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Nano-urea improves yield attributes, yield and active ingredients in Ashwagandha (*Withania somnifera* (L.) Dunal)

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Abstract
Ashwagandha (*Withania somnifera* (L.) Dunal) is an important medicinal plant of the Solanaceae family. In Ayurveda, its roots, leaves, seeds, and even orange-red fruit have been used for hundreds of years for medicinal functions. Due to high demand, there is an urgent need to expand the cultivation of this important medicinal plant to meet the industrial and domestic demands. The present experiment was conducted in Randomized Block Design during *Kharif* season of the year 2021- 22 (August 2021 to February, 2022) with nine treatments and three replications. T_1 - Untreated (Control), T_2 - Split application nitrogen @ 40 Kg ha^{-1} (50% dose at 45 days after sowing and rest at 90 DAS), T_3 - Split application nitrogen @ 60 Kg ha^{-1} (50% dose at 45 DAS and rest at 90 DAS), T_4 - Split application nitrogen @ 80 Kg ha^{-1} (50% dose at 45 DAS and rest at 90 DAS), T_5 - Foliar spray @ 0.2% nano-Urea (at 45 DAS and 90 DAS), T_6 - Foliar spray @ 0.4% n-Urea (at 45 DAS and 90 DAS), T_7 - Foliar spray @ 0.6% n-Urea (at 45 DAS and 90 DAS), T_8 - Foliar spray @ 0.8% n-Urea (at 45 DAS and 90 DAS), T_9 - Foliar spray @ 1.0% n-Urea (at 45 DAS and 90 DAS). Foliar spray of Nano-Urea @ 0.6% outperformed in all treatment achieving overall performance including economic yield and root quality trait. It is efficiently effective in reducing input cost or increasing benefit cost ratio by reducing losses This treatment is better than conventional urea application in terms of leaf, root, biological yield, Harvest index (Root), Protein and Withanolide content in *Withania somnifera.* Seed yield and Harvest index for seed is better for 0.4% n-Urea and 80 Kg ha^{-1} Nitrogen respectively.

Keywords: Ayurveda, herbal industry, demand, supply, qualitative analysis

Introduction

Ashwagandha (*Withania somnifera* (L.) Dunal) is a member of the Solanaceae family, mainly found in subtropical regions in India (Gupta and Rana, 2007; Tripathi *et al*. 2018) [6, 23].

This plant has been treasured for ages in the hallowed halls of Indian Ayurveda, having been hailed as a potent Rasayana in the ancient Nighanta wisdom writings. Its well-deserved term belies its potent restorative properties; it is a tonic that gives life and gradually leads the body toward optimal health and restored well-being. In Ayurveda, its roots, leaves, seeds, and even orange-red fruit have been used for hundreds of years for medicinal functions to possess health maintenance, restoration properties and treat widely recognized body ailments in distinctive approaches due to presence of two main alkaloids namely withanine and somniferine. Both of these bioactive compounds have properties like "Ginseng" so, Ashwagandha is popularly called "Indian ginseng" (Gaurav *et al*. 2023) [5].

Pandemic worries are driving up demand for the age-old Ayurvedic medicine ashwagandha. Millions of individuals worldwide find hope in its potent immunomodulatory and stress-relieving qualities, demonstrating its significance in the quest for wellbeing. In India, 10780 hectares cultivated with the production of 8429 tonnes. Based on the current trend the demand for Ashwagandha would be around 12500 tonnes (Shalini *et al*., 2017) [18]. Therefore, there is an urgent need to expand the cultivation of this important medicinal plant to meet the industrial and domestic demands. Therefore, some varieties of Ashwagandha like 'Jawahar Ashwagandh-20', 'Jawahar Ashwagandh-134', 'Vallabh Ashwagandha-1', 'Anand *Withania somnifera*-1' and 'Poshita' (Khabiya *et al*. 2023) [9]. Productivity result of any crop depends on numerous factors interacting throughout the life cycle of that crop. Higher economic yield may be achieved through a combination of ideal fertilizer association along with environment-friendly agronomic practices (Ngoune and Shelton, 2020; Rebouh *et al*. 2023) [13, 16].

A barrier to attaining the best yield from ashwagandha growing is the scarcity of inorganic fertilizers. Innovative methods are needed for contemporary, sustainable agriculture since traditional chemical fertilizers have been criticized for damaging the environment and lowering the quality of agricultural goods.

Since nanoscale fertilizers offer increased efficiency and nutrient delivery, they represent a paradigm change in crop management. These fertilizers reach plants through stomata and trichomes, circumventing the conventional absorption pathways by encasing vital nutrients within nanoparticles. Compared to traditional fertilizers, this focused technique increases absorption and minimizes nutrient losses, resulting in superior plant development at lower treatment rates. The effectiveness of nanoscale fertilizers is further enhanced by their enormous surface area and regulated release characteristics, guaranteeing a more economical and sustainable method of agricultural production. This cutting-edge technology has enormous potential to maximize resource use and advance environmental sustainability in the agricultural sector.

Previous studies proved the beneficial influence of nano-fertilizers and suggested that the foliar applications of liquid nano-fertilizer could improve the plant growth and yield (Ekinci *et al*. 2014) [3]. At the same manner, using of nano-scale iron oxide particles promoted the photosynthesis, growth, yield, and quality of peanut (Liu *et al*. 2005) [12], soybean (Sheykhbaglou *et al*. 2010) [19], wheat (Harsini *et al*. 2014) [7] and pearl millet (Kumar *et al*. 2022) [11]. So, on the basis of above background the present investigation was conducted to analyze the effects of nano-urea (n-Urea) on productivity and quality of roots of Ashwagandha.

Materials and Methods

The present experiment was conducted at Research Field Medicinal Garden, Department of Plant Physiology, College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, India (411.78 m above msl, 23'213'' N, 79'958'' E) during *Kharif* season of the year 2021- 22 (August 2021 to February, 2022). The annual rainfall was about 1284 mm of which 90% was received between June 2021 and September 2021. The average temperature was 29 °C, and varies from 20 °C to 45 °C.

The experiment was laid out in RBD (Randomized Block Design) design with nine treatments comprising and three replications. Ashwagandha seeds at 10 kg ha^{-1} were sown in line 40 cm apart, seedling depth being 1-2 cm with the size of each treatment plot 8.4 m^2 (2.8 m x 3.0 m). Treatments were as follows: T_1 - Untreated (Control), T_2 - Split application nitrogen @ 40 Kg ha^{-1} (50% dose at 45 days after sowing and rest at 90 DAS), T_3 - Split application nitrogen @ 60 Kg ha^{-1} (50% dose at 45 DAS and rest at 90 DAS), T_4 - Split application nitrogen @ 80 Kg ha^{-1} (50% dose at 45 DAS and rest at 90 DAS), T_5 - Foliar spray @ 0.2% nano-Urea (at 45 DAS and 90 DAS), T_6 - Foliar spray @ 0.4% n-Urea (at 45 DAS and 90 DAS), T_7 - Foliar spray @ 0.6% n-Urea (at 45 DAS and 90 DAS), T_8 - Foliar spray @ 0.8% n-Urea (at 45 DAS and 90 DAS), T_9 - Foliar spray @ 1.0% n-Urea (at 45 DAS and 90 DAS). All the recommended cultural and plant protection operation were followed to raise healthy crop. Thinning, gap filling and hand sowing was done to ensure the desired population. The field was irrigated twice during the life cycle of crop. Data on Plant height, number of leaves, number of branches plant^{-1}, number of capsules plant^{-1}, root length was recorded on 5 randomly selected plants. Selected plant partitioning was done in plant parts. After sun drying, number of seeds capsules^{-1} (berries) and seed yield was recorded. After removal of calyx persistence number of seeds capsule^{-1}, Seed yield, leaf biomass, root yield was recorded treatment wise. Analysis of observations taken on different variables was carried out to know the degree of variation among all the treatments. The data was statistically analyzed through Randomized Block Design (Fisher, 1967) [4].

Results and Discussion

Yield components maybe the resultant of different physiological metabolic and biochemical process materialized by the plant. Soil application of Nitrogen as urea and foliar spray of Nano-Urea significantly (p<0.01) increased the plant height than that obtained in control (Table 1). Among the fertilizer doses, soil application of 60 Kg N ha^{-1} (T_3) gave the highest plant height (51.67 cm). Desai *et al.* (2017) [2] stated that increase in Nitrogen concentration up to a limit increases plant height. Sultana *et al.* (2005) also reported the similar results on plant height due to increasing N fertilizer. Different nitrogen sources cause plants to grow taller because nitrogen encourages plant development and lengthens and multiplies internodes, both of which lead to a continual increase in plant height (Amin, 2011) [2].

Number of branching was influenced by the application of Nitrogen as Urea more effective than control. Maximum number of branches per plant (3.17) was found by the soil application of Nitrogen as urea 80 kg ha^{-1}. This increase may be due to the role of N in promoting vegetative growth of the plants. Our research data concur with the literature reporting that increasing nitrogen rates increased number of branches per plant (Pandey *et al.* 2006; Desai *et al.* 2017) [14, 2].

It can be seen from the Table 1 that the increasing doses of soil application of nitrogen as urea and foliar spray of nano-urea up to 0.6% resulted in progressive (p<0.01) increase in number of capsules plant^{-1}, number of seeds capsule^{-1} and test weight (1000 seed). Highest number of capsules plant^{-1} (446.67), number of seeds capsule^{-1} (32.67) and test weight (160.9 mg) were observed in soil application of Nitrogen as urea 80 kg ha^{-1}. Test weight is generally correlated with number of capsules and number of capsules are generally correlated with number of branches. Our results were confirmed by the findings of Pandey *et al*. (2006) [14] and Jain (2006) [8].

Root length (cm) is an important factor of Ashwagandha productivity. Application of Nitrogen as Urea and Nano-Urea was found effective in increasing the root length over control. Maximum root length (16.977 cm) was found in application of Nitrogen @ 80 kg ha^{-1}. But the collar diameter or the root of crop was found in Nano- Urea @ 0.6%. Interaction of root length and collar diameter was the resultant of root yield. The result was justified by Pandey *et al*. (2006) [14] and Kubsad *et al*. (2009) [10]. Increase in root length and diameter with applications of fertilizers may be due to increased photosynthesis.

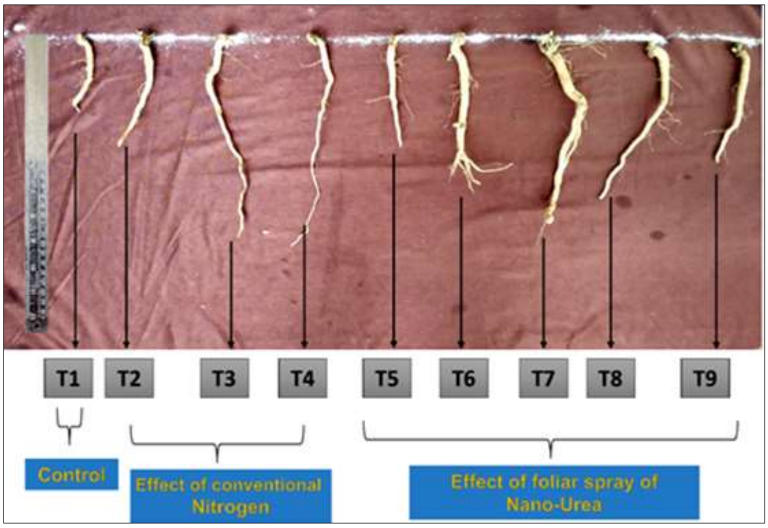

Fig 1: Measurement of root length Influenced by soil application of conventional Nitrogen as Urea and foliar spray Nano-Urea along with control.

Table 1: Yield Component in Ashwagandha as influenced by soil application of Nitrogen and foliar spray of nano- Urea treatment

Treatment	Yield Component						
	Plant Height (cm)	Number of leaves Plant^{-1}	Number of Branches Plant^{-1}	No. of capsules Plant^{-1}	No. of seeds Capsule^{-1}	Test weight (1000 seed) mg	Length of Root (cm)
T_1	31.48	27.15	1.63	164.00	20.33	77.0	8.680
T_2	48.99	33.81	2.50	252.67	24.33	121.8	15.333
T_3	51.67	43.54	2.88	396.33	29.33	146.7	17.353
T_4	48.52	36.21	3.17	446.67	32.67	160.9	17.960
T_5	37.17	35.18	1.83	192.33	24.00	87.8	9.413
T_6	34.14	53.33	2.02	225.67	27.00	112.9	11.360
T_7	38.35	61.99	2.19	295.00	29.00	142.8	15.473
T_8	37.78	56.78	2.45	271.00	26.00	135.9	13.837
T_9	36.65	59.04	2.87	248.00	25.00	113.7	12.447
S.Em ±	1.86	1.07	0.11	2.70	0.60	0.01	0.830
CD at 5%	5.63	3.23	0.32	8.16	1.82	0.02	2.511

Table 2: Yield Component in Ashwagandha as influenced by soil application of Nitrogen and foliar spray of nano- Urea treatment

Treatment	Leaf Biomass Kg ha^{-1}	Root Yield Kg ha^{-1}	Seed Yield Kg ha^{-1}	Economical Yield (Root + Leaf)	Biological yield Kg ha^{-1}	Harvest Index of leaf (%)	Harvest Index of root (%)	Harvest Index of seed (%)
T_1	245.33	348.67	420.00	594.00	2036.67	12.05	17.12	20.63
T_2	351.81	530.67	687.07	882.48	2828.21	12.44	18.76	24.29
T_3	568.37	701.47	786.93	1269.84	3763.44	15.10	18.64	20.91
T_4	498.15	567.73	844.68	1065.88	3059.89	16.28	18.55	27.61
T_5	336.17	445.88	548.71	782.05	2292.09	14.67	19.45	23.94
T_6	643.53	728.00	687.87	1371.53	3455.40	18.62	21.07	19.91
T_7	777.27	931.33	721.33	1708.60	3983.27	19.51	23.38	18.11
T_8	586.45	694.67	749.33	1281.12	3530.45	16.61	19.68	21.22
T_9	496.00	603.09	629.33	1099.09	3225.76	15.38	18.70	19.51
S. Em ±	1.79	3.30	4.63	3.74	11.50	0.08	0.11	0.20
CD at 5%	5.36	9.84	13.88	11.16	34.34	0.23	0.32	0.59

Table 3: Yield Component in Ashwagandha as influenced by soil application of Nitrogen and foliar spray of nano- Urea treatment

Treatment	Withanolide content	Proline Content (mg g^{-1})	Moisture %	Protein %	Fat %	Fibre %	Ash %	Carbohydrate %
T_1	0.029	251.48	4.573	7.273	0.544	54.413	9.873	23.310
T_2	0.035	347.84	3.737	9.495	0.495	55.469	8.390	22.430
T_3	0.047	384.71	2.843	11.753	0.478	59.464	7.510	17.550
T_4	0.063	416.98	3.597	14.910	0.462	64.643	9.167	9.840
T_5	0.044	267.74	1.175	8.443	0.482	55.903	8.897	23.557
T_6	0.065	295.48	1.393	9.690	0.452	56.363	7.913	22.980
T_7	0.083	324.21	1.815	13.408	0.394	57.157	5.403	24.600
T_8	0.084	335.56	2.247	11.797	0.435	61.755	7.863	16.967
T_9	0.062	448.47	2.780	11.227	0.443	63.309	8.600	13.283
S.Em ±	0.0047	2.075	0.044	0.563	0.006	0.948	0.032	0.032
CD at 5%	0.0141	6.273	0.132	1.689	0.019	2.867	0.096	0.095

Number of leaves per plant (Table 1) and leaf biomass g plant^{-1} (Table 2) influenced by soil application of nitrogen as Urea and foliar spray of Nano-Urea. At harvest, the number of leaves (61.99 plant^{-1}) and leaf biomass (777.27 kg ha^{-1}) was noted more in foliar application of Nano-Urea @ 0.6%. Rostami *et al.* (2017) [17] observed that Foliar applied nitrogen nano fertilizer increased the leaf dry weight of peppermint by 165 percent over control.

Root length and root diameter decide root yield of Ashwagandha. The superior performance of foliar application of Nano-Urea @ 0.6% was found for Root yield (931.33 kg ha^{-1}). Whereas, seed yield (844.68 kg ha^{-1}) was found superior in nitrogen @ 80 kg ha^{-1} as Urea. Solanki and Shaktawat (1999) [21] found similar results that the N levels increased to 45 kg N ha^{-1} significantly increased seed yield, over control. Maximizing seed yield may be resultant in a greater number of branches and higher photosynthate accumulation in seed. The result confirmed by Pawar *et al.* (2000) [15].

Economic yield was a combination of leaf yield and root yield because they are used for medicinal purposes. Application of Nitrogen as Urea and Nano-Urea was found effective in affecting the economic yield kg per hectare. Foliar spray of Nano- Urea @ 0.6% was found maximum economic yield (1708.60 kg ha^{-1}). Similarly, maximum biological yield (3983 kg ha^{-1}) was also found in same treatment foliar spray of Nano-Urea @ 0.6%. This result justifies the finding of Abdel-Aziz *et al.* (2016) that nano-fertilizers are quickly absorbed by the epidermis of leaves and translocated to stems, facilitating active molecule absorption and improving Ashwagandha growth and yield.

A significant difference was observed for the harvest index of leaves among the treatments. Treatment foliar spray of Nano-Urea @ 0.6% exhibited maximum harvest index of leaf. The range for harvest index of leaf is 12.05-19.51. Similarly, foliar spray of Nano-Urea @ 0.6 exhibited maximum harvest index of root (23.38). Whereas, soil applications of nitrogen as Urea @ 80 kg ha^{-1} exhibited maximum harvest index of seed (23.38) in our experiment.

The most critical measure determining farmer economic returns is root dry weight in Ashwagandha. However, at the harvesting stage, the chemical composition of root is usually ignored. Both farmers and commercial processors would profit from optimal production quality and chemical composition at the harvesting stage. In our study, the total Withanolide content (0.083) was significantly higher with foliar spray of Nano-Urea @ 0.8% but statistically it was similar with foliar spray of Nano-Urea @ 0.6% which was 0.083.

The biochemical estimations were also made on Ashwagandha roots for other macromolecules like carbohydrates, protein and fixed fat (Table 3). Data obtained during the present study indicated that treatment control (4.573%) recorded the highest moisture percentage and Nano-Urea @ 0.2% (1.175%) lowest moisture contents respectively. Proline increases proportionately faster than other amino acids in plants under water stress, has been suggested as an evaluating parameter for irrigation scheduling. It was discovered that increasing stress intensity resultant in increasing proline content. Proline content (mg g^{-1}) was both Nitrogen is Urea and Nano-Urea effectively. Maximum proline content (448.47 mg g^{-1}) was found in Nano-Urea @ 1.0%. Higher concentration of Nitrogen causes more accumulation of proline protein in leaves (Spormann *et al.* 2023) [22].

Soil application of Nitrogen is Urea and foliar spray of Nano-Urea was found effective in affecting ash contents present in the roots. Control (9.873%) recorded the highest and Nano-Urea @ 0.6% (5.403%) lowest ash contents, respectively. Similarly, maximum (0.544) fat % was found in control whereas the lowest (0.394%) was found in Nano-Urea @ 0.6%.

Soil application of Nitrogen is Urea and foliar spray of Nano-Urea was found effective in affecting the crude fibre present in the roots. Maximum crude fiber (64.643) was found in Nitrogen @ 80 Kg ha^{-1} as compared to other treatments. Whereas, highest protein (64.643) was found in Nitrogen @ 80 Kg ha^{-1} which was statistically similar Nano-Urea @ 0.6%a (13.408%) had the higher magnitudes for protein contents, whereas treatment control recorded the minimum (7.273%). It is justified by Singh and Kumar (2003) that an increase in Nitrogen concentration results in an increment in the protein content.

Conclusion

Foliar spray of Nano-Urea @ 0.6% outperformed in all treatment achieving overall performance including root yield (highest at 1708 kg ha^{-1}), economic yield and root quality trait. It works effectively to lower input costs or raise the benefit-cost ratio by minimising losses. In terms of Withania somnifera leaves, roots, biological yield, Harvest index (root), protein, and Withanolide content, this therapy is superior than traditional urea administration. Higher nitrogen levels (80 kg ha^{-1}) and 0.4 % n-urea result in higher seed yield and harvest index, respectively.

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