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Comparative efficacy of biofertilizers, FYM and inorganic nitrogen on productivity and withanolides yield of *Withania somnifera* (L.) Dunal

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Abstract
Withania somnifera (L.) Dunal, commonly known as Ashwagandha, is a widely cultivated medicinal plant in Ayurveda, valued for its broad-spectrum therapeutic properties attributed to its bioactive constituents, particularly alkaloids and withanolides. A field experiment was carried out during the Rabi season of 2022-2023 at the Herbal Garden, Department of Plant Physiology, College of Agriculture, JNKVV, Jabalpur (M.P.). The trial was laid out in a Completely Randomized Block Design (CRBD) with eight treatments and three replications, comprising: Control (T_1), PSB @ 5 kg ha^{-1} (T_2), FYM @ 10,000 kg ha^{-1} (T_3), PSB + FYM (T_4), Azotobacter + PSB + FYM (T_5), Urea @ 130 kg ha^{-1} (T_6), Nano urea @ 2 L ha^{-1} (T_7), and Azotobacter @ 5 kg ha^{-1} (T_8). Among the treatments, Azotobacter + PSB + FYM (T_5) significantly enhanced root yield (943 kg ha^{-1}), fruit yield (1430 kg ha^{-1}), seed yield (853.33 kg ha^{-1}), and total biological yield (3783.33 kg ha^{-1}). The highest stem yield (1033 kg ha^{-1}) was observed with PSB + FYM (T_4). Biochemical analysis revealed that Withanolide A content in roots ranged from 0.0082% to 0.0223%, with T_5 showing the maximum Withanolide A content (0.0223%). Withanolide B content in roots ranged from 0.0001% to 0.0142% the highest Withanolide B content (0.0142%) were recorded with PSB @ 5 kg ha^{-1} (T_2). The findings demonstrate that integrated application of biofertilizers (Azotobacter and PSB) along with organic variations i.e., (FYM) effectively improves biomass production and withanolide accumulation in Ashwagandha, suggesting a sustainable alternative to chemical fertilizers for enhanced medicinal quality and yield.

Keywords: Ashwagandha, FYM, azotobacter, PSB, nano-urea, alkaloids, withanoilides

1. Introduction

Withania somnifera (L.) Dunal, commonly known as Ashwagandha, Indian winter cherry, is a medicinally important plant belonging to the family Solanaceae (Mir *et al*., 2013) [6]. It is indigenous to the Mediterranean region of North Africa, and is widely distributed across arid and semi-arid regions of North-Western and Central India. In India, two species of the genus are commonly found, with major cultivation in Madhya Pradesh, Rajasthan, Gujarat, Maharashtra, Punjab, and Uttar Pradesh. Ashwagandha is predominantly grown as a late kharif crop under rainfed conditions, thriving in low-irrigation and dry climates.

Nationally, the crop is cultivated on approximately 15,000 hectares, producing an estimated 60,000 quintals with an average productivity of 4.0 q ha^{-1} (Patidar, 2012) [10]. In Madhya Pradesh alone, cultivation spans over 8,000 hectares, yielding 32,000 quintals. In Ayurvedic and Unani systems of medicine, Ashwagandha is classified as an adaptogenic herb, often compared to Panax Jinseng due to its rejuvenating and restorative properties. The plant parts particularly the roots are used for treating various disorders including leprosy, arthritis, neurological diseases, respiratory ailments, ulcers, and joint inflammation. Ashwagandha contains a wide array of bioactive compounds, mainly steroidal lactones (withanolides) and alkaloids, which are distributed in the roots, leaves, and berries. The root also contains 40-65% starch, 40-65% fiber, 0.4-1.2% alkaloids, and trace amounts of essential oils. Withanolides are the principal pharmacologically active compounds that confer its therapeutic effects.

Modern agronomic strategies now emphasize the use of biofertilizers microbial-based inputs that promote plant growth through enhanced nutrient availability and uptake.

The integration of biofertilizers has been shown to improve crop yield and reduce dependency on chemical fertilizers (Subba Rao *et al*., 1980) [13]. Despite their benefits, limited research exists on the application of biofertilizers in Ashwagandha cultivation, particularly in relation to their effect on yield and withanolide accumulation. Therefore, the present investigation was undertaken to evaluate the impact of biofertilizers, FYM, and nitrogen sources on biomass production and secondary metabolite content in Ashwagandha.

2. Materials and Methods

The pot experiment was conducted at the Herbal Garden, Department of Plant Physiology, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, during the Rabi season of 2022-23. Jabalpur is geographically located at 23˚90' N latitude and 79˚58' E longitude, characterized by a semi-humid subtropical climate with pronounced winters and summers. The region has an average annual temperature of 24.6 °C and receives an average annual rainfall of 1107.1 mm, predominantly during the monsoon season from mid-June to the end of October, with occasional showers occurring during other seasons. Overall, the climate is mild, warm, and temperate.

The experiment was arranged using a Completely Randomized Design (CRD) with three replications and eight treatments, including: control (T_1), PSB at 5 kg ha^{-1} (T_2), FYM at 10,000 kg ha^{-1} (T_3), combined PSB at 5 kg ha^{-1} and FYM at 10,000 kg ha^{-1} (T_4), combined Azotobacter at 5 kg ha^{-1}, PSB at 5 kg ha^{-1} and FYM at 10,000 kg ha^{-1} (T_5), urea at 130 kg ha^{-1} (T_6), Nano urea at 2 L ha^{-1} (T_7), and Azotobacter at 5 kg ha^{-1} (T_8). Each replication comprised four polybags per treatment, totalling 96 polybags for the entire study. Each polybag had an area of 0.05 m² and a diameter of 0.25 m. Polybags were filled with a soil and FYM mixture at a 2:1 ratio and arranged according to the randomized layout generated using a random number table.

Seeds of Jawahar Ashwagandha-20 were manually sown in each pot at a rate of 8 kg ha^{-1} on 17 October 2022. Standard agronomic practices were consistently applied throughout the crop growth period, including immediate irrigation after sowing. Yield attributes and total yields were recorded after harvesting. Roots, considered the primary economic yield when cultivated specifically for root production, were harvested, cleaned, and dried for yield measurement. Similarly, seed yield, occasionally considered an economic yield for seed-oriented production, was recorded post-harvest after cleaning and drying. The total produce per treatment was measured using a weighing balance and expressed in kg plot^{-1}. Biomass components, including stem, leaf, fruit, and root yields collectively represented the biological yield. Withanolide content was determined using High-Performance Thin-Layer Chromatography (HPTLC) analysis.

3. Results and Discussion

3.1 Effect of Integrated Biofertilizer, FYM, and Nitrogen Management on Yield Attributes of Ashwagandha

Yield attributes, including stem yield, root yield, leaf yield, fruit yield, seed yield, and biological yield, were significantly influenced by different combinations of biofertilizers, FYM, and nitrogen treatments. Data obtained from five randomly selected plants revealed notable differences among the treatments. The maximum stem yield (1033.33 kg ha^{-1}) was recorded in treatment T_4 (PSB @ 5 kg ha^{-1} + FYM @ 10,000 kg ha^{-1}), closely followed by T_3 (FYM @ 10,000 kg ha^{-1}; 1030.00 kg ha^{-1}) and T_2 (PSB @ 5 kg ha^{-1}; 1020.00 kg ha^{-1}). The minimum stem yield (763.33 kg ha^{-1}) was observed under the control treatment (T_1). This enhancement in stem yield might be attributed to improved nutrient availability facilitated by organic amendments (FYM) and biofertilizers, promoting better vegetative growth. Similar observations were reported by Shrivastava *et al*. (2012) [12].

Root yield was highest in treatment T_5 (Azotobacter @ 5 kg ha^{-1} + PSB @ 5 kg ha^{-1} + FYM @ 10,000 kg ha^{-1}; 943.33 kg ha^{-1}), significantly surpassing the control (600.00 kg ha^{-1}). Enhanced root biomass under integrated nutrient management indicates improved soil fertility and nutrient uptake efficiency. Leaf yield followed a similar trend, with the highest leaf yield recorded in treatment T_5 (436.67 kg ha^{-1}) compared to the lowest in T_1 (293.33 kg ha^{-1}). The integrated nutrient supply likely enhanced leaf area development and photosynthetic efficiency.

Fruit and seed yields also showed significant variations. Maximum fruit yield (1430.00 kg ha^{-1}) and seed yield (853.33 kg ha^{-1}) were both obtained from treatment T_5, which substantially higher than other treatments, including the control (Table 1). The combined application of biofertilizers, FYM, and inorganic nutrients likely promoted enhanced flowering, fruiting, and seed development through increased metabolic activities and protein synthesis, supporting findings reported by Panchabhai *et al*. (2005) [9].

Biological yield was maximum (3783.33 kg ha^{-1}) under treatment T_5, reflecting overall positive effects on vegetative and reproductive growth. The lowest biological yield (2486.67 kg ha^{-1}) was recorded in the control treatment. These findings agree with previous research by Patidar *et al*. (2014) [11] and Productivity result of any crop depends on numerous factors interacting throughout the life cycle of that crop. This result highlighting the role of balanced nutrient supply in achieving maximum biomass production (Table 1).

Table 1: Effect of Integrated Application of Biofertilizers, Farmyard Manure (FYM), and Nitrogen on Yield Attributes of Ashwagandha (*Withania somnifera*): Stem, Root, Leaf, Fruit, Seed and Biological Yield.

Treatments	Stem (kg ha^{-1})	Root (kg ha^{-1})	Leaf (kg ha^{-1})	Fruit (kg ha^{-1})	Seed (kg ha^{-1})	Biological yield (kg ha^{-1})
T_1	763.33	600.00	293.33	830.00	523.33	2486.67
T_2	1020.00	703.33	346.67	886.67	646.67	2956.67
T_3	1030.00	796.67	393.33	1276.67	583.33	3496.67
T_4	1033.33	880.00	326.67	1163.33	673.33	3403.33
T_5	973.33	943.33	436.67	1430.00	853.33	3783.33
T_6	1000.00	740.00	310.00	1033.33	616.67	3083.33
T_7	926.67	776.67	353.33	956.67	646.67	3013.33
T_8	920.00	843.33	406.67	1060.00	713.33	3230.00
Sem±	19.508	25.55	19.08	18.67	20.17	39.70
CD at 5%	58.48	76.60	57.19	55.98	60.48	119.03

3.2 Effect of Integrated Biofertilizer, FYM, and Nitrogen Management on Withanolide Content and Yield Attributes in Ashwagandha.

The withanolide content varied significantly among different treatments. The highest Withanolide A content (0.0223%) was recorded in treatment T_5, whereas the lowest was noted in treatment T_7 (Nano urea @ 2 L ha^{-1}; 0.0082%). For Withanolide B, maximum content (0.0142%) was observed in treatment T_2, while the minimum (0.0001%) was in treatment T_8 (Azotobacter @ 5 kg ha^{-1}). These results suggest that biofertilizers and organic amendments play a crucial role in modulating secondary metabolite biosynthesis, possibly due to improved nutrient assimilation and enhanced stress tolerance, as previously discussed by Jain *et al*. (2014). [4]

Withanolide A yield was found to be highest under treatment T_5, reaching 943.36 kg ha^{-1}, whereas the lowest yield was observed in the control treatment, recording only 600.01 kg ha^{-1}. Similarly, withanolide B yield varied significantly among treatments, ranging from a maximum of 9.97 kg ha^{-1} in treatment T_2 to a minimum of 0.11 kg ha^{-1} in treatment T_8. These marked differences in withanolide yields among treatments emphasize the pivotal role of nutrient management practices in modulating secondary metabolite accumulation in Ashwagandha (Table 2). The present findings are in consonance with the results reported by Choudhary *et al*. (2017) [3], who also documented the substantial influence of organic fertilizer application on the biosynthesis of alkaloids and withanolides in *Withania somnifera*. An increase in leaf number potentially enhances biomass production, alkaloid, and withanolide synthesis, subsequently maximizing their yield if efficiently partitioned (Baraiya *et al*., 2012) [2]. Taking root as economic yield showing improvement in partitioning of assimilates and withanolides towards economic sink, i.e., root (Mohare *et al*., 2023) [7]. This highlights the importance of integrating suitable nutrient management strategies to optimize the therapeutic potential and commercial value of Ashwagandha crops.

Table 2: Effect of Biofertilizer, FYM, and Inorganic Nitrogen Treatments on Withanolide A and B Content and Yield in Ashwagandha Roots.

Treatments	Withanolide A (%)	Withanolide B (%)	Withanolide A (kg ha^{-1})	Withanolide B (kg ha^{-1})
T_1	0.0115	0.0108	600.01	6.49
T_2	0.0209	0.0142	703.35	9.97
T_3	0.0217	0.0077	796.69	6.12
T_4	0.0144	0.0107	880.01	9.38
T_5	0.0223	0.0096	943.36	9.02
T_6	0.0145	0.0037	740.01	2.71
T_7	0.0082	0.0096	776.67	7.45
T_8	0.0097	0.0001	843.34	0.11
Sem±	0.00021	0.00024	25.55	0.35
CD at 5%	0.00063	0.00073	76.06	1.05

4. Conclusion

The present investigation clearly suggest that the integrated application of biofertilizers, farmyard manure (FYM), and inorganic nitrogen substantially enhances both yield and withanolide content in Ashwagandha (*Withania somnifera*). Among the evaluated treatments, the combined application of Azotobacter, phosphate solubilizing bacteria (PSB), and FYM (T_5) was found to be the most efficacious, resulting in significant improvements across all major yield parameters as well as Withanolide A content. In contrast, the application of PSB alone (T_2) was particularly effective in increasing Withanolide B content. These findings emphasize the pivotal role of biofertilizers in enhancing nutrient availability, improving soil health, and stimulating the biosynthesis of secondary metabolites. The utilization of biofertilizers not only presents a cost-effective alternative to conventional chemical fertilizers but also contributes to environmentally sustainable crop production. Thus, the adoption of integrated nutrient management strategies centered on biofertilization can be considered a viable and sustainable approach for enhancing both the productivity and quality of Ashwagandha, thereby supporting long-term agricultural and environmental goals.

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