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Research article

Partial substitution of conventional nitrogen fertilizers with nano urea and plant growth-promoting rhizobacteria in fodder oats

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Abstract
Oats are an important winter-season cereal fodder crop in India. The field trial was conducted to evaluate the effect of nano urea and plant growth-promoting rhizobacteria on the fodder oats during the *Rabi* season of 2021-22 to 2022-23. A field experiment was laid out in a randomized block design with eight treatments and four replications. Results of two years experiment revealed that different nutrient management practices caused significant variations in growth as well as green and dry fodder yield. Application of 75% RDN+ PGPR+ nano urea sprays recorded statistically similar results concerning plant height, number of tillers per running metre, number of leaves per plant, leaf length, stem growth, crop growth rate, relative growth rate, green and dry fodder yields with 100% RDN and 100% RDN+ PGPR during first and second fodder cutting.Treatment 75% RDN+ PGPR+ nano urea obtained 49.15% higher green fodder yield over control. Nano urea and PGPR achieved equal yields with 100% RDN by reducing 25% RDN.

Keywords: Fodder oats, Foliar nutrition, Green fodder, Nano urea, PGPR

Introduction
According to the 20th livestock census, India is home to 535.78 million livestock population (Anonymous, 2019). Present fodder crops cultivated area is merely 5.4% (8.4 M ha) of the total cultivated area in the country and leads to fodder deficiency of 11.24% for green and 23.4% for dry matter (Roy et al., 2019). The major constraint for poor productivity of livestock was the unavailability of good quality green fodder. The unavailability of green and dry fodder accounts for 12.3 and 11.6% losses in dairy milk production, respectively, in India (Brithal and Jha, 2005). Oats are one of the important winter cereal fodder crops in the north, central and west zones of the country (Priyanka et al., 2022). It has an excellent growth habit and is an economical source of dietary energy (Singhal et al., 2022). It provides soft, palatable quality fodder rich in crude protein (10–12%). It contains 55 to 63% neutral detergent fiber (NDF), 30 to 32% acid detergent fiber (ADF), 22 to 23.5% cellulose, 17 to 20% hemicellulose when harvested at 50% of flowering stage of plants (Kumar et al., 2012).

Prevalent commercial farming systems utilize conventional fertilizers to meet the essential plant nutrient requirement for maximum productivity to feed burgeoning populations. In India, it is very difficult to sustain crop productivity without fertilizer input (Tarafdar *et al*. 2014). Approximately 35 to 40% of the crop productivity is attributed to fertilizer application. In India, the current NPK consumption ratio is 7.7:3.1:1 over 4:2:1 is evident for unchecked use of chemical fertilizers for crop production. Nitrogen is considered to be the major element of chlorophyll, which aids in photosynthesis. In the year 2019-20, worldwide nitrogen demand was 107 million tonnes and out of it, 76.5% was supplied through urea (FAO, 2019). Most of the applied nitrogen fertilizers lost through volatilization, nitrification and denitrification release N_2O, which leads to environmental pollution (Mahmud *et al*., 2021). Thus, nitrogen use efficiency is low (30–40%) under field conditions. Ensuring sufficient quality fodder and environmental sustainability for the growing livestock population using fewer resources is a major challenge in successful animal husbandry. Currently, nanotechnology has provided the feasibility of exploring nanostructured materials as fertilizer carriers or controlled-release vectors for the building of smart fertilizers to enhance the production nutrient use efficiency and reduce the extent of environmental pollution (Chinnamuthu and Boopathi, 2009). Recently, IFFCO-Nano Biotechnology Research Centre Kalol, Gujarat, developed liquid nano urea through indigenous proprietary patented technology. This liquid nano urea is the world's first liquid nano fertilizer, which the Fertilizer Control Order, Government of India, has notified. It contains 4% total nitrogen on weight by volume basis with particle size 20 to 50 nm and has 10,000 times greater surface area to volume size than granular urea particles. These particles are evenly dispersed in water. Foliar application at critical crop growth stages sufficiently meets nitrogen requirementsresulting in increased crop productivity and quality of fodder. It is well suited for precision agriculture practices, as its controlled release pattern allows farmers to accurately target the nutrient needs of specific crop stages, ensuring maximum nutrient absorption. Plant growth-promoting rhizobacteria (PGPR) represents a consortium of beneficial soil microorganisms that habitats in the rhizosphere and stimulate plant growth. Growth-promoting rhizobacteria increase nutrient uptake by improving their availability in the soil through atmospheric fixation and soil mobilization. Besides, it regulates hormonal and nutritional balance, induces resistance against plant pathogens and stimulates ion transport systems in the root through symbiotic association. In food crops, PGPR has demonstrated its potential to enhance plant growth, nutrient uptake and resistance to various stresses. So, integrating these cutting-edge technologies into fodder production systems can offer sustainable solutions to address the challenges faced by farmers in fodder production. After critically analyzing problems and research gaps, a two-year field study was carried out to evaluate the efficacy of nano urea and PGPR on growth parameters and fodder yield of oats.

Materials and Methods

Experiment site: A field trial was conducted to evaluate the effect of nano urea and PGPR on the performance of fodder oats for two consecutive years during the *Rabi* season of 2021-22 to 2022-23 at the Research Farm, Agronomy Section, ICAR-National Dairy Research Institute, Karnal. The soil of the experiment site was clay loam in texture, slightly alkaline pH (7.62) and normal in electrical conductivity (0.283 dS m^{-1}), medium organic carbon 0.644% and low in available nitrogen (190.42 kg ha^{-1}), medium phosphorus (12.92 kg P_2O_5 ha^{-1}) and potassium status (163.73 kg K_2O ha^{-1}).

***Experiment details and treatments*:** The field experiment was laid out in a randomised block design with eight treatments and four replications. Treatments comprise a combination of nano urea and PGPR as substitution of nitrogen at different levels of the recommended dose of nitrogen as follows,

Treatment No.	Treatment details
T_1	: Control
T_2	: 100% RDN
T_3	: 100% RDN + PGPR
T_4	: 75 % RDN + Nano urea
T_5	: 75% RDN + PGPR + Nano urea
T_6	: 75% RDN + Conventional urea spray @ 2.5%
T_7	: 50% RDN + Nano urea
T_8	: 50% RDN + PGPR + Nano urea

***Cultural operations*:** Pre-sowing irrigation was given 7 days before sowing. The experiment field was prepared by a disc plow followed by a disc harrow and rotavator at optimum moisture. The field was laid out according to experimental design, having eight treatments and four blocks with prominent bunds between plots. Fodder oats cv 'kent' was sown at the seed rate of 100 kg ha^{-1} during 2^{nd} fortnight of November under irrigated conditions, keeping row to row spacing of 30 cm. The recommended dose of fertilizers (RDF) for dual cut fodder oats is 120:40:40 N: P_2O_5: K_2O kg ha^{-1} was applied according to schedule (50% nitrogen at basal application and remaining N at two equal splits during 1^{st} irrigation and after 1^{st} cutting). Phosphorus and potassium were applied uniformly to all the treatments as per recommendation as basal dose. Fodder oats were harvested for two cuttings, first at 60 DAS and second cutting at 50% flowering.

***Nano urea and PGPR*:** Nano urea was applied at 30, 45 days after sowing (DAS) as well as 15, 30 days after the first cutting of fodder oats @ 4 ml L^{-1} while conventional urea @ 2.5%. The foliar application was carried out using hand-operated knapsack sprayers equipped with flat fan nozzles to ensure complete coverage of the foliage during evening hours, ensuring that no dew was present on the foliage. In the present study, a PGPR formulation 'PUSA Sampoorna' was obtained from the Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi. It is an eco-friendly biofertilizer having a consortium of three bacteria strains, namely, *Azotobacter chroococcum* (N_2 fixing bacteria), *Pseudomonas striata* (P-solubilizing bacteria), and *Bacillus decolorations* (K-solubilizing bacteria). Seeds were treated with PGPR formulation and dried under shade before sowing.

***Measurement of crop growth attributes and yield*:** Biometric observations of plant growth such as plant height, number of tillers per plant, number of leaves, leaf length and stem girth were recorded from five tagged plants at 30 DAS, I and II cut and expressed as a mean value. Green fodder yield was recorded by weighing green biomass from the net plot and converting it to a hectare basis. The dry fodder yield was worked out by multiplying green fodder yield with the dry matter content of their respective treatments. The increase in plant biomass per unit area and time or crop growth rate (CGR) and relative growth rate (RGR) were calculated as per the equation given by Radford (1967).

$$\mathrm{CGR}\ (\mathrm{g\ m^{-2}\ day^{-1}}) = \frac{1}{P} \times \frac{(W_2 - W_1)}{(t_2 - t_1)}$$

$$\mathrm{RGR}\ (\mathrm{mg\ g^{-1}\ day^{-1}}) = \frac{(\log_e W_2 - \log_e W_1)}{(t_2 - t_1)}$$

***Statistical analysis*:** Two years recorded data was analyzed for analysis of variance (ANOVA) as per randomized block design (Gomez and Gomez, 1984) and tabulated as two years mean. Recorded data was. The significance of the treatments was tested using an F test with a 5% level of significance ($P \leq 0.05$). Graphs were plotted using MS Excel.

Results and Discussion

***Growth parameters of fodder oats*:** Results from Tables 1 and 2 revealed that the growth parameters of fodder oats did not differ significantly at 30 DAS. This might be due to the initial slow absorption of applied nutrients, slow initial crop establishment, lack of complete treatment imposition and also the inefficiency of microbes to function at their full potential. However, growth parameters *viz*., plant height, number of tillers per plant, number of leaves, leaf length and stem girth of fodder oats showed significant differences among treatments for nano urea sprays and PGPR seed treatment under different recommended nitrogen levels at first and second fodder cuttings (Tables 1 and 2). Among treatments, T_3: 100% RDN+PGPR, T_2: 100% RDN and T_5: 75% RDN + nano urea sprays+ PGPR recorded significantly higher growth parameters and showed statistically on par results to each other during I and II harvest as compared to other remaining treatments and also T_4: 75% RDN + nano urea produced statistically similar results to T_2: 100% RDN with respect to plant height, number of leaves, leaf length. Significantly lower growth parameters were recorded in the control treatment (T_1) during both cuttings. The percent plant height increased by about 31.74, 28.64 and 27.60% in T_3, T_2 over T_5 during I harvest and 34.45, 32.71 and 31.47% during II harvest in T_3, T_2 and T_5 over control (T_1), respectively. The percentage of increment in number of leaves per plant was about 37.02, 33.62 and 32.58% during I harvest and 46.06, 43.80 and 42.52% during II harvest in T_3, T_2 and T_5, respectively over T_1. The percentage of increment in leaf length was around 35.80, 28.29 and 25.68% in T_3, T_2 and T_5 over T_1 during I harvest of fodder oats. Whereas, it was about 38.02, 30.33 and 28.08% during II harvest over T_1, respectively.

Application 75% RDN+ PGPR with two nano urea sprays each for I and II fodder cuts (T_5) recorded statistically on par results with T_3 and T_2 in terms of growth parameters of fodder oats during both the years of study. This might be due to the initial application of fertilizers helps in early crop growth besides foliar nutrition of nano urea to foliage, leading to direct penetration through stomatal pores and passage through plasmodesmata (Kumar *et al*., 2021). Translocated nitrogen increased photosynthesis, chlorophyll production and enhancement of growth parameters. Seed treatment with PGPR accelerated the mineralization and mobilization of nutrients, making increased availability for subsequent growth stages which increased photosynthesis, leading to increased dry matter production. Srivani *et al*. (2022) reported that the application of nitrogen employing both granular urea and nano urea in combination significantly increased growth parameters over control. Foliar spray of nano urea at critical stages had a significant impact on the growth parameters of rice (Velmurugan *et al*., 2021). Nano urea spray might be attributed to increased protein synthesis due to better availability of nitrogen, which plays a key role in cell division and plant growth (Samanta *et al*., 2022). Our results were reliable with Kumar *et al*. (2023), who also reported similar results with 100% RDN and 75% RDN with 25% nano nitrogen in fodder oats and Upadhyay *et al*. (2023a), who reported two foliar sprays of nano urea with $N_{75}PK$ and $N_{100}PK$ with or without nano urea showed on par results in terms of growth parameters in different crops like wheat, maize, mustard and pearl millet.

***Dry matter accumulation ($g\ m^{-2}$)*:** Significantly higher dry matter accumulation was witnessed in the treatment receiving T_3:100% RDN + PGPR (606.39 and 472.57 g m^{-2}) at the first and second harvest stage, respectively, than in other treatments. It remained on par withT_2:100% RDN (575.05 and 450.22 g m^{-2}) and T_5:75% RDN + nano urea sprays + PGPR (564.15 and 436.79 g m^{-2}) during the first and second cutting, respectively (Table 2). However, significantly lower dry matter accumulated per m^2 was observed in the control (381.39 and 328.07 g m^{-2}) during the first and second harvests, respectively. The percentage of increase in dry matter accumulation was about 58.99, 50.77 and 47.92% in T_3, T_2 and T_5 over T_1 during the first harvest of fodder oats, whereas it was about 45.18, 38.31 and 34.19% during the second harvest. Foliar nutrition of nano urea during 30 and 45 DAS on foliage leads to quick absorption through stomatal pores and passage through plasmodesmata (Kumar *et al*., 2021). Translocated

Table 1. Effect of nano urea and PGPR on growth parameters of fodder oats (two years mean data)

Treatments	Plant height (cm)			Number of leaves per plant			Number of tillers per running meter			Leaf length (cm)		
	30 DAS	I Cut	II Cut	30 DAS	I Cut	II Cut	30 DAS	I Cut	II Cut	30 DAS	I Cut	II Cut
T_1	42.50	109.54	85.78	29.82	40.72	37.37	25.56	84.09	56.28	29.82	40.72	37.37
T_2	47.68	140.91	113.84	32.50	52.24	48.71	33.26	117.50	83.45	32.50	52.24	48.71
T_3	48.33	144.56	115.33	32.80	55.30	51.59	34.05	120.53	84.85	32.80	55.30	51.59
T_4	45.33	136.97	109.50	31.78	49.82	46.39	30.90	111.73	79.73	31.78	49.82	46.39
T_5	46.70	140.02	112.78	32.46	51.17	47.87	31.78	116.20	81.83	32.46	51.17	47.87
T_6	46.25	134.12	106.38	31.27	48.04	45.06	30.87	106.45	75.33	31.27	48.04	45.06
T_7	45.33	128.84	104.18	30.40	47.00	43.51	27.79	103.70	71.74	30.40	47.00	43.51
T_8	46.18	133.18	104.79	30.75	47.27	43.99	28.65	105.24	74.15	30.75	47.27	43.99
SEM±	1.23	2.22	2.02	0.71	1.57	1.51	1.86	2.21	2.84	0.71	1.57	1.51
LSD ($p \leq 0.05$)	NS	6.54	5.95	NS	4.63	4.45	NS	6.51	8.35	NS	4.63	4.45

Table 2. Effect of nano urea and PGPR on stem girth, dry matter production, green and dry fodder yield of oats (two years mean data)

Treatments	Stem girth (cm)			Dry matter production (g m^{-2})			Green fodder yield (t ha^{-1})			Dry fodder yield (t ha^{-1})		
	30 DAS	I Cut	II Cut	30 DAS	I Cut	II Cut	I Cut	II Cut	Total	I Cut	II Cut	Total
T_1	0.55	1.34	1.32	196.79	381.39	328.07	21.81	14.84	36.65	3.30	2.09	5.39
T_2	0.59	1.52	1.73	206.31	575.05	450.22	33.85	22.39	56.24	5.69	3.50	9.19
T_3	0.60	1.56	1.75	206.99	606.39	472.57	34.15	23.13	57.28	5.94	3.63	9.57
T_4	0.57	1.46	1.68	204.45	510.43	425.23	31.01	20.61	51.62	5.09	3.19	8.28
T_5	0.58	1.51	1.69	205.23	564.15	436.79	33.79	22.27	56.05	5.68	3.48	9.15
T_6	0.57	1.46	1.65	203.77	489.21	403.05	30.29	19.28	49.56	4.89	2.94	7.83
T_7	0.56	1.45	1.65	200.27	448.77	398.20	27.86	18.91	46.78	4.48	2.88	7.35
T_8	0.57	1.46	1.65	201.83	472.73	402.89	29.18	19.43	48.60	4.73	2.97	7.71
SEM±	0.01	0.03	0.04	2.19	18.11	12.30	0.99	0.65	1.57	0.21	0.11	0.33
LSD ($p \leq 0.05$)	NS	0.09	0.10	NS	53.26	36.17	2.92	1.91	4.62	0.62	0.31	0.97

nitrogen helps in increased photosynthesis, chlorophyll production, cell expansion and enlargement, ultimately on dry matter production. Seed treatment with PGPR accelerates the mineralization and mobilization of nutrients and makes availability for subsequent growth stages which increases photosynthesis, leading to increased dry matter production.

***Crop growth rate (CGR) and relative growth rate*:** Results from Figures 1 and 2 indicated that CGR and RGR varied significantly for the application of different treatments during 30 DAS to harvest of I cut. However, CGR showed no significant difference during 0-30 DAS. Treatment with application of 100% RDN+ PGPR(T_3) recorded significantly higher CGR (13.31 g m^{-2} day^{-1}) and RGR (15.56 mg g^{-1} day^{-1}) during 30 DAS to harvest stage and it was remained on par with T_2: 100% RDN (12.29 g m^{-2} day^{-1} and 14.77 mg g^{-1} day^{-1}), T_5: 75% RDN + nano urea + PGPR (11.96g m^{-2} $day^{-1}$and 14.63 mg g^{-1} day^{-1}) 30 DAS to harvest stage in terms of CGR and RGR, respectively. However, the control treatment (T_1) recorded the least CGR (6.15 g m^{-2} day^{-1}) and RGR (9.58 mg g^{-1} day^{-1}) in the same period. The percent of increment in CGR was about 116.36, 99.75 and 94.43%; RGR was about 62.39, 54.18 and 52.76% during 30DAS-I cut in T_3, T_2 and T_5 over absolute control. Kumar *et al*. (2022) reported application of PGPR with RDF improves growth parameters, CGR and RGR in fodder oats.

***Green fodder and dry fodder yield*:** The green fodder yield of fodder oats includes the above-ground total biomass produced by the plant. The data pertaining to green and dry fodder yield of fodder oats differed significantly for the application of various treatments

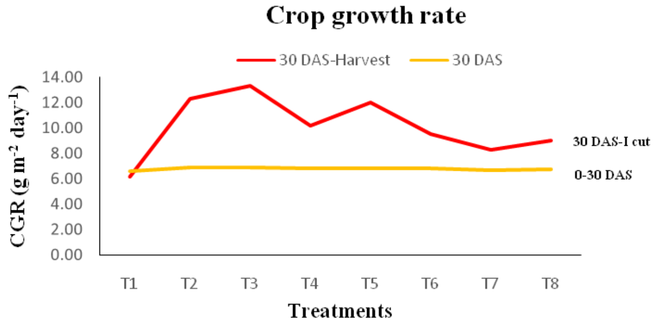

Fig 1. Effect of nano urea and PGPR on crop growth rate of fodder oats (two years mean data)

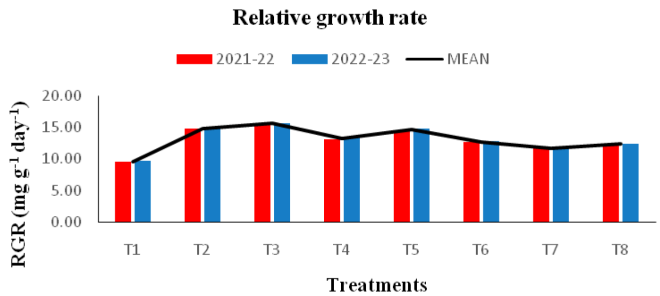

Fig 2. Effect of nano urea and PGPR on relative growth rate of fodder oats during 30 DAS-I cut (two years mean data)

at I and II cuts (Table 2). Significantly higher green and dry fodder yield was recorded in the treatment receiving T_3 over other treatments. However, it remained on par with T_2 and T_5 during I and II harvest and total fodder and treatment T_4:75% RDN + nano urea produced a statistically similar yield with T_2:100% RDN. Treatment receiving conventional urea sprays shows statistically similar results with nano urea spray treatment under equal nitrogen levels, *i.e.*, T_6 and T_4. The percentage of increase in green fodder yield was about 58.94, 50.68 and 49.15% in total GFY in T_3, T_2 and T_5, respectively, over T_1. It is a dependent factor of the shoot and root parameters, both parameters play a key role in the total green fodder production.

Application of 75% RDN+ PGPR+ nano urea sprays showed statistically similar green fodder and dry fodder yields to 100% RDN and 100% RDN+ PGPR. Nano urea helps in achieving yields similar to 100% RDN by reducing 25% RDN without compromising the yields. Rajesh *et al.* (2022) also reported that 75% RDN and nano urea treatment was found statistically on par with 100% RDN in terms of green and dry fodder yield of oats. Similarly, higher green and dry fodder yield of oats recorded with PGPR inoculation along with reduced RDF might be due to a significant increase in growth attributes like plant height, number of leaves, number of tillers per plant, and dry matter accumulation (Rajesh *et al.* 2022). Foliar spraying of nano urea at critical stages has favorable impacts on cell elongation, differentiation, and co-enzymes, enhanced operation of meristematic tissues, and photosynthetic area reflected on better growth parameters, ultimately on fodder yield. Raj *et al.* (2024) stated nano urea has been testified to improve plant growth parameters, plant dry weight, and yield attributes, contributing to increased grain and stover yield in maize. Higher nitrogen content in plants can have a positive impact on chlorophyll biosynthesis, leading to increased photosynthesis rates (Sun *et al.*, 2020). Upadhyay *et al.* (2023b) reported two foliar sprays of nano urea with N_{75}PK exhibits on par with N_{100}PK with or without nano urea in terms of yields of different crops like wheat, maize, mustard and pearl millet. Similar results were reported by Saklani andPal (2022); and Sankar *et al.* (2020).

Foliar application of nano fertilizers during critical stages combined with reduced levels of conventional fertilizers increases crop yields similar to normal soil application dosage of granular fertilizers (Rajesh *et al.*, 2021). PGPR-employed treatments showed improved results with respect to green and dry fodder yield of oats. Andrade *et al.* (2023) stated PGPR uses its metabolism to fix nitrogen, solubilize phosphates, and produce hormones and they can directly affect plant metabolism when applied as seed inoculation. Ramanjaneyalu *et al.* (2012) also reported that 50% RDF along with biofertilizers produced significantly higher fresh and dry weight of fodder sorghum than that of 50% RDF alone and control treatments.

Conclusion

Results of the present study revealed that the application of 100% RDN+ PGPR treatment found higher growth parameters in green, dry fodder yield. However, the application of 75% RDN, two foliar sprays of nano urea and PGPR seed treatment also produces statistically similar results. It could be concluded that two sprays of nano urea in fodder by reducing 25% RDN is equally effective. However, no superior results were obtained from nano urea application over 100% RDN. So, employing nano urea sprays and PGPR sustains fodder yield with a 25% reduction in conventional fertilizer usage.

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