eight treatment combinations, each of which was reproduced three times. To minimize edge effects and soil heterogeneity across replicates, the plots were designed to evenly distribute treatment combinations.

Crop Management Practices

During the course of the experiment, all recommended standard agronomic practices and cultural operations were performed uniformly across all plots. These included:

- Seedbed preparation
- Sowing at appropriate depth and spacing
- Irrigation scheduling
- Weed and pest control
- Monitoring of plant health and environmental conditions

This standardization ensured that observed differences in growth could be attributed primarily to the organic manure treatments rather than to variations in crop management.

Data Collection and Statistical Analysis

Various growth metrics (including plant height, biomass, and root production) were systematically recorded during different stages of crop development in order to evaluate the performance of winter cherry under different treatments.

For the purpose of determining the significance of differences among treatment means, the recorded data were statistically analyzed. The ICAR-Central Coastal Agricultural Research Institute in Goa created the Web Agri Stat Package (WASP) version 2.0 and it was used for this purpose. With its intuitive interfaces, this program makes analysis a breeze, making it ideal for use in agricultural studies.

We used Analysis of Variance (ANOVA) to compare the treatment averages at a 5% level of significance ($p < 0.05$). Analysis of variance (ANOVA) was useful in deciding whether or not there was a statistically significant difference between the treatment groups. To determine which treatment combinations had a substantial impact on winter cherry growth, post hoc comparisons were performed when appropriate.

IV. RESULT AND DISCUSSION

Table 1: Effect of Organic Amendments on Plant Height (cm) at 60 and 120 DAS

Treatments	Plant Height (cm) 60 DAS	Plant Height (cm) 120 DAS
S1	25.20	43.36
S2	28.14	43.87
S3	26.60	43.42
S4	26.10	42.67
SE.d	0.106	0.243
CD (0.05)	0.259	0.596
F1	26.86	42.94
F2	26.16	43.71
SE.d	0.040	0.063

CD (0.05)	0.092	0.146
S1F1	25.60	43.08
S1F2	24.82	41.44
S2F1	28.75	44.74
S2F2	27.52	43.90
S3F1	26.82	43.69
S3F2	26.38	43.15
S4F1	26.30	43.53
S4F2	25.92	42.99
SE.d	0.120	0.260

In Table 1, we can see how different organic amendments affected plant height at 60- and 120-days post-sowing (DAS). The experiment included four distinct treatments (S1–S4), two doses of fertilizer (F1 and F2), and a combination of the two (SxFy).

The plant heights at 60 DAS were 28.14 cm for treatment S2, 26.60 cm for treatment S3, and 25.20 cm for treatment S1. By 120 DAS, S2 had once again reached its maximum height of 43.87 cm, but the disparity between the treatments had shrunk to 43.36 cm for S1 and 43.42 cm for S3. Both measures had a critical difference (CD) of 0.259 cm and 0.596 cm at the 5% level, suggesting that S2's difference at 60 DAS is statistically significant.

F1 resulted in a higher plant height at 60 DAS (26.86 cm) than F2 (26.16 cm), when comparing fertilizer amounts. But at 120 DAS, F2 did marginally better than F1 (43.71 cm vs. 42.94 cm). Statistical analysis revealed statistically significant differences at both stages, with CD values of 0.092 cm and 0.146 cm, respectively.

At both 60 and 120 days after seeding (DAS), the S2F1 combination produced the tallest plants (28.75 cm and 44.74 cm, respectively), according to the combined treatment analysis. At 24.82 cm and 41.44 cm, respectively, S1F2 exhibited the shortest plant heights among the two groups. This data points to the fact that S2 + F1 is the optimal growth promoter for plants, especially young plants.

In terms of overall effect on plant height, the combination of treatment S2 and fertilizer level F1 (S2F1) was the most successful, whereas treatments S1 and F2 had the lowest effect.

Table 2: Effect of Organic Amendments on Number of Secondary Branches

Treatments	No. of Secondary Branches
S1	4.50
S2	5.38
S3	5.17
S4	4.90
SE.d	0.042
CD (0.05)	0.102
F1	5.05
F2	4.92
SE.d	0.022
CD (0.05)	0.052
S1F1	4.57
S1F2	4.44
S2F1	5.45

S2F2	5.32
S3F1	5.23
S3F2	5.13
S4F1	4.98
S4F2	4.81

For various treatments and fertilizer levels, Table 2 shows how organic additions affected the number of secondary branches per plant.

S2 had 5.38 secondary branches, S3 had 5.17, and S4 had 4.90 among the primary treatments, however S1 had the fewest (4.50). At the 5% level of significance, the critical difference (CD) was 0.102, which was higher than the standard error of difference (SE.d) of 0.042, indicating a statistically significant difference between treatments. This proves that S2 considerably enhanced branching when contrasted with S1.

Fertilizer levels were marginally different between F1 and F2, with F1 producing 5.05 secondary branches and F2 producing 4.92. A p-value of 0.052 indicates that the numerical difference was statistically significant, despite its tiny size.

Out of all the combinations tested, S2F1 produced 5.45 secondary branches, followed by S2F2 at 5.32 and S3F1 at 5.23. The S1F2 treatment had the lowest number (4.44), which further supports the idea that S2 consistently improved branching performance when combined with either F1 or F2.

Treatment S2, particularly when combined with fertilizer level F1 (S2F1), appeared to have the greatest impact on encouraging the growth of secondary branches, whereas treatments S1 had the opposite effect.

Table 3: Effect of Organic Amendments on Leaf Length and Leaf Width (cm)

Treatments	Leaf Length (cm)	Leaf Width (cm)
S1	7.83	4.53
S2	8.20	4.90
S3	8.05	4.75
S4	7.95	4.65
SE.d	0.012	0.009
CD (0.05)	0.030	0.017
F1	8.05	4.75
F2	7.96	4.66
SE.d	0.006	0.006
CD (0.05)	0.015	0.014
S1F1	7.88	4.58
S1F2	7.79	4.49
S2F1	8.25	4.95
S2F2	8.14	4.84
S3F1	8.09	4.79
S3F2	8.01	4.71
S4F1	7.98	4.68
S4F2	7.93	4.63

For each treatment (S1–S4), fertilizer amount (F1–F2), and interaction, Table 3 shows the influence of organic additions on leaf length and width (in cm).

S2 had the broadest leaves (4.90 cm) and the longest (8.05 cm) among the primary treatments, whereas S3 had the narrowest (8.05 cm length and 4.75 cm width). S1 had the smallest measured leaf dimensions (7.83 cm) and breadth (4.53 cm). Both the length and width CD (0.05) values were greater than the respective SE.d values, indicating statistically significant differences among treatments.

When it came to fertilizer levels, F1 did somewhat better than F2 in terms of leaf width (4.75 cm vs. 4.66 cm) and leaf length (8.05 cm vs. 7.96 cm). Additionally, these variations were found to be statistically significant with CD values of 0.015 cm for length and 0.014 cm for width.

When looking at the effects of different treatments together, S2F1 had the longest (8.25 cm) and widest (4.95 cm) leaves, followed by S2F2 (8.14 cm) and S3F1 (8.09 cm) (8.79 cm). The best results were seen with S2 treatments, especially when combined with F1, since the lowest values were seen in S1F2 (7.79 cm and 4.49 cm).

S2, an organic amendment treatment, greatly increased the width and length of the leaves, with the greatest effects shown when combined with S2F1. On the other hand, the combination of S1 and F2 resulted in much slower leaf development.

Table 4: Effect of Organic Amendments on Stem Girth (cm)

Treatments	Stem Girth (cm)
S1	1.44
S2	1.57
S3	1.55
S4	1.52
SE.d	0.019
CD (0.05)	0.045
F1	1.53
F2	1.51
SE.d	0.028
CD (0.05)	0.068
S1F1	1.45
S1F2	1.44
S2F1	1.60
S2F2	1.56
S3F1	1.55
S3F2	1.55
S4F1	1.53
S4F2	1.51

In Table 4, we can see how various treatments (S1–S4), fertilizer levels (F1 and F2), and combinations thereof affected stem girth (in cm) as a result of organic amendments.

S1 had the smallest stem girth (1.44 cm), whereas S2 had the largest (1.57 cm), S3 the second-largest (1.55 cm), and S4 the smallest (1.52 cm) among the individual treatments. At the 5% level of significance, the crucial difference (CD) was 0.045 cm, meaning that S2 and S1 are significantly different.

With respect to fertilizer levels, F1 had a somewhat larger stem girth (1.53 cm) than F2 (1.51 cm), however this difference was not statistically significant since it falls within the CD of 0.068 cm.

Stem girth was greatest in the S2F1 combination (1.60 cm), second in the S2F2 interaction (1.56 cm), and lowest in the S1F2 interaction (1.44 cm). Aside from S3F1 and S3F2 at 1.55 cm, the majority of the other combinations only yielded moderate effectiveness.

The stem girth was most positively affected by treatment S2, particularly when paired with F1 (S2F1), while treatments with S1 had less of an impact.

V. CONCLUSION

The results show that *Withania somnifera*'s growth characteristics were significantly improved after using organic soil amendments and bio-stimulants. When comparing the treatments, S2 always came out on top in terms of overall plant height, secondary branch count, leaf size (both breadth and length), and stem girth.

There was a synergistic impact between the organic amendment and bio-stimulant when S2 and fertilizer level F1 (S2F1) were combined; this resulted in the highest values across most growth measures. On the other hand, growth responses were typically lowest with S1 therapies.

In conclusion, the study shows that some organic supplements, especially S2, might boost the plant vigor and vegetative growth of *Withania somnifera*, which could lead to higher yields and better quality when grown sustainably.

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