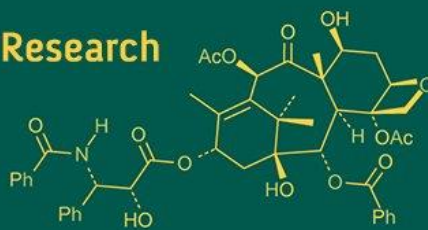


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(11): 17-20
www.biochemjournal.com
Received: 20-08-2025
Accepted: 23-09-2025

Manish Bamniya
Department of Plant
Physiology, College of
Agriculture, JNKVV,
Jabalpur, Madhya Pradesh,
India

Gyanendra Tiwari
Department of Plant
Physiology, College of
Agriculture, JNKVV,
Jabalpur, Madhya Pradesh,
India

Subrata Sharma
Department of Plant
Physiology, College of
Agriculture, JNKVV,
Jabalpur, Madhya Pradesh,
India

Aradhna Kumari
Department of Plant
Physiology, College of
Agriculture, JNKVV,
Jabalpur, Madhya Pradesh,
India

Sachin Nagre
Department of Plant
Physiology, College of
Agriculture, JNKVV,
Jabalpur, Madhya Pradesh,
India

Jay Surya
Department of Plant
Physiology, College of
Agriculture, JNKVV,
Jabalpur, Madhya Pradesh,
India

Ankita Singh
Department of Plant
Physiology, College of
Agriculture, JNKVV,
Jabalpur, Madhya Pradesh,
India

Corresponding Author:
Ankita Singh
Department of Plant
Physiology, College of
Agriculture, JNKVV,
Jabalpur, Madhya Pradesh,
India

Biofertilizer and vermicompost application affect root yield withanolide accumulation and other biochemical parameters of Ashwagandha [*Withania somnifera* (L.) Dunal.]

Manish Bamniya, Gyanendra Tiwari, Subrata Sharma, Aradhna Kumari, Sachin Nagre and Jay Surya and Ankita Singh

DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i11Sa.6175>

Abstract

The present study was conducted to evaluate the effect of biofertilizer and vermicompost applications on the biochemical composition and withanolide accumulation in *W. somnifera* under controlled conditions. during *rabi*, the experiment was arranged in a Completely Randomized Design (CRD) with five treatments: T₁ (Control), T₂ (Azotobacter @ ½ RDBF), T₃ (Azotobacter @ RDBF), T₄ (Azotobacter @ 2× RDBF), and T₅ (Vermicompost @ 3.5 kg per 50 kg soil) and four replications. Observations were recorded for withanolide A (%), withanolide B (%), total carbohydrate (%), protein (%), fat (%), fiber (%), ash (%), and moisture (%). The results revealed significant differences among treatments for all biochemical parameters. Maximum withanolide A content (0.064%) was recorded in T₂, while withanolide B (0.071%) was highest in T₃. The T₄ showed the highest protein content (11.83%) in T₄, which also exhibited overall superior biochemical quality. Vermicompost treatment (T₅) enhanced fat (0.553%) and fiber (27.83%) contents, whereas the control (T₁) showed the highest total carbohydrate (54.49%) but lower withanolide accumulation. The combined use of Azotobacter and vermicompost improved nutrient assimilation and secondary metabolism, resulting in enhanced biochemical quality and market value of Ashwagandha. The findings suggest that the integrated application of biofertilizers and vermicompost effectively balances primary and secondary metabolism, improves protein synthesis and withanolide biosynthesis, enhances soil health, and supports sustainable, eco-friendly production of high-quality *W. somnifera* under dryland conditions.

Keywords: *Withania somnifera*, biofertilizer, vermicompost, withanolide accumulation, biochemical composition

Introduction

Ashwagandha (*Withania somnifera* L. Dunal), a member of the *Solanaceae* family, is an important medicinal plant native to the Indian subcontinent. It grows erectly to a height of about 35-75 cm and features small green flowers along with orange-red fruits when ripe. Commonly known as 'winter cherry' or 'Indian ginseng,' it is well adapted to dryland conditions (Khanna *et al.*, 2006; Kulkarni and Dhir, 2008) [8, 10]. The roots, the most valuable part of the plant, are widely used in Ayurvedic and Unani medicines for their adaptogenic, anti-stress, and revitalizing properties (Sivanandhan *et al.*, 2011) [14]. The plant's therapeutic potential is primarily attributed to steroidal lactones known as withanolides, including withanolide A, withaferin A, and withanone, which exhibit diverse pharmacological activities. The leaves also contain withanolides with antibiotic, antitumor, and immunosuppressive properties, while the roots possess alkaloids like withanine and somniferine, with total alkaloid content ranging from 0.13% to 0.31% (Choudhary *et al.*, 2005; Kaileh *et al.*, 2007) [3, 6].

India is the principal producer and global supplier of high-quality Ashwagandha roots (Kissu, 2023; Smith *et al.*, 2022) [9, 15]. During 2022-23, Neemuch district in Madhya Pradesh, the major cultivation hub of fine-quality Ashwagandha, produced an estimated 1,519 MT from 2,170 ha of cultivated area. Across India, the average annual production of dry Ashwagandha roots was around 6,720.7 MT from approximately 10,000 ha of land

spread across major states such as Madhya Pradesh, Rajasthan, Uttar Pradesh, Andhra Pradesh, and Karnataka, against a global demand estimated at 8,000-10,000 MT (Kissu, 2023) ^[9]. Overall, Ashwagandha is cultivated over nearly 15,000 ha in India, with a production of 60,000 tons and a productivity of about 4.0 q/ha. The main producing states include Madhya Pradesh, Gujarat, Haryana, Maharashtra, Punjab, Rajasthan, and Uttar Pradesh. Despite the high medicinal value and skyrocketing market price of the roots of this crop, there is a need to increase average productivity through the inclusion of recently developed agrotechniques (Anonymous, 2020) ^[11].

Organic nutrient sources such as farmyard manure, vermicompost, and vermiwash play a vital role in enhancing soil fertility and promoting sustainable crop growth, as they release essential nutrients gradually throughout the plant growth period (Pal, 2013) ^[13]. The application of these organic inputs not only improves the yield and quality of medicinal and aromatic plants but also enhances their resistance to pests and diseases (Khan *et al.*, 2015; Awasthi *et al.*, 2011) ^[7, 21].

Thus, the use of biofertilizers and organic manures is crucial for maintaining soil health, improving crop productivity, and ensuring sustainable cultivation of Ashwagandha.

Materials and Methods

A study entitled "Effect of bio-fertilizer and vermicompost biochemical parameters of Ashwagandha [*Withania somnifera* (L.) Dunal.]" was conducted during the *rabi* of 2024-25 at the herbal garden, Department of Plant Physiology, College of Agriculture, JNKVV, Jabalpur (M.P.), to evaluate the influence of bio-fertilizer and vermicompost on biochemical composition of Ashwagandha. The experiment was laid out in a completely randomized design (CRD), with five treatments and four replications, viz., Control, Azotobacter @ 1/2 RDBF per 50 kg soil (0.75 kg), Azotobacter @ RDBF per 50 kg soil (1.5 kg), Azotobacter @ 2× RDBF per 50 kg soil (3.0 kg), and Vermicompost @ (3.5 kg) per 50 kg soil with four replications.

The harvested samples were oven-dried, powdered, and subjected to biochemical estimations as per standard protocols. Observations were recorded for the following biochemical parameters after harvesting of the crop.

Quantitative estimation of Withanolide A and Withanolide B was carried out using the High Performance Liquid Chromatography (HPLC) procedure as described by Ganzera *et al.* (2003). The purified extracts were analyzed against known standards, and the results were expressed as percentage per plant. Total carbohydrate content in the sample was determined using the hydrolysis method described in AOAC (1984). The absorbance was read spectrophotometrically, and the concentration was calculated using a standard curve. Fat content was estimated using a Pelican Equipment Socs Plus system based on the principle of Soxhlet extraction as outlined in AOAC (1980). Petroleum ether was used as the solvent for extraction.

Moisture percentage was measured by oven-drying the sample at 105 ± 2 °C until constant weight was achieved, following the standard procedure of AOAC (1980). Crude protein content was estimated using the Kjeldahl method according to AOAC (1965), as per the procedure suggested by Gopalan *et al.* (1985). Nitrogen content was obtained by titration, and protein percentage was calculated using a

conversion factor of 6.25. Ash content of the seed sample was determined by igniting the sample in a muffle furnace at 550±5 °C, as per the standard method described in AOAC (1980). The residual inorganic matter was expressed as a percentage. Crude fiber in the sample was estimated as per AOAC (1980), involving acid and alkaline digestion followed by ignition in a muffle furnace. The loss in weight on ashing was expressed as the crude fiber percentage.

Data recorded on different biochemical parameters were analyzed using a Completely Randomized Design (CRD) as per Fisher (1972). Analysis of variance (ANOVA) was performed to test treatment differences at a 5% level of significance.

Results and Discussion

The results revealed significant differences among treatments (bio-fertilizer and vermicompost) in biochemical parameters (Table 1 & Fig. 1 and Table 2 & Fig. 2-3). The withanolide A content (%) of plant⁻¹ was significantly influenced due to different treatments. The maximum withanolide A content (%) plant⁻¹ (0.064%) was noted in T₂, which was statistically at par with the T₄ (0.062%) and followed by the T₅ (0.052%). However, the minimum withanolide A content (%) plant⁻¹ (0.042%) was observed in T₁, followed by T₃ (0.051%). Similar results were reported by Gopal and Natarajan (2009) ^[4], Jain *et al.* (2014) ^[5], and Tiwari *et al.* (2025) ^[16], who revealed that it effectively improves biomass production and withanolide accumulation in Ashwagandha.

The withanolide B content (%) plant⁻¹ was significantly influenced due to different treatments. The maximum withanolide B content (%) plant⁻¹ (0.071%) was noted in T₃, followed by T₄ (0.042%) and T₁ (0.038%). However, the minimum withanolide A content (%) plant⁻¹ (0.022%) was observed in T₅, which was at par with T₂ (0.035%). Similar results were reported by Gopal and Natarajan (2009) ^[4], Jain *et al.* (2014) ^[5], and Tiwari *et al.* (2025) ^[16], and revealed that it effectively improves biomass production and withanolide accumulation in Ashwagandha.

The total carbohydrate (%) was significantly influenced due to different treatments. For total carbohydrate (%) maximum (54.49%) was recorded in T₁, which was statistically at par with T₃ (54.48%) and significantly followed by T₅ (53.47%). The minimum total carbohydrate (%) (44.40%) was noticed in T₄, which was at par with T₂ (45.02%). These findings align with earlier reports by Ningaraju *et al.* (2018) and revealed that biofertilizers improve nutrient utilization efficiency, resulting in optimum mineral accumulation rather than excessive deposition.

The ash (%) was significantly influenced due to different treatments. With respect to ash (%), the highest (12.008%) was recorded in T₁, significantly followed by T₃ (9.845%) and T₂ (6.418%), whereas the minimum ash (%) (4.143%) was observed in T₅, followed by T₄ (6.318%).

The moisture (%) was significantly influenced due to different treatments. For moisture (%), the maximum (6.20%) was noted in T₁, followed by T₃ (5.54%) and T₂ (5.34%), whereas the moisture (%) (4.38%) was observed in T₄, which was at par with T₅ (4.63%). These findings were reported by Kumar *et al.* (2017) ^[11].

The protein (%) was significantly influenced due to different treatments. In terms of protein (%), the highest (11.83%) occurred in T₄, significantly followed by T₃ (10.72%) and T₅ (8.99%), whereas the minimum protein (%) (6.58%) was

recorded in T₁, which was at par with T₅ (8.21%).

The fat (%) was significantly higher (0.553%) in T₅, which was statistically at par with T₃ (0.498%) and significantly followed by T₄ (0.470%), whereas the minimum fat (%) was recorded in T₁ (0.398%), which was at par with T₂ (0.443%).

The fiber (%) was significantly influenced due to different treatments. For fiber (%), the maximum (32.98%) was noted in T₃, significantly followed by T₄ (28.96%) and T₅ (27.83%), whereas the minimum fiber (%) (21.17%) was observed in T₁, which was at par with T₂ (22.31%). Similar findings were reported by Khanna *et al.* (2006) [8].

The root yield (g plant⁻¹) was significantly influenced due to different treatments. The maximum root yield (g plant⁻¹) (4.36 g plant⁻¹) was noted in T₄ followed by T₅ (3.86 g plant⁻¹) and T₃ (3.04 g plant⁻¹). However minimum root yield (g plant⁻¹) was observed in T₁ (2.06 g plant⁻¹) which followed by T₂ (2.84 g plant⁻¹). Similar findings were reported by Morya and Tuteja (2022) [12] and Tiwari *et al.*

(2025) [16].

The root yield (kg ha⁻¹) was significantly influenced due to different treatments. The maximum root yield (kg ha⁻¹) (2882.74 kg ha⁻¹) was noted in T₄, followed by T₅ (2576.10 kg ha⁻¹), and T₃ (2013.96 kg ha⁻¹) significantly. However, the minimum root yield (kg ha⁻¹) (1367.20 kg ha⁻¹) was observed in T₁, followed by T₂ (1880.80 kg ha⁻¹). Similar findings were reported by Morya and Tuteja (2022) [12] and Tiwari *et al.* (2025) [16].

These findings collectively highlight that the application of biofertilizers and vermicompost plays an important role in balancing primary and secondary metabolism, improving protein synthesis, enhancing fiber and fat content, and optimizing withanolide accumulation, thereby improving both quality and market value of *W. somnifera*. Hence, adopting a combination of biofertilizers and vermicompost (T₄) appears to be a sustainable and effective strategy for achieving higher productivity along with superior biochemical quality in commercial cultivation.

Table 1: Impact of biofertilizer and vermicompost on root yield and withanolide A and B content on plant⁻¹ on *Withania somnifera*

Treatment	Withanolide A content (%) plant ⁻¹	Withanolide B content (%) plant ⁻¹	Root dry wt. (kg ha ⁻¹)	Root yield (g plant ⁻¹)
(T ₁) Control	0.042	0.038	1365.80	2.06
(T ₂) Azotobacter @ 1/2 RDBF per 50 kg soil (0.75 kg)	0.064	0.035	1883.31	2.84
(T ₃) Azotobacter @ RDBF per 50 kg soil (1.5 kg)	0.051	0.071	2015.73	3.04
(T ₄) Azotobacter @ 2 × RDBF per 50 kg soil (3.0 kg)	0.062	0.042	2863.77	4.36
(T ₅) Vermicompost @ (3.5 kg) per 50 kg soil	0.052	0.022	2573.70	3.86
SE (m)	0.001	0.001	14.947	0.013
CD	0.003	0.002	45.056	0.038

Table 2: Impact of biofertilizer and vermicompost on moisture, protein, ash, total carbohydrate, fat, and fiber percentage on *Withania somnifera*

Treatment	Moisture (%)	Protein (%)	Ash (%)	Total carbohydrate (%)	Fat (%)	Fiber (%)
(T ₁) Control	6.20	6.58	12.008	54.49	0.398	21.17
(T ₂) Azotobacter @ 1/2 RDBF per 50 kg soil (0.75 kg)	5.34	8.21	6.418	45.02	0.443	22.31
(T ₃) Azotobacter @ RDBF per 50 kg soil (1.5 kg)	5.54	10.72	9.845	54.48	0.498	32.98
(T ₄) Azotobacter @ 2 × RDBF per 50 kg soil (3.0 kg)	4.38	11.83	6.318	44.40	0.470	28.96
(T ₅) Vermicompost @ (3.5 kg) per 50 kg soil	4.63	8.99	4.143	53.47	0.553	27.83
SE (m)	0.22	0.16	0.190	0.28	0.023	0.53
CD	0.66	0.49	0.573	0.86	0.069	1.59

Conclusion

The present investigation demonstrated that the combined application of biofertilizer and vermicompost significantly influenced the biochemical composition and secondary metabolite profile of *Withania somnifera* (L.) Dunal. The treatments enhanced withanolide accumulation, protein synthesis, and fiber and fat content, while optimizing carbohydrate and ash levels. Among the treatments, the integrated application (T₄: Azotobacter @ 2 × RDBF per 50 kg soil + Vermicompost @ 3.5 kg per 50 kg soil) proved most effective, resulting in superior biochemical quality and enhanced withanolide A and B contents. The findings clearly indicate that organic and biofertilizer-based nutrient management systems play a pivotal role in promoting balanced primary and secondary metabolism in *W. somnifera*. This integrated approach improves nutrient use efficiency, enhances soil biological activity, and supports the sustainable production of high-quality medicinal raw materials. Therefore, the incorporation of biofertilizers in combination with vermicompost offers a viable, eco-friendly, and economically sustainable alternative to conventional chemical fertilizers for commercial

Ashwagandha cultivation under dryland conditions.

References

- Anonymous. Ashwagandha crop productivity and growing states. Agricultural Statistics at a Glance. Ministry of Agriculture, Government of India; 2020.
- Awasthi SK, Rai VP, Tripathi A. Biofertilizer-mediated enhancement of medicinal plants. Biol Agric Hortic. 2011;27(4):337-346.
- Choudhary A, Pal A, Choudhary S. Withanolide content and antioxidant activity of *Withania somnifera* L. Dunal. J Ethnopharmacol. 2005;99(2):373-379.
- Gopal N, Natarajan S. Combined inoculation of *Azospirillum lipoferum* AAs-11, *Azotobacter* AAz-3, *Bacillus* APb-1 and *Pseudomonas fluorescens* APs-1 with panchakavya improves biochemical constituents and rhizosphere activity of *Withania somnifera* L. Dunal. J Med Plants Res. 2009;3(6):489-496.
- Jain S, Mehta VK, Singh AK. Influence of biofertilizers on withanolide content in roots of *Withania somnifera* L. Dunal. Phytochemistry. 2014;102:120-125.

6. Kaileh M, Bergman M, Xiao H. Withanolide D induces apoptosis and cell cycle arrest in human cancer cells. *Mol Cancer Ther.* 2007;6(10):3003-3010.
7. Khan MN, Bano A, Guha AK. Organic cultivation of medicinal plants: an overview. *Indian J Tradit Know.* 2015;14(2):225-234.
8. Khanna P, Singh R, Sharma V. Influence of *Azotobacter chroococcum* and *Azospirillum brasilense* on biomass and biochemical composition of *Withania somnifera* L. Dunal. *Indian J Plant Physiol.* 2006;11(3):189-195.
9. Kissu P. Status and market demand of fine quality Ashwagandha roots. *Agricultural Statistics at a Glance.* Ministry of Agriculture, India; 2023.
10. Kulkarni SK, Dhir A. *Withania somnifera*: an Indian ginseng. *Fitoterapia.* 2008;79(3):287-289.
11. Kumar M, Kumar K, Kumar K, Patel A. Impact of different manures on Ashwagandha (*Withania somnifera* Dunal.) production under rainfed conditions. *Int J Curr Microbiol Appl Sci.* 2017;6(10):5043-5059.
12. Morya SK, Tuteja SS. Effect of integrated nutrient management on growth, yield, and quality of Ashwagandha (*Withania somnifera* L. Dunal). *J Pharmacog Phytochem.* 2022;11(2):125-130.
13. Pal T. Vermiwash and plant growth. *J Environ Biol.* 2013;34(2):325-330.
14. Sivanandhan G, Kumar RS, Venkatesalu V. Pharmacognostic and antimicrobial studies on *Withania somnifera* L. Dunal. *Pharmacogn Mag.* 2011;7(26):37-42.
15. Smith S. India's role as the leading producer of fine root quality Ashwagandha. *Agricultural Statistics at a Glance.* Ministry of Agriculture, India; 2022.
16. Tiwari A, Goud P, Nagre SP, Tiwari G. Comparative efficacy of biofertilizers, FYM, and inorganic nitrogen on productivity and withanolides yield of *Withania somnifera* (L.) Dunal. *Int J Res Agron.* 2025;8(7):1085-1089.