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Response of foliar application of cow-urine and nitrogen fixing bacterial inoculants on quality of cauliflower (*Brassica oleracea* L. var. *botrytis*) cv. White crystal grown under naturally ventilated polyhouse condition

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Abstract
The present investigation "Response of Foliar Application of Cow-urine and Nitrogen Fixing Bacterial Inoculants on Quality of Cauliflower (*Brassica oleracea* L. var. *botrytis*) cv. White Crystal Grown under Naturally Ventilated Polyhouse Condition" was carried out in the poly-house at the Centre of Excellence on Protected Cultivation and Precision Farming (COEPCPF), IGKV, Raipur, (Chhattisgarh) during the academic years 2022-2023 and 2023-2024 in *Rabi* season. The experiment was laid out in completely randomized design (CRD) in three replications with nine treatments. The results of the present study showed that the quality parameters such as chlorophyll content in leaves (60.26), nitrogen content in leaves (2.23%), vitamin C (60.02 mg /100 g), β- carotene content (13.02 μg/100 g), vitamin A content (21.70 μg/100 g), protein content (15.33%), nitrogen content (2.46 %), phosphorus content (0.26%), potassium content (3.38%), reducing sugar (2.10 %), non-reducing sugar (0.24 %), total sugar (2.35%) and total soluble solids (7.83%) in curd were found superior in treatment T_9 - Foliar application of foliar *Azotobacter* and foliar *Azospirillum* + FS of 10% Cow-urine + 0.1 % Molasses.

Keywords: Cow-urine, nitrogen fixing bacterial inoculants, foliar application, quality, poly-house and cauliflower

Introduction

Cauliflower (*Brassica oleracea* L. var. *botrytis*) belongs to the brassicaceae family and it has chromosome number 2n = 18. Cauliflower is a cold-tolerant crop that grows best in a moist, cool climate. This crop originated in Cyprus in the Mediterranean region and was introduced in India from England in 1822 by Dr. Jemson (Swarup and Chatterjee, 1972) [18].

Cauliflower has high protein content and is exceptional for retaining vitamin C after cooking. When consumed uncooked, cauliflower is a good source of vitamins and minerals. In 100 g of cauliflower protein (2.60 g), calcium (33.0mg), carbohydrates (4.0 g), vitamin C (56.0 mg), vitamin K (16 μg), folate (57.0 μg), vitamin B6 (0.2 mg), and fiber (2.5 g) are all present (Kapusta-Duch *et al.*, 2019) [10].

In India, cauliflower is cultivated in an area of 473000 hectare with a production of 9283000 tonnes and productivity 19.62 T/ha (Anonymous, 2021-22) [1]. In Chhattisgarh it is cultivated in about 24260 hectare with a production of 484250 tonnes and productivity 19.96 T/ha (Anonymous, 2022-23) [2].

Polyhouse cultivation of vegetables offers distinct advantages of quality, productivity and favourable market prices to the growers. It allows precision farming and overcomes limitations of space and disadvantages of climate change. In these systems various factors of the environment are controlled and to minimize or eliminate insects and diseases problems so that plants can be grown pesticide free under covered conditions.

Bio fertilizers are eco-friendly and cheapest source of nutrients. Among the nitrogen fixing bacteria, *Azotobactor* is a bio-inoculants that works in a non-symbiotic relationship with non-leguminous crops to fix nitrogen in the environment. *Azotobacter* inoculation saves roughly 20 to 40 kg nitrogen per hectare (Ashokan *et al.*, 2000) [3]. The beneficial effects of *A. chroococcum* are attributed to production of plant growth hormones such as IAA and GA, improved nutrient uptake and antagonistic effect on plant pathogens (Parmar and Dadarwal, 1997) [14]. It can also provide protection against drought and some soil-borne diseases.

Azospirillum biofertilizer is an associative symbiotic nitrogen fixing bacteria. One main property of *Azospirillum* relies on the synthesis of phytohormones and other compounds, including auxins, cytokinins, gibberellins, abscisic acid, ethylene, and salicylic acid. Phytohormones increases root growth, resulting in improvements in uptake of moisture and nutrients. Some *Azospirillum* strains can solubilize inorganic phosphorus, making it more readily available to the plants and resulting in higher yields (Fukami *et al.*, 2018) [7].

Cow urine is a source to improve soil fertility, productivity, and the quality of vegetables. The use of cow urine corrects micronutrient deficiency and makes phytohormones available to plants (Ghosh *et al.*, 2018) [8]. Therefore, cow urine is an effective tool to correct nutritional deficiencies.

The nutrient content in cow urine, especially nitrogen, is much higher than other locally available manures. Cow urine contains 95% water, 2.5% urea and 2.5% minerals, 24 types of salts, hormones and 2.5% enzymes (Mandavgane & Kulkarni, 2020) [13]. It is rich source of macro & micro nutrients, disinfectant and prophylactic properties thus purify the atmosphere and improve soil fertility. To maintain long term soil health and productivity there is a need for nutrient management through cow urine and bio-fertilizers apart from costly chemical fertilizers for better yield of the crop (Dikshit, 2015) [6].

Materials and Methods

The present investigation was carried out during *Rabi* season of 2022-2023 and 2023-2024 at the Centre of Excellence on Protected Cultivation and Precision Farming (COEPCPF), IGKV, Raipur, (C. G.). The soil of experimental field is clayey in texture, with soil ph 7.12, low in availability of nitrogen (220.5 kg/ha), medium in Phosphorus (10.72 kg/ha) and high in potassium status (315 kg/ha). The experiment was conducted in a CRD with 09 treatments and replicated thrice. The net plot size was 3.24 m^2 (2.4 m × 1.35 m). Cauliflower seed rate is 380 g/ha with a spacing of 60 cm (Row to Row) X45 cm (Plant to Plant).

Table 1: Quantity of solution applied as per treatments in per hectare

Treatment		First foliar spray (400 Litre solution) – 15 DAT Second foliar spray (400 Litre solution)- 30 DAT Third foliar spray (400 Litre solution)- 45 DAT
T_1	Control	-
T_2	FS of *Azotobacter* and *Azospirillum*	400 ml *Azotobacter* +400 ml *Azospirillum* + 399 litre water
T_3	FS of foliar *Azotobacter* and foliar *Azospirillum*	400 ml foliar *Azotobacter* +400 ml foliar *Azospirillum* + 399 litre water + 80 gm CMC
T_4	Foliar Spray of 10% Cow-urine	40 litre cow-urine + 360 litre water
T_5	Foliar Spray of 10% Cow-urine + 0.1 % Molasses	40 litre cow-urine + 400 gm molasses + 360 litre water
T_6 (T_2+T_4)	FS of *Azotobacter* and *Azospirillum* + Foliar Spray of 10% Cow-urine	400 ml *Azotobacter* +400 ml *Azospirillum* + 40 litre cow-urine + 360 litre water
T_7 (T_3+T_4)	FS of foliar *Azotobacter* and foliar *Azospirillum* + Foliar Spray of 10% Cow-urine	400 ml foliar *Azotobacter* +400 ml foliar *Azospirillum* + 80 gm CMC + 40 litre cow-urine + 360 litre water
T_8 (T_2+T_5)	FS of *Azotobacter* and *Azospirillum* + Foliar Spray of 10% Cow-urine + 0.1 % Molasses	400 ml *Azotobacter* +400 ml *Azospirillum* + 40 litre cow-urine + 400 gm molasses + 360 litre water.
T_9 (T_3+ T_5)	FS of foliar *Azotobacter* and foliar *Azospirillum* + Foliar Spray of 10% Cow-urine + 0.1 % Molasses	400 ml foliar *Azotobacter* +400 ml foliar *Azospirillum* + 80 gm CMC + 40 litre cow-urine + 400 gm molasses + 360 litre water.

Results and Discussion

Quality attributes

The data on quality attributes were presented in Table 1(a, b, c).

Chlorophyll content in leaves (SPAD Value)

The statistical data revealed that the application of nitrogen fixing bacterial inoculants and cow-urine was significantly increased the chlorophyll content in leaves. In the case of pooled mean, the maximum chlorophyll content in leaves was measured under treatment T_9 (60.26), which was statistically superior as compared to all treatments but statistically *at par* with treatment T_8 (59.89), T_7 (59.66), T_6 (59.25) and T_5 (58.78). However, the minimum chlorophyll content in leaves was recorded under treatment T_1 (53.69).

The maximum chlorophyll content may be due to the increase in nitrogen content in the plant by nitrogen fixing bacterial inoculants because nitrogen is a major component of chlorophyll. A similar finding has been reported by Chandra *et al.* (2022) [4] in broccoli, Kaur *et al.* (2020) [11] in cauliflower and Chaudhary *et al.* (2017) [5] in knolkhol.

Nitrogen content in leaves (%)

The nitrogen content in leaves was significantly influenced by the different treatments. Pooled analysis of the data showed highest nitrogen content in leaves was recorded in treatment T_9 (2.23 %), which was statistically *at par* with treatment T_8 (2.21%) and T_7 (2.18%), where as it was found significantly differ with rest of the other treatment. However, the nitrogen content in leaves was observed lowest in treatment T_1 (2.08 %) and this was statistically inferior to all other treatments.

The maximum value of N content might be due to increased supply of N through Cow-urine and fixation of atmospheric nitrogen by *Azotobacter* and *Azospirillum.* A similar finding has been reported by Chaudhary *et al.* (2017) [5] in knolkhol, Zahraa *et al.* (2020) [20] in cauliflower

Total soluble solid (%)

In the case of pooled mean, the maximum TSS of curd was measured under treatment T_9 (7.83%), which was statistically superior as compared to all treatments but statistically *at par* with treatment T_8 (7.70%) and T_7

7.53%). However, the minimum TSS of curd was recorded under treatment T_1 (7.17%).

This enhancement in TSS content by bio-fertilizers may be due to higher mobility and availability of essential nutrients that might have hastened the breakdown of complex polysaccharides into simple sugars and destined their accumulation in developing curds. These findings are in resemblance Shivran *et al.* (2017) [16] in broccoli and Kaur *et al.* (2020) [11] in cauliflower

Sugar content in curd (%)

Non-significant differences were exhibited among different treatments with respect to the total sugar, reducing sugar and non reducing sugar in curd. Pooled analysis of the data showed maximum total sugar, reducing sugar and non reducing sugar in the curd was recorded with treatment T_9 (2.35%, 2.10 %, and 0.24 %, respectively) followed by treatment T_8 (2.33%, 2.09 % and 0.24 %, respectively), while minimum total sugar, reducing sugar and non reducing sugar in the curd was measured under control treatment T_1 (2.17%, 1.96 % and 0.22 %, respectively).

β- Carotene content (µg/100 g)

The β- carotene content (µg/100 g) in curd was significantly influenced by the different treatments. Pooled analysis of the data showed maximum β- carotene content (µg/100 g) in curd was recorded in treatment T_9 (13.02 µg), which was statistically *at par* with treatment T_8 (12.84 µg), T_7 (12.70 µg) and T_6 (12.60 µg), where as it was found significantly differ with rest of the other treatment. However, the β-carotene content (µg/100 g) in curd was observed lowest in treatment T_1 (12.04 µg) and this was statistically inferior to all other treatments.

Vitamin A (µg/100 g) in curd

The statistical data revealed that the significant difference exhibited among the treatments with respect to the vitamin A content (µg/100 g) in curd. In case of pooled data mean, the maximum vitamin A content (µg/100 g) in curd was recorded under treatment T_9 (21.70 µg), which was statistically *at par* with treatment T_8 (21.40 µg), T_7 (21.16 µg) and T_6 (21.00 µg), where as it was found significantly differ with rest of the other treatment. However, the vitamin A content (µg/100 g) in curd was observed lowest in treatment T_1 (20.06 µg) and this was statistically inferior.

Talat *et al.* (2014**)** [19] reported that use of bio-fertilizers increases vitamin A content in cole crop, which may be attributed to better mobilization and supply of available phosphorus and nitrogen; as it is associated with several vital functions, such as the proper distribution of sugar and starch and its consumption, better photosynthesis activity and root growth is achieved. A similar finding was reported by Kachari and Korla (2012) [9] in cauliflower. The higher availability and uptake of different essential micro-nutrients may be one of the reasons for improved vitamin A content in sprouting broccoli (Kumar *et al*., 2017) [12].

Vitamin C content (mg/100 g) in curd

The statistical data revealed that the application of nitrogen fixing bacterial inoculants and cow-urine was significantly increased the vitamin C content (mg/100 g) in curd. Pooled analysis of the data showed maximum vitamin C content (mg/100 g) in curd was recorded under treatment T_9 (60.02 mg), which was statistically superior as compared to all treatments but statistically *at par* with treatment T_8 (59.53 mg), T_7 (59.12 mg), T_6 (58.78 mg) and T_5 (58.38 mg). However, the minimum vitamin C content (mg/100 g) in curd was recorded under treatment T_1 (54.55 mg).

More vitamin C content in this treatment combination might be due to the biosynthesis of auxin and gibberellins by bacteria. Further, the gibberellins could either augment the biosynthesis of ascorbic acid or block oxidation of synthesized ascorbic acid content. A similar finding has been reported by Sindhu *et al.* (2024) [17] in sprouting broccoli, Kachari and Korla 2012 [9] and Shivran *et al.* (2017) [16] in cauliflower.

Table 1(a): Effect of nitrogen fixing bacterial inoculants and cow-urine on quality parameters of cauliflower

Treatments		Chlorophyll content in leaves (SPAD Value)			Nitrogen content in leaves (%)			pH			TSS (%)			Total sugar (%)		
		2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled
T_1	Control	54.81	52.57	53.69	2.10	2.07	2.08	6.27	6.29	6.28	7.20	7.13	7.17	2.19	2.16	2.17
T_2	FS of *Azotobacter* and *Azospirillum*	57.19	55.73	56.46	2.12	2.10	2.11	6.38	6.34	6.36	7.23	7.20	7.22	2.23	2.21	2.22
T_3	FS of foliar *Azotobacter* and foliar *Azospirillum*	58.81	58.03	58.42	2.13	2.11	2.12	6.43	6.38	6.40	7.27	7.27	7.27	2.24	2.22	2.23
T_4	FS of 10% Cow-urine	56.60	54.33	55.47	2.15	2.13	2.14	6.33	6.31	6.32	7.30	7.33	7.32	2.21	2.19	2.20
T_5	FS of 10% Cow-urine + 0.1 % Molasses	59.16	58.41	58.78	2.16	2.14	2.15	6.47	6.41	6.44	7.40	7.33	7.37	2.27	2.26	2.27
T_6 ($T_{2+}T_4$)	FS of *Azotobacter* and *Azospirillum* + FS of 10% Cow-urine	59.83	58.67	59.25	2.16	2.15	2.15	6.50	6.48	6.49	7.47	7.43	7.45	2.28	2.29	2.28
T_7 ($T_{3+}T_4$)	FS of foliar *Azotobacter* and foliar *Azospirillum* + FS of 10% Cow-urine	60.00	59.33	59.66	2.19	2.17	2.18	6.52	6.51	6.51	7.60	7.47	7.53	2.31	2.30	2.31
T_8 ($T_{2+}T_5$)	FS of *Azotobacter* and *Azospirillum* + FS of 10% Cow-urine + 0.1 % Molasses	60.37	59.41	59.89	2.21	2.20	2.21	6.56	6.55	6.55	7.73	7.67	7.70	2.34	2.32	2.33
T_9 ($T_{3+}T_5$)	FS of foliar *Azotobacter* and foliar *Azospirillum* + FS of 10% Cow-urine + 0.1 % Molasses	60.83	59.70	60.26	2.23	2.22	2.23	6.58	6.56	6.57	7.87	7.80	7.83	2.35	2.34	2.35
S.Em (±)		1.11	1.17	1.11	0.02	0.02	0.02	0.10	0.11	0.10	0.12	0.12	0.12	0.03	0.03	0.03
CD (5%)		3.20	3.48	3.20	0.07	0.07	0.07	NS	NS	NS	0.36	0.38	0.36	NS	NS	NS

Table 1(b): Effect of nitrogen fixing bacterial inoculants and cow-urine on quality parameters of cauliflower

Treatments		Reducing sugar (%)			Non reducing sugar (%)			β- Carotene content in curd (µg/100 g)			Vitamin A (µg/100 g) in curd			Vitamin C content (mg/100 g) in curd		
		2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled
T_1	Control	1.97	1.95	1.96	0.22	0.21	0.22	12.07	12.01	12.04	20.11	20.02	20.06	54.92	54.18	54.55
T_2	FS of *Azotobacter* and *Azospirillum*	1.99	1.97	1.98	0.24	0.23	0.24	12.30	12.28	12.29	20.49	20.46	20.48	57.67	55.90	56.78
T_3	FS of foliar *Azotobacter* and foliar *Azospirillum*	2.02	2.00	2.01	0.22	0.21	0.22	12.41	12.38	12.39	20.68	20.63	20.65	58.17	56.83	57.50
T_4	FS of 10% Cow-urine	1.99	1.97	1.98	0.22	0.22	0.22	12.17	12.20	12.18	20.28	20.33	20.31	56.33	55.33	55.83
T_5	FS of 10% Cow-urine + 0.1 % Molasses	2.03	2.05	2.04	0.23	0.22	0.22	12.52	12.44	12.48	20.87	20.74	20.80	59.00	57.75	58.38
T_6 $(T_{2+}T_4)$	FS of *Azotobacter* and *Azospirillum* + FS of 10% Cow-urine	2.04	2.07	2.05	0.24	0.22	0.23	12.62	12.59	12.60	21.03	20.98	21.00	59.23	58.33	58.78
T_7 $(T_{3+}T_4)$	FS of foliar *Azotobacter* and foliar *Azospirillum* + FS of 10% Cow-urine	2.06	2.07	2.06	0.25	0.24	0.25	12.71	12.69	12.70	21.18	21.14	21.16	59.40	58.83	59.12
T_8 $(T_{2+}T_5)$	FS of *Azotobacter* and *Azospirillum* + FS of 10% Cow-urine + 0.1 % Molasses	2.08	2.09	2.09	0.25	0.23	0.24	12.86	12.82	12.84	21.43	21.36	21.40	59.73	59.33	59.53
T_9 $(T_{3+}T_5)$	FS of foliar *Azotobacter* and foliar *Azospirillum* + FS of 10% Cow-urine + 0.1 % Molasses	2.10	2.10	2.10	0.26	0.24	0.24	13.07	12.97	13.02	21.78	21.61	21.70	60.07	59.97	60.02
S.Em (±)		0.03	0.03	0.03	0.01	0.008	0.009	0.17	0.16	0.16	0.29	0.26	0.27	0.82	0.82	0.82
CD (5%)		NS	NS	NS	NS	NS	NS	0.51	0.47	0.48	0.86	0.79	0.80	2.46	2.45	2.37

Table 1(c): Effect of nitrogen fixing bacterial inoculants and cow-urine on quality parameters of cauliflower

Treatments		Protein content (%) in curd			Nitrogen content (%) in curd			Phosphorus content (%) in curd			Potassium content (%) in curd		
		2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled
T_1	Control	13.98	13.92	13.95	2.24	2.23	2.23	0.21	0.20	0.21	3.09	3.05	3.07
T_2	FS of *Azotobacter* and *Azospirillum*	14.06	13.98	14.02	2.25	2.24	2.24	0.22	0.21	0.22	3.19	3.17	3.18
T_3	FS of foliar *Azotobacter* and foliar *Azospirillum*	14.27	14.33	14.30	2.28	2.29	2.29	0.22	0.23	0.23	3.21	3.20	3.20
T_4	FS of 10% Cow-urine	14.10	14.04	14.07	2.26	2.25	2.25	0.23	0.24	0.23	3.24	3.23	3.24
T_5	FS of 10% Cow-urine + 0.1 % Molasses	14.67	14.58	14.63	2.35	2.33	2.34	0.24	0.24	0.24	3.27	3.25	3.26
T_6 $(T_{2+}T_4)$	FS of *Azotobacter* and *Azospirillum* + FS of 10% Cow-urine	14.92	14.75	14.83	2.39	2.36	2.37	0.25	0.25	0.25	3.29	3.28	3.28
T_7 $(T_{3+}T_4)$	FS of foliar *Azotobacter* and foliar *Azospirillum* + FS of 10% Cow-urine	15.08	15.00	15.04	2.41	2.40	2.41	0.25	0.25	0.25	3.30	3.31	3.31
T_8 $(T_{2+}T_5)$	FS of *Azotobacter* and *Azospirillum* + FS of 10% Cow-urine + 0.1 % Molasses	15.29	15.21	15.25	2.45	2.43	2.44	0.26	0.25	0.25	3.34	3.33	3.34
T_9 $(T_{3+}T_5)$	FS of foliar *Azotobacter* and foliar *Azospirillum* + FS of 10% Cow-urine + 0.1 % Molasses	15.35	15.33	15.34	2.46	2.45	2.46	0.26	0.26	0.26	3.39	3.37	3.38
S.Em (±)		0.33	0.31	0.32	0.05	0.05	0.05	0.006	0.006	0.006	0.05	0.06	0.05
CD (5%)		0.98	0.92	0.92	0.14	0.14	0.14	0.01	0.01	0.01	0.15	0.18	0.16

Protein content (%)

Significant differences were exhibited among different treatments with respect to the protein content in curd. In the case of pooled mean, the maximum protein content in curd was recorded under treatment T_9 (15.34 %), which was statistically superior as compared to all treatments but statistically *at par* with treatment T_8 (15.25 %), T_7 (15.04 %), T_6 (14.83 %) and T_5 (14.63 %). However, the minimum protein content in curd was recorded under treatment T_1 (13.95 %).

Higher protein is due to the improved nutritional environment in the phyllosphere as well as faster utilization of nutrients by the plant system. A similar finding has been reported by Sable and Bhamare (2007) [15] in cauliflower.

NPK content (%) in curd

The statistical data revealed that the application of nitrogen fixing bacterial inoculants and cow-urine was significantly increased the nitrogen, phosphorus and potassium content in curd. In the case of pooled mean, the maximum nitrogen, phosphorus and potassium content in curd was recorded under treatment T_9 (2.46 %, 0.26 % and 3.38 %, respectively) followed by treatment T_8 (2.44 %, 0.25 % and 3.34 %, respectively). However, the minimum nitrogen, phosphorus and potassium content in curd were recorded under treatment T_1 (2.23 %, 0.21 % and 3.07 %, respectively).

Conclusion

Based on the present study it can be concluded that the foliar application of foliar *Azotobacter* and foliar *Azospirillum* + FS of 10% Cow-urine + 0.1 % Molasses showed superior performance over other treatments recording significantly higher values for all the quality parameters such as chlorophyll content in leaves, nitrogen content (%) in leaves, total soluble solids (%), nitrogen content (%) , phosphorus content (%) and potassium content (%) in curd, protein content (%) in curd, vitamin C content (mg/100 g) in curd, β- carotene content (µg/100 g) and vitamin A (µg/100 g) in curd.

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