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Impact of Plant–Microbe Interactions on The Yield, Quality, and Physiological Parameters of Oat (*Avena Sativa* L.) Fodder

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ABSTRACT

Oat (*Avena sativa* L.) is an important cool-season fodder crop, and improving its productivity and nutritional quality is essential for sustainable livestock production. This study evaluated the effects of two plant growth-promoting rhizobacteria (PGPR) strains, *Enterobacter cloacae* and *Lysinibacillus macroides*, and foliar-applied tryptamine, individually and in combination, on the agronomic, forage quality, physiological, and biochemical attributes of Faisalabad Oat 2021. The field experiment was conducted during 2024–2025 at the Fodder Research Sub-station, Ayub Agricultural Research Institute, Faisalabad, Pakistan. Eight treatments comprised an untreated control, individual bacterial inoculations, foliar application of tryptamine at 10^{-5} M, combined bacterial inoculation, and combinations of tryptamine with either or both bacterial strains. Among the treatments, T8 (PGPR1 + PGPR2 + tryptamine) recorded the highest green fodder yield (74.08 t ha^{-1}), dry matter yield (12.10 t ha^{-1}), plant height (138.10 cm), tiller density (755.21 tillers m^{-2}), SPAD value (44.24), chlorophyll content (1.72 mg g^{-1}), crude protein concentration (8.43%), soluble protein content (9.91 mg g^{-1}), and catalase activity (25.36). T6 recorded the lowest neutral detergent fiber concentration (58.50%). Compared with the untreated control, T8 increased green fodder yield by 10.37%, dry matter yield by 12.04%, crude protein concentration by 16.76%, and catalase activity by 35.40%. These findings indicated that the combined application of *E. cloacae*, *L. macroides*, and tryptamine showed promise for improving oat fodder productivity and several forage quality and biochemical attributes under the tested field conditions. Further evaluation across seasons and locations is recommended to validate these responses.

Introduction

Oat (*Avena sativa* L.) is an important cool-season cereal crop widely cultivated for green fodder, hay, silage, and grain production. It plays a vital role in livestock-based farming systems by providing palatable, digestible, and nutritionally valuable forage during the winter season, when the availability of other green fodder crops is often limited. Oat forage supplies dry matter, crude protein, and essential nutrients required for livestock growth, milk production, and maintenance. In addition to its fodder value, oats contribute to soil conservation, nutrient cycling, and sustainable crop rotations. Their relatively rapid growth, adaptability to different agroclimatic conditions, and potential for substantial biomass production make oats particularly valuable in regions where livestock productivity depends on a reliable supply of quality fodder. In Pakistan, improving oat productivity and nutritional quality is therefore important for addressing seasonal fodder shortages and supporting sustainable livestock production. However, achieving high fodder yields without compromising nutritional quality requires suitable cultivars and effective crop-management strategies (Buerstmayr *et al*., 2007; Suttie & Reynolds, 2004; Kour *et al*., 2024).

Plant growth-promoting rhizobacteria (PGPR) have attracted considerable attention as biological tools for improving crop growth, nutrient acquisition, and productivity while supporting sustainable agricultural practices. Depending on the bacterial strain, PGPR may promote plant development through biological nitrogen fixation, phosphate solubilization, siderophore production, phytohormone modulation, and improved root growth (Kelbessa *et al*., 2023). These mechanisms can improve nutrient availability and plant physiological functioning, although their effectiveness depends on microbial identity, soil conditions, crop genotype, and environmental interactions. Consequently, different bacterial strains may produce distinct effects on plant growth and development, making the evaluation of individual strains and their combined application important for developing crop-specific management strategies. Recent reviews have emphasized the potential of PGPR-based approaches to support sustainable agricultural production under changing climatic conditions, while also highlighting the need for field-based validation (Kelbessa *et al*., 2023).

In addition to microbial inoculation, the application of specific growth-regulating compounds may influence plant development and physiological processes. Tryptamine is an indole-related compound associated with auxin biosynthesis and metabolism and may influence plant development through interactions with auxin-related pathways. Its effects can depend on concentration, application method, plant species, and environmental conditions. Foliar application offers a practical approach for investigating its potential effects on crop growth and physiological performance. Moreover, combining tryptamine application with PGPR inoculation may produce complementary responses through the interaction of microbial effects on nutrient availability and plant development with the potential growth-regulatory effects of tryptamine. However, the outcomes of such combinations cannot be assumed to be additive or beneficial in all circumstances and therefore require systematic evaluation under field conditions.

Although PGPR have been investigated in different crops, their effectiveness cannot be generalized across species, genotypes, bacterial strains, and production environments. Similarly, the effects of tryptamine application may vary with the crop and application conditions. Comparative assessment of individual PGPR strains, their combined inoculation, foliar tryptamine application, and the integration of tryptamine with individual or combined bacterial strains is therefore important for determining their potential contribution to fodder production. In oat, such an assessment should consider not only green fodder yield (GFY) and dry matter (DM) production but also plant height (PH), tiller density, forage quality, chlorophyll status, soluble protein content, and antioxidant activity. Evaluating these traits together can provide a more comprehensive understanding of treatment responses than yield measurements alone.

Climate change has increased the challenges associated with maintaining agricultural productivity through changes in temperature, rainfall patterns, atmospheric carbon dioxide concentration, and the frequency of extreme weather events. These changes can alter crop growth, nutrient acquisition, soil microbial activity, and biomass production, thereby increasing the need for sustainable crop-management strategies (IPCC, 2022; Kelbessa *et al*., 2023). Forage crops are particularly important in mixed crop–livestock production systems because their productivity and nutritional quality directly influence livestock feeding, milk production, and farm profitability. In Pakistan, where livestock production contributes substantially to agricultural livelihoods, the availability of high-quality fodder remains an important production constraint. Oat is cultivated in irrigated and rainfed environments and provides a valuable source of winter and spring forage (Food and Agriculture Organization of the United Nations [FAO], 2011). Nevertheless, its productivity and nutritional quality depend on genotype, nutrient availability, environmental conditions, and crop management. Green fodder yield alone does not adequately describe forage value; DM accumulation, crude protein (CP), neutral detergent fiber (NDF), and physiological and biochemical characteristics must also be considered. Improved agronomic practices and nutrient management have been reported to enhance oat productivity, forage quality, and nutrient-use efficiency (Kour *et al*., 2024). Research examining oat responses to elevated temperature and carbon dioxide has also highlighted the importance of understanding crop performance under future

climatic conditions (Tomar *et al*., 2024). Although the present study did not directly evaluate climate stress, identifying effective PGPR-based and tryptamine application strategies may contribute to the development of improved fodder-production practices.

The present study evaluated two PGPR strains, *Enterobacter cloacae* and *Lysinibacillus macroides*, applied individually and in combination, together with foliar tryptamine at (10^{-5}) M. Eight treatments were compared, including an untreated control, each bacterial strain individually, tryptamine alone, combined inoculation of both bacterial strains, and combinations of tryptamine with either individual strain or both strains. Conducted under field conditions with Faisalabad Oat 2021 at Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan, the study provided a comparative assessment of these treatments across agronomic, forage-quality, physiological, and biochemical traits. The objectives were to (i) evaluate the effects of individual and combined applications of the two PGPR strains and tryptamine on oat growth and fodder productivity, and (ii) assess treatment-associated changes in forage quality, physiological attributes, and biochemical responses. The findings were intended to identify promising treatment approaches for improving oat fodder production and quality under the agroclimatic conditions of Faisalabad.

Materials and Methods

The field experiment was conducted during the Rabi season of 2024–2025 at the Fodder Research Sub-station, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. The oat (*Avena sativa* L.) cultivar Faisalabad Oat 2021 was evaluated under eight treatments (Table 1). The experiment was established during the recommended autumn sowing period for fodder oats under the agroclimatic conditions of Punjab, Pakistan (Food and Agriculture Organization of the United Nations [FAO], 2011).

Table No. 1: Treatment details for evaluating fodder yield, forage quality, and biochemical traits of oat (*Avena sativa L.*).

Sr. No	Treatment	Designation
1	Control	T1
2	PGPR1 (*Enterobacter cloacae*)	T2
3	PGPR2 (*Lysinibacillus macroides*)	T3
4	Tryptamine @ 10^{-5} M (Foliar spray)	T4
5	PGPR1+ PGPR2	T5
6	PGPR-1 + Tryptamine @ 10^{-5} M (Foliar spray)	T6
7	PGPR-2 + Tryptamine @ 10^{-5} M (Foliar spray)	T7
8	PGPR-1+ PGPR-2 + Tryptamine @ 10^{-5} M (Foliar spray)	T8

Data Recording

Green fodder yield (GFY), dry matter (DM), plant height (PH), and tiller density were recorded to assess crop growth and productivity. Relative leaf greenness was assessed using a portable SPAD chlorophyll meter, which provided a nondestructive estimate of leaf chlorophyll status (Markwell *et al*., 1995; Peltonen *et al*., 1995). Total chlorophyll content was determined spectrophotometrically following pigment extraction, using the procedure described by Arnon (1949). Catalase (CAT) activity was determined by monitoring the decomposition of hydrogen peroxide spectrophotometrically, following Aebi (1984). Total soluble protein (SOL) was quantified using a colorimetric protein assay, following Bradford (1976). The measured physiological and biochemical parameters were used to evaluate treatment-associated variation in leaf pigment status, protein accumulation, and antioxidant enzyme activity.

Statistical Analysis

The experimental data were analyzed by analysis of variance (ANOVA) using Statistix version 8.1, and treatment means were evaluated using an appropriate mean-comparison procedure. Graphs were prepared in Microsoft Excel and arranged for presentation in Microsoft PowerPoint.

Results and Discussion

Analysis of Variance (ANOVA)

The analysis of variance (ANOVA) revealed significant treatment effects for most measured traits of oat (*Avena sativa* L.). Green fodder yield (GFY; MSS = 11.7809), plant height (PH; 15.18), and tiller density (2,842.74) showed significant variation, with PH and tiller density marked as highly significant. These results indicated that individual and combined applications of *Enterobacter cloacae*, *Lysinibacillus macroides*, and tryptamine influenced important growth and fodder-yield attributes. Similar variations

in oat growth and yield in response to crop management have been reported by Bhilare and Joshi (2008), Ahmad *et al.* (2013), and Chand *et al.* (2025).

Treatment effects were also significant for dry matter (DM; MSS = 2.87), crude protein (CP; 4.51), and neutral detergent fiber (NDF; 2.18), suggesting that the treatments affected both biomass accumulation and forage composition. These changes are important because forage quality depends on nutritional composition as well as total fodder yield. Previous studies likewise reported treatment-related variation in oat forage yield, protein concentration, and fiber composition (Kumari *et al*., 2014; Zakirullah *et al*., 2017).

Among the biochemical traits, catalase (CAT) activity (MSS = 7.98), chlorophyll content (0.02), and soluble protein (SOL; 0.89) showed significant or highly significant treatment effects according to the supplied ANOVA table. These responses suggested that the PGPR strains and tryptamine, applied individually or in combination, influenced biochemical attributes associated with plant physiological functioning. Fertilizer management has similarly been reported to affect chlorophyll-related traits and biomass production in oat (Zhang *et al*., 2024). However, SPAD (MSS = 5.04) was not marked as significant; therefore, no significant treatment effect should be inferred for this trait. Overall, the ANOVA indicated that the tested treatments affected most growth, forage-quality, and biochemical traits, although the magnitude and direction of individual treatment responses must be established from treatment means and appropriate mean-comparison tests.

Table No. 2: Analysis of variance (ANOVA) for fodder yield, forage quality, physiological, and biochemical traits of oat (*Avena sativa* L.) under different treatments.

Sr No	Parameters	MSS (Treatment)
1	Catalases	7.98*
2	Dry Matter	2.87*
3	Crude protein	4.51*
4	Plant Height	15.18**
5	Tillers/m²	2842.74**
6	Chlorophyll Contents	0.02*
7	Neutral Detergent Fiber	2.18*
8	Total soluble Protein	0.89**
9	SPAD	5.04
10	Green Fodder Yield	11.7809**

Correlation Analysis

The correlation analysis revealed strong relationships among growth, forage quality, physiological, and biochemical traits of oat (*Avena sativa* L.) under the evaluated treatments. Catalase (CAT) activity was strongly positively correlated with tiller number (NT; $r = 0.99$), SPAD ($r = 0.95$), chlorophyll content (CC; $r = 0.81$), and plant height (PH; $r = 0.71$). Similarly, CC was positively correlated with NT ($r = 0.99$), SPAD ($r = 0.95$), and PH ($r = 0.76$), while PH was strongly associated with NT ($r = 0.96$). These relationships suggested coordinated variation in vegetative growth and physiological attributes across the observations. Similar associations among growth, yield, and physiological traits have been reported in oats (Bukhari *et al*., 2009; Ahmad *et al*., 2013; Chand *et al*., 2025).

Forage quality traits also showed notable associations. Crude protein (CP) was positively correlated with dry matter (DM; $r = 0.67$) but negatively correlated with neutral detergent fiber (NDF; $r = -0.94$). NDF was also negatively associated with DM ($r = -0.55$), green fodder yield (GFY; $r = -0.69$), PH ($r = -0.61$), CC ($r = -0.70$), and SPAD ($r = -0.69$). These relationships indicated that higher CP concentration tended to coincide with lower NDF concentration in the observed data. Similar variation in oat forage yield, protein, and fiber composition has been reported under different management practices and among cultivars (Kumari *et al*., 2014; Zakirullah *et al*., 2017; *Optimal fertilizer rate and sowing density*, 2025).

Soluble protein (SOL) was positively correlated with CP ($r = 0.86$), GFY ($r = 0.82$), CC ($r = 0.78$), and CAT activity ($r = 0.76$), but negatively correlated with NDF ($r = -0.89$). DM showed a moderate positive correlation with CP ($r = 0.67$) and CC ($r = 0.51$), whereas its association with GFY was weak ($r = 0.2547$). These findings suggested that soluble protein, forage quality, and biomass-related traits varied together to different degrees across the observations. Related relationships between oat forage yield and quality have been discussed by Zhang *et al.* (2025) and Jia *et al.* (2026).

Overall, the correlation patterns highlighted associations among CAT, CC, SPAD, and tillering, as well as an inverse relationship between CP and NDF. These traits may therefore be useful for jointly assessing oat growth and forage quality under the tested

PGPR and tryptamine treatments. However, correlation coefficients indicate association rather than causation, and their interpretation should consider the number of independent observations and the statistical significance of the coefficients.

Table No. 3: Pearson's correlation coefficients among fodder yield, forage quality, physiological, and biochemical traits of oat under different treatments of PGRP and Bio Stimulants (*Avena sativa* L.)

	CAT	CP	CC	DM	GFY	PH	NDF	SPAD	SOL
CP	0.67**								
CHL	0.81*	0.68**							
DM	0.51**	0.67**	0.51*						
GFY	0.59*	0.67*	0.62*	0.2547					
PH	0.71*	0.58**	0.76*	0.44*	0.51				
NDF	-0.66	-0.94*	-0.70*	-0.55*	-0.69*	-0.61*			
SPAD	0.95*	0.7075	0.95**	0.60	0.53*	0.93	-0.69*		
SOL	0.76*	0.86*	0.78*	0.45*	0.82	0.69	-0.89	0.69	
NT	0.99*	0.67*	0.99*	0.53*	0.61*	0.96*	-0.68	0.95	0.76

CP= Crude Protein Contents, CHL= Chlorophyll contents, DM= Dry Matter, GFY= Green Fodder Yield, PH= Plant height, NDF= Neutral detergent fiber, SPAD= Soil Plant Analysis Development

Mean Data Presentation

The treatments differed in their effects on fodder yield and growth attributes of oat. T8 recorded the highest green fodder yield (74.08 t ha^{-1}), followed by T7 (72.81 t ha^{-1}), whereas T1 produced the lowest yield (67.12 t ha^{-1}). Compared with T1, T8 increased green fodder yield by 10.37%. T8 also produced the highest dry matter yield (12.10 t ha^{-1}), representing an increase of 12.04% over T1 (10.80 t ha^{-1}). Similarly, T8 recorded the maximum plant height (138.10 cm) and tiller density (755.21 tillers m^{-2}), compared with 131.10 cm and 658 tillers m^{-2}, respectively, in T1. These improvements indicated that T8 promoted vegetative growth and tillering, contributing to greater fresh fodder and dry matter production. The superior performance of T8 suggested that the combined application of PGPR1, PGPR2, and tryptamine was promising for improving oat productivity under the conditions of this experiment. Similar variations in oat fodder yield in response to variety, nitrogen management, and harvesting practices were reported by Bhilare and Josh (2008), Zakirullah *et al.* (2017), and Biswal *et al.* (2025) (Fig. 1).

T8 recorded the highest crude protein concentration (8.43%), followed by T6 (7.96%), whereas T1 had the lowest value (7.22%). The crude protein concentration under T8 was 16.76% higher than that under T1, indicating improved forage protein concentration. T8 also recorded the highest soluble protein concentration (9.91 mg g^{-1}), compared with 8.23 mg g^{-1} in T1, representing an increase of 20.41%. However, the response of neutral detergent fiber (NDF) differed from that of the other quality attributes. T6 recorded the lowest NDF concentration (58.50%), followed by T2 (59.84%), whereas T8 had the highest value (61.25%). Thus, although T8 showed superior crude and soluble protein concentrations, it did not improve all forage quality attributes simultaneously. Lower NDF may favor voluntary feed intake, although digestibility and animal performance were not directly evaluated in this study. These findings agreed with previous reports indicating that oat genotype, nitrogen supply, and harvesting stage influence forage protein concentration and cell-wall composition (Kumari *et al.*, 2014; Kir, 2021) (Fig. 1).

T8 exhibited the strongest performance across the measured physiological and biochemical attributes. It recorded the highest SPAD value (44.24), chlorophyll concentration (1.72 mg g^{-1}), soluble protein concentration (9.91 mg g^{-1}), and catalase activity (25.36). Compared with T1, the SPAD value increased by 10.52%, while chlorophyll concentration increased by 20.28%. Soluble protein concentration and catalase activity increased by 20.41% and 35.40%, respectively. The higher SPAD and chlorophyll values indicated greater leaf greenness and pigment accumulation, which may have supported photosynthetic performance and biomass production. Similarly, the higher soluble protein concentration suggested enhanced protein accumulation, while greater catalase activity indicated an increased capacity for hydrogen peroxide detoxification. Nevertheless, catalase activity alone cannot confirm reduced oxidative damage because reactive oxygen species and lipid peroxidation were not measured. Variation in chlorophyll and other physiological traits among oat genotypes has also been documented by Ahmad *et al.* (2013) (Fig. 1).

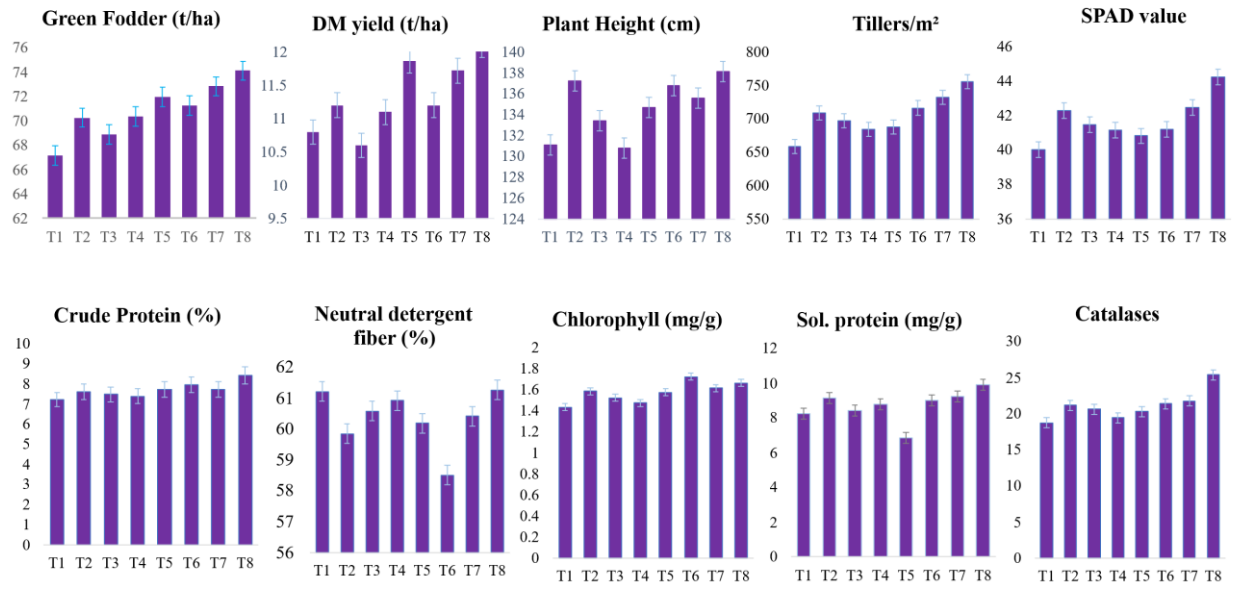


Figure No. 1: Effects of different treatments on fodder yield, forage quality, physiological, and biochemical traits of oat (*Avena sativa* L.).

Conclusion

The results indicated that the combined application of PGPR and tryptamine (T8) was the most promising treatment for improving green fodder yield (45.43 t ha^{-1}), crude protein content (8.43%), soluble protein content (9.97 mg g^{-1}), and catalase activity (24.00) in oat. The combined application of PGPR and tryptamine showed potential for improving oat fodder productivity, forage quality, and biochemical performance under the tested field conditions. Further evaluation across seasons and locations is recommended to validate these findings.

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