Impact of Organic and Inorganic Amendments on Peroxidase Activity, Lipid Peroxidation, and Antibacterial Potential of Trigonella foenum-graecum Grown in Polluted Soil

[1]Dr. Payal Jaiswal, [2]Prachi Srivastava, [3]Pratibha Sharma

1Assistant Professor, Institute of Sciences, SAGE University, Indore, Madhya Pradesh, India

2Assistant Professor, Institute of Sciences, SAGE University, Indore, Madhya Pradesh, India

3Assistant Professor, Institute of Sciences, SAGE University, Indore, Madhya Pradesh, India

1jpayal96@gmail.com

2srivastava.prachi529@gmail.com

3pratibharoma@gmail.com

Abstract

Soil pollution is a major environmental problem that negatively affects plant metabolism and biochemical functions. The present study aimed to evaluate the effect of different organic and inorganic soil amendments on the biochemical and antibacterial responses of Trigonella foenum-graecum (fenugreek) cultivated in polluted fields. Peroxidase (POD) activity, malondialdehyde (MDA) levels, and antibacterial activity were assessed to determine the physiological status of plants under various treatments—vermicompost, compost by IMC, N.P.K. fertilizer, and untreated control—across two polluted field sites (T_1 and T_2), compared with an organic control field.

Results revealed a significant decline in peroxidase activity across all treatments when compared with the control, with the lowest activity observed in plants grown without fertilizer. Vermicompost treatment showed the least reduction in peroxidase activity (0.54%– 2.43%), indicating its protective effect against oxidative stress. Conversely, MDA levels, an indicator of lipid peroxidation, increased significantly under N.P.K. and untreated conditions, suggesting higher oxidative damage. The antibacterial activity of methanolic extracts also decreased in all treatments compared to the positive control (Gentamicin), with vermicompost-treated plants exhibiting better retention of antimicrobial potential.

Overall, organic amendments such as vermicompost and compost significantly mitigated the adverse effects of soil pollution by enhancing antioxidant defense and preserving antibacterial activity. These findings highlight the importance of using organic fertilizers for sustainable cultivation of medicinally important plants in contaminated soils.

Keywords: Trigonella foenum-graecum; Peroxidase; Malondialdehyde (MDA); Antibacterial activity; Vermicompost; Compost; N.P.K.; Soil pollution; Oxidative stress; Sustainable agriculture.

1. Introduction:

Soil contamination by industrial discharges, heavy metals, and excessive use of synthetic fertilizers has emerged as a major global concern affecting soil fertility and plant productivity. Polluted soils not only impair plant growth but also induce oxidative stress, leading to the overproduction of reactive oxygen species (ROS). These reactive molecules can damage cellular structures, disturb membrane integrity, and alter metabolic processes in plants.

Plants have evolved a complex antioxidant defense mechanism to combat oxidative stress. Among these, peroxidase (POD) plays a vital role by catalyzing the reduction of hydrogen peroxide (H_2O_2) using various electron donors. Enhanced or suppressed peroxidase activity serves as a biochemical indicator of the plant’ s stress response capacity. Simultaneously, lipid peroxidation is a common outcome of oxidative stress that results in the formation of malondialdehyde (MDA), a by-product reflecting the extent of membrane damage and lipid degradation.

Trigonella foenum-graecum (fenugreek) is a leguminous plant widely used in traditional medicine due to its antioxidant, antimicrobial, and nutritional properties. However, when cultivated in polluted environments, its biochemical and pharmacological potential may be significantly altered. Therefore, evaluating the influence of different soil amendments on its physiological and bioactive responses is essential.

Organic fertilizers such as vermicompost and compost have gained attention for their ability to improve soil structure, nutrient content, and microbial activity while reducing the toxic effects of pollutants. In contrast, chemical fertilizers like N.P.K. may increase plant yield but often exacerbate oxidative stress under contaminated conditions.

The present study investigates the impact of organic (vermicompost and compost) and inorganic (N.P.K.) fertilizers, along with untreated soil, on peroxidase activity, malondialdehyde levels, and antibacterial potential of Trigonella foenum-graecum grown in polluted and organic fields. The objective is to understand the role of organic amendments in enhancing plant defense mechanisms and maintaining medicinal value in stressed environments.

2. Materials and Methods:

2.1. Plant Material and Experimental Design: Healthy seeds of Trigonella foenum-graecum (fenugreek) were sown in both organic and polluted fields. The polluted sites were designated as T_1 and T_2, representing two industrially affected locations. The experimental setup included four treatments in each polluted field and one organic control group as follows:

- Control (Organic field) – No pollution, organically maintained soil.
- Treatment 1 (Vermicompost) – Application of organic vermicompost to polluted soil.
- Treatment 2 (Compost by IMC) – Application of compost prepared by Indore Municipal Corporation (IMC).
- Treatment 3 (N.P.K.) – Application of chemical fertilizer (Nitrogen, Phosphorus, Potassium).
- Treatment 4 (Without Fertilizer) – Polluted soil without any additional fertilizer.

All treatments were carried out under uniform agronomic conditions. After maturation, plant samples were collected and used for biochemical and antimicrobial assays.

2.2. Estimation of Peroxidase Activity: Peroxidase (POD) activity was measured according to the method of Summer et al.

Principle: O-dianisidine was used as a hydrogen donor and H_2O_2 as an electron donor. The rate of formation of yellow-orange colored dianisidine dehydrogenation product was recorded spectrophotometrically at 430 nm, representing peroxidase activity.

Calculation:

$$\text{Peroxidase activity} = \text{O.D of test} \times \frac{1}{\text{Time in min.}} \times \frac{\text{Total volume of extract}}{\text{Vol. of extract use}} \times \frac{1}{\text{Wt. of sample in gm}}$$

2.3. Estimation of Lipid Peroxidation (MDA Content) Lipid peroxidation was estimated following the method of Heath and Packer (1968).

Principle: Malondialdehyde (MDA), the final product of peroxidized polyunsaturated fatty acids, reacts with Thio-barbituric acid (TBA) to produce a pink-colored chromogen measured at 532 nm.

Calculation:

Concentration of MDA was calculated by using the extinction coefficient of $155 mM^{-1} cm^{-1}$ MDA at 532nm using the formula-

A= 1c

Where, A= absorbance at specific wavelength.

= Extinction coefficient

1= length of cell (1cm)

c= concentration

2.4. Estimation of Antibacterial Activity: Antibacterial activity of methanolic leaf extracts of T. foenum-graecum was determined by the agar well diffusion method.

Principle: The inhibition of bacterial growth surrounding the well indicates the presence of antibacterial compounds. The zone of inhibition was measured in millimeters (mm) using a zone reader. Gentamicin served as a positive control.

2.5. Statistical Analysis: All experiments were conducted in triplicate and expressed as mean ± standard deviation (SD). Statistical significance was analyzed using Student' s t-test with $p < 0.05$ considered significant, and $p > 0.05$ as non-significant (NS).

3. Results and Discussion

3.1. Effect of Treatments on Peroxidase Activity: The peroxidase activity of T. foenum-graecum showed a noticeable decline under all polluted field treatments compared to the organic control (Table 5.14). The control plants exhibited a POD activity of 20.12 unit/min/mg, while polluted field (T_1) plants treated with vermicompost showed only a 0.54% decrease, which was statistically non-significant ($p \geq 0.05$). In contrast, significant reductions were recorded in compost (1.98%), N.P.K. (4.37%), and untreated (13.22%) samples.

A similar trend was observed in polluted field (T_2), where peroxidase activity decreased by 2.43–9.99%, with the lowest values in plants without fertilizer ($p < 0.05$).

These findings indicate that vermicompost effectively maintained peroxidase activity, suggesting enhanced oxidative stress tolerance. The organic amendments likely improved soil microbial activity and nutrient availability, leading to stronger enzymatic defense systems.

Table No.3.1: Effect of different treatment on Peroxidase activity of *Trigonella foenum-graecum*

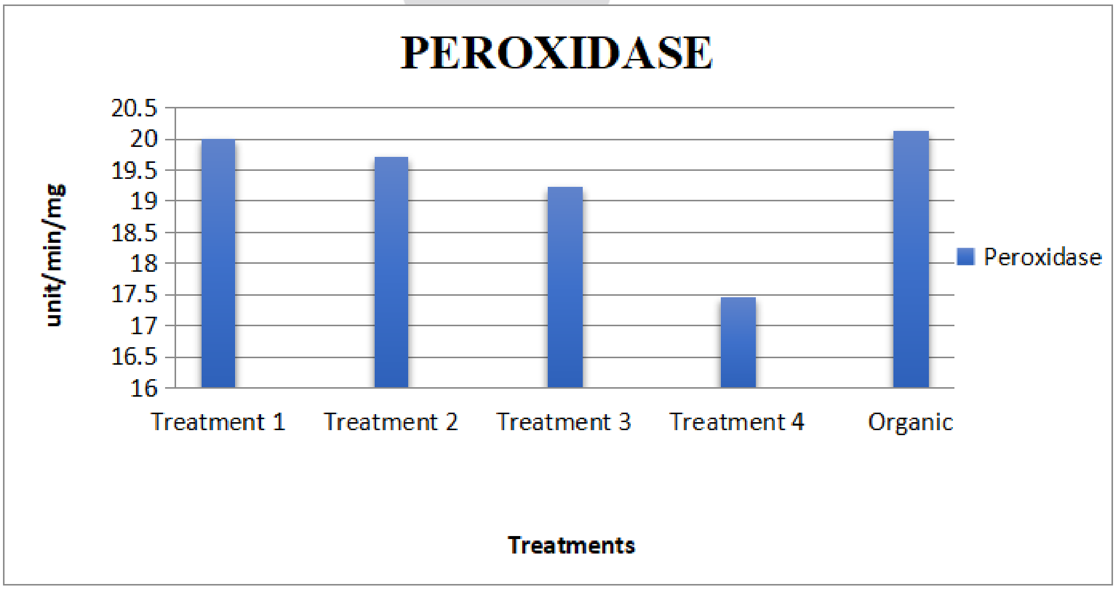
S.NO.	Different treatments	Peroxidase	% Change	p-value
Organic field (control)	Organic field	20.12±0.025	0	0
Polluted field (T1)	Vermicompost (Treatment 1)	20.01±0.16	0.54↓	0.31
	Compost by IMC (Treatment 2)	19.72±0.025	1.98↓	4.10 e-0.5
	N.P.K. (Treatment 3)	19.24±0.045	4.37↓	7.9 e-06
	Without Fertilizer (Treatment 4)	17.46±0.57	13.22↓	2.103 e-07
23Polluted field (T2)	Vermicompost (Treatment 1)	19.63±0.23	2.43↓	0.021
	Compost by IMC (Treatment 2)	19.59±0.11	2.78↓	0.001
	N.P.K. (Treatment 3)	19.13±0.11	4.92↓	0.0001
	Without Fertilizer (Treatment 4)	18.11±0.015	9.99↓	3.08 e-08

Values expressed are means ± standard deviation.

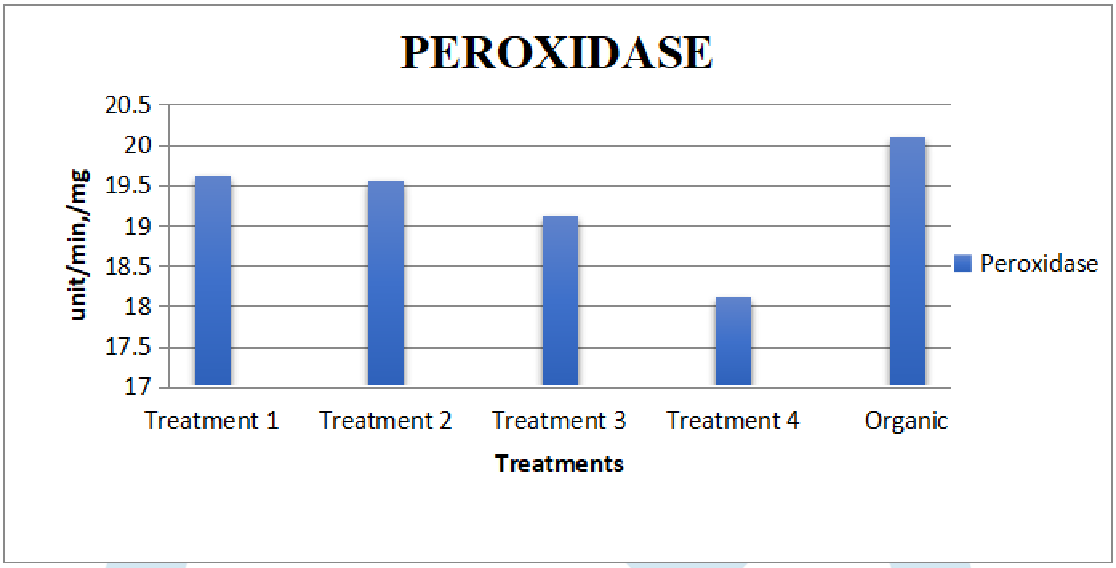
*Indicates $p<0.05$ and is significant.

^{NS} Indicates $p> 0.05$ and is not significant.

↑↓ Indicates percent increase and decrease as compared to control.



Graph No.3.1: Effect of different treatments on Peroxidase activity (unit/min/mg) of *Trigonella foenum-graecum* (polluted field T1)



Graph No.3.2: Effect of different treatments on Peroxidase activity (unit/min/mg) of *Trigonella foenum-graecum* (polluted field T2)

3.2. Effect of Treatments on Lipid Peroxidation (MDA Levels): MDA levels, indicative of lipid peroxidation and membrane damage, varied significantly among treatments (Table 5.15). The control group recorded an MDA level of 0.42 mM/g, while polluted field (T_1) plants treated with vermicompost showed a 2.38% decrease ($p \geq 0.05$), implying reduced oxidative damage. Conversely, treatments with compost (21.42%), N.P.K. (42.85%), and no fertilizer (33.33%) exhibited significant increases in MDA levels ($p < 0.05$).

In polluted field (T_2), MDA levels increased across all treatments, most prominently in the N.P.K. group (42.8%), reflecting higher lipid peroxidation and oxidative injury.

Thus, vermicompost and compost treatments mitigated lipid peroxidation, possibly by improving antioxidant enzyme efficiency and maintaining cellular integrity, while N.P.K. exacerbated stress-induced lipid damage.

Table No.3.2: Effect of different treatment on MDA level of *Trigonella foenum-graecum*

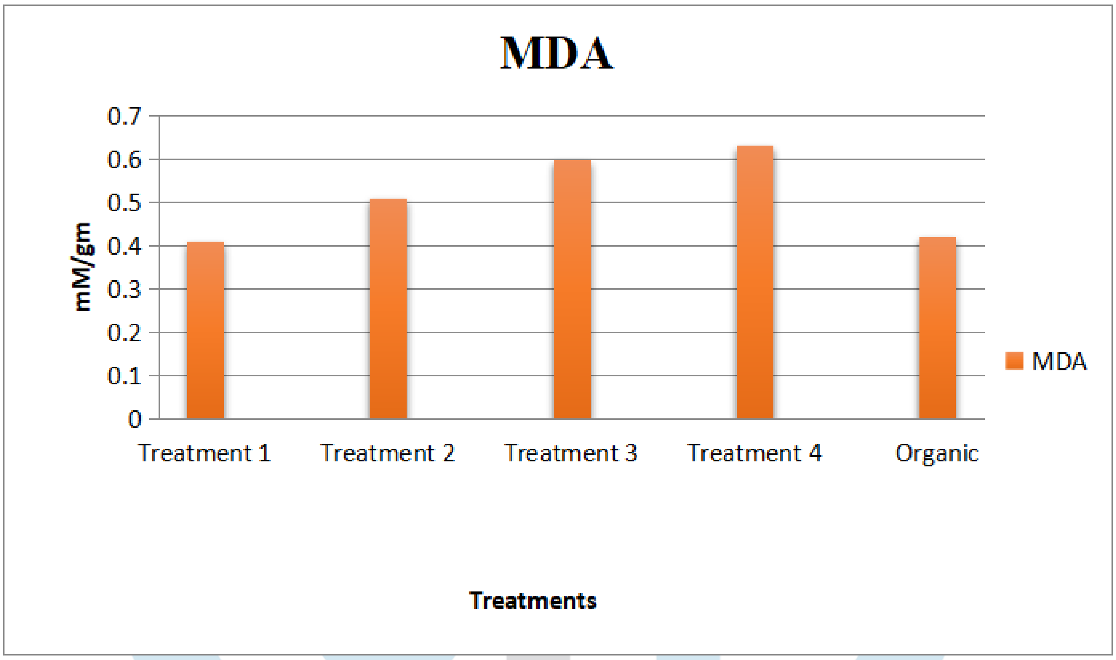
S.NO.	Different treatments	MDA	% Change	p-value
Organic field (control)	Organic field	0.42±0.005	0	0
Polluted field (T1)	Vermicompost (Treatment 1)	0.41±0.015	2.38↓	0.51
	Compost by IMC (Treatment 2)	0.51±0.01	21.42↑	0.0002
	N.P.K. (Treatment 3)	0.60±0.015	42.85↑	1.62 e-05
	Without Fertilizer (Treatment 4)	0.63±0.04	33.33↑	0.0009
Polluted field (T2)	Vermicompost (Treatment 1)	0.46±0.17	9.5↑	0.025
	Compost by IMC (Treatment 2)	0.51±0.015	21.42↑	0.0005
	N.P.K. (Treatment 3)	0.58±0.005	38.09↑	4.16 e-06
	Without Fertilizer (Treatment 4)	0.60±0.005	42.8↑	2.6 e-06

Values expressed are means ± standard deviation.

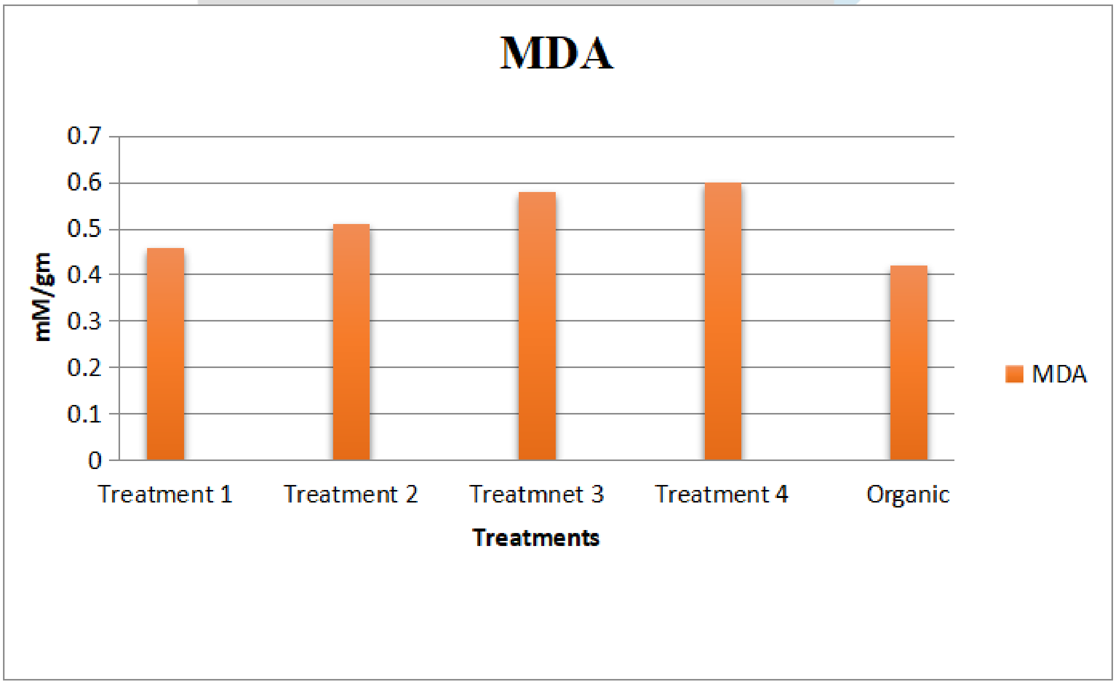
*Indicates p<0.05 and is significant.

NS Indicates p> 0.05 and is not significant.

↑↓ Indicates percent increase and decrease as compared to control.



Graph No.3.3: Effect of different treatments on MDA level (mM/gm) of *Trigonella foenum-graecum* (polluted field T1)



Graph No.3.4: Effect of different treatments on MDA level (mM/gm) of *Trigonella foenum-graecum* (polluted field T2)

3.3. Effect of Treatments on Antibacterial Activity: Antibacterial activity, determined by the zone of inhibition, also showed a decreasing pattern with soil pollution (Table 5.25). The positive control (Gentamicin) exhibited a 23 mm inhibition zone, while organic control plants showed 22 mm (4.34% decrease). Polluted field (T_1) plants with vermicompost displayed 19 mm (17.39% decrease), whereas compost, N.P.K., and unfertilized treatments recorded progressively lower activities (35.29%, 47.82%, and 52.17% decreases, respectively). Similar results were obtained for polluted field (T_2), with the lowest antibacterial activity (9 mm; 60.86% decrease) observed in untreated plants.

This demonstrates that organic fertilizers help preserve the antibacterial potential of T. foenum-graecum by maintaining its secondary metabolite profile even under stress conditions, whereas chemical fertilizer and polluted soil drastically reduce bioactivity.

Table No.3.3: Effect of different treatment on antibacterial activity of *Trigonella foenum-graecum*

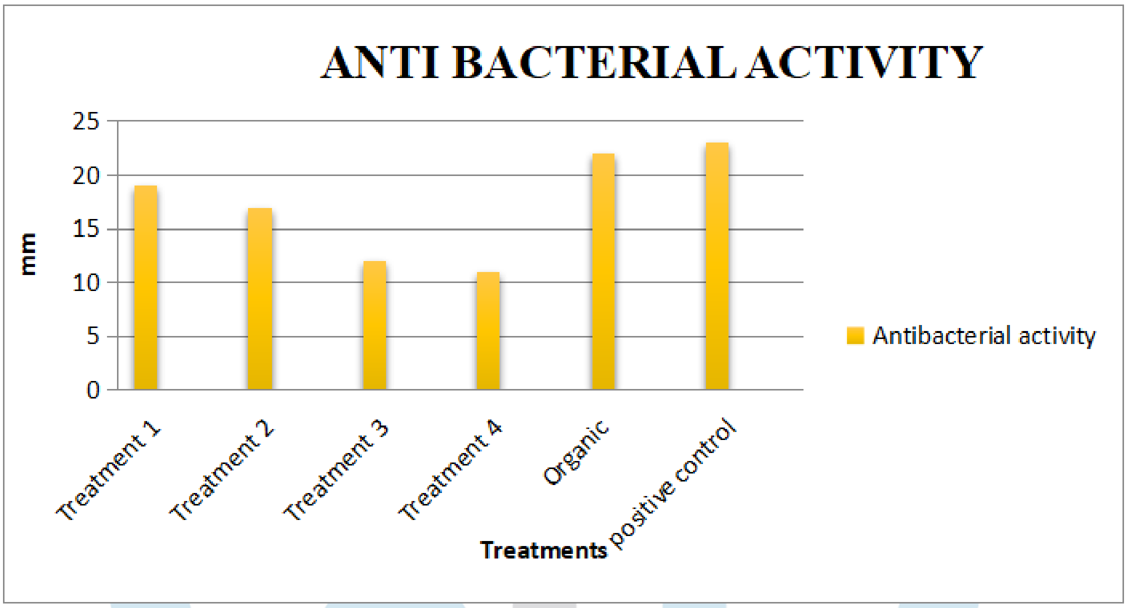
S.NO.	Different treatments	Antimicrobial activity (zone of inhibition in mm)	% Change	p-value
Positive control	Gentamicin	23±0.57	0	**0**
Organic field (control)	Organic field	22±0.5	4.34↓	0.00096
Polluted field (T1)	Vermicompost (Treatment 1)	19±0.5	17.39↓	0.00016
	Compost by IMC (Treatment 2)	17±0.2	35.29↓	0.007
	N.P.K. (Treatment 3)	12±0.57	47.82↓	0.001
	Without Fertilizer (Treatment 4)	11±0.5	52.17↓	0.0006
Polluted field (T2)	Vermicompost (Treatment 1)	20±0.28	13.04↓	0.007
	Compost by IMC (Treatment 2)	15±0.5	34.78↓	0.0001
	N.P.K. (Treatment 3)	10±0.28	56.52↓	0.0009
	Without Fertilizer (Treatment 4)	9±0.28	60.86↓	0.0009

Values expressed are means ± standard deviation.

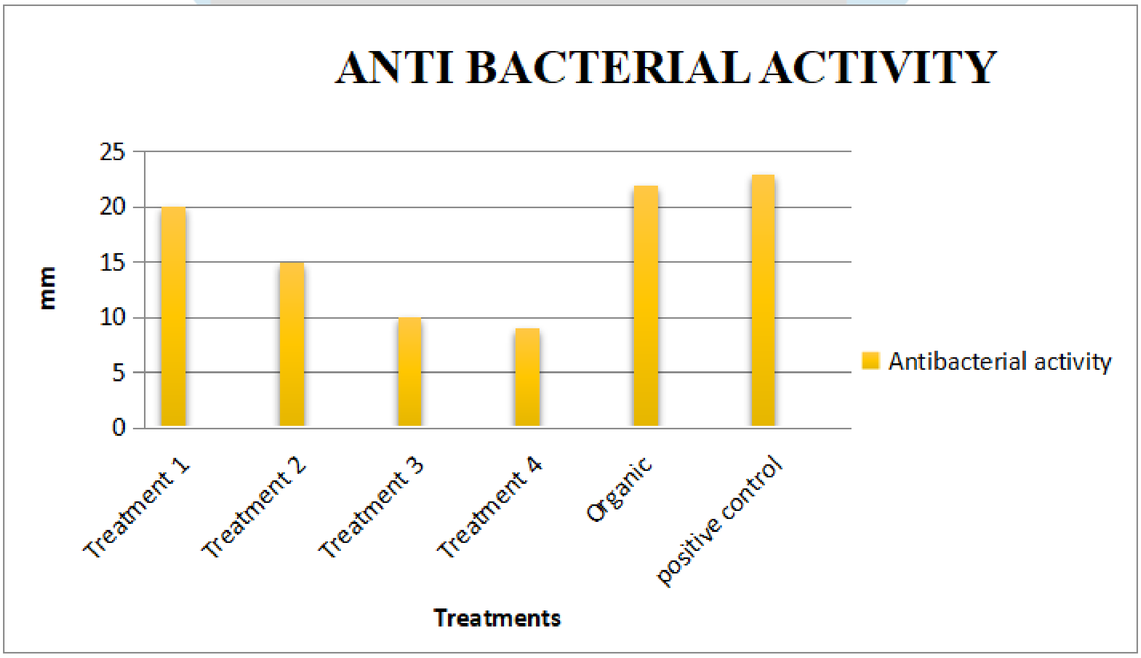
*Indicates p<0.05 and is significant.

[NS] Indicates p> 0.05 and is not significant.

↑↓ Indicates percent increase and decrease as compared to control.



Graph No.3.5: Effect of different treatments on antimicrobial activity (mm) of *Trigonella foenum-graecum* (polluted field T1)



Graph No.3.6: Effect of different treatments on antimicrobial activity (mm) of *Trigonella foenum-graecum* (polluted field T2)

The study clearly demonstrates that organic treatments—particularly vermicompost—ameliorate the negative effects of soil pollution on T. foenum-graecum. Enhanced peroxidase activity, reduced lipid peroxidation, and better antibacterial retention reflect improved physiological resilience and bioactive compound stability.

In contrast, N.P.K. fertilizer and untreated soils showed elevated oxidative stress markers and reduced bioactivity, emphasizing the ecological and biochemical advantages of organic soil management for medicinal plant cultivation in contaminated environments.

4.Conclusion: The present investigation demonstrates that soil pollution significantly influences the biochemical and antimicrobial properties of Trigonella foenum-graecum. Plants grown in polluted fields exhibited a decline in peroxidase activity, increased lipid peroxidation, and reduced antibacterial potential compared to those cultivated in organic soil. Among the various treatments, vermicompost proved most effective in mitigating oxidative stress, maintaining higher peroxidase activity, lowering MDA levels, and preserving antibacterial efficacy.

The enhanced performance of vermicompost- and compost-treated plants may be attributed to improved soil health, microbial activity, and nutrient balance, which collectively strengthen the plant' s defense system. In contrast, chemical fertilizers (N.P.K.) and untreated soils intensified oxidative stress, leading to biochemical imbalance and loss of therapeutic potential.

These findings underline the importance of using organic amendments for cultivating medicinal plants in polluted or stressed environments. Vermicompost and compost not only improve soil quality but also safeguard the biochemical integrity and pharmacological properties of T. foenum-graecum. Future studies should focus on identifying the specific bioactive compounds affected by pollution and exploring molecular mechanisms underlying antioxidant defense enhancement through organic management.

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