# STUDIES ON THE EFFECT OF FERTILIZERS AND HUMIC ACID ON SOIL MICROBIAL POPULATIONS OF FOXTAIL MILLET CROP

**Abstract:** A field experiment was conducted during the kharif and rabi seasons of 2020–21 and 2021–22 at the College Farm, Agricultural College, Mahanandi (ANGRAU), to assess the impact of humic acid and Recommended Dose of Fertilizers (RDF) on soil health. The experiment was laid out in Split plot design with three replications with four main plots and six sub plots total twenty four treatments *viz.,* Control (M1), 50 % RDF (M2) , 75 % RDF (M3) and 100% RDF (M4) as main plots and six humic acid levels to foxtail millet crop comprising of No Humic acid application (S1), 10 kg ha-1 Humic acid as soil application (S2) , 20 kg Humic acid as soil application (S3), 0.2% of foliar application of Humic acid (S4), 10 kg ha-1Humic acid as soil application + 0.2% foliar application of Humic acid (S5) and 20 kg ha-1Humic acid as soil application + 0.2% foliar application of Humic acid (S6) as sub- plot treatments to evaluate the effect of fertilizers and humic acid on soil biological properties of foxtail millet crop. The application of 100% RDF (M4) resulted in the highest microbial populations (bacteria, fungi, and actinomycetes), statistically similar to 75% RDF (M3) in all four growing seasons. Among the humic acid treatments, S6 (20 kg ha⁻¹ soil application + 0.2% foliar spray) led to significantly improved higher microbial populations, comparable to S3 (20 kg ha⁻¹ soil application only).However, no significant interaction was observed between RDF levels and humic acid treatments during either year. The results demonstrate that humic acid application, particularly when used both as a soil amendment and foliar spray, enhances soil fertility and microbial activity. Moreover, integrating 75% RDF with humic acid offers a promising strategy for reducing chemical fertilizer use while maintaining soil health and promoting sustainable agricultural practices.

**Key words:** Humic acid, In-Organic fertilizers- Soil microbial and enzyme activity of foxtail millet -Foxtail millet-Bengalgram cropping system.

**Introduction: “**Humic substances (H.S) are major components of soil organic matter and are widely recognized as natural and effective growth promoters in sustainable agriculture. Formed through the decomposition of organic matter, HS are increasingly used as soil amendments to enhance soil structure, nutrient availability, and microbial activity. Recent research using integrated approaches has aimed to uncover the relationship between the chemical structure of humic substances and their biological effects on plants. Findings suggest that specific functional groups within HS can initiate local and systemic physiological responses in plants, potentially mediated by complex, hormone-like signaling pathways” (Nardi *et al.,* 2021). “Although soil microbes constitute only a small fraction of the total mass of soil organic matter, they play a pivotal role in essential soil processes such as organic matter decomposition, nutrient cycling, and overall soil health. Microbial activity is a key driver of ecosystem functions, with estimates indicating that 80–90% of soil processes are mediated by microorganisms” (Nannipieri & Badalucco, 2003). “Enzyme activities in soil, such as urease and dehydrogenase, serve as sensitive indicators of microbial function and community dynamics. These enzymes reflect changes in biochemical processes and the dynamics of soil organic matter in response to both natural and human-induced environmental factors” (Trasar-Cepeda *et al.,* 2008).

Understanding the factors that influence soil microbial populations—including their size, diversity, and activity—is essential, given their role in regulating key processes such as nutrient cycling and organic matter breakdown (Masto *et al.,* 2006). Soils with high microbial diversity typically exhibit healthier soil-plant interactions and are more resilient to environmental changes, while soils with reduced microbial diversity often show impaired ecosystem function. Most of the current research focuses on the physicochemical state of humic acid to improve the soil quality while few studies have been conducted to investigate the infuence of humic acid on soil microbes. According to Yan Li *et al.* (2019), soil microbes are central to nutrient transformation, humus formation, and organic matter breakdown. Introducing humic acid into the soil modifies microbial behavior in multiple ways as soil bacteria can utilize humic acid as a carbon source, promoting growth even at low concentrations,  Humic acid often acts as a microbial stimulant—in one study, it increased growth in 41% of tested bacterial strains and it selectively enhances beneficial microbial taxa, such as *Pseudomonas* spp., which aid in phosphorus solubilization and plant hormone production . In long-term field experiments humic acid shifted microbial composition towards more fungal diversity (Basidiomycota, Mortierellomycota) and away from pathogenic Ascomycota, alongside boosting soil enzyme activity.

**Material and Methods:** A field experiment was conducted at the college farm, Agricultural College, Mahanandi, ANGRAU during *kharif & rabi* seasons of 2020-21 and 2021-22. The experimental site was geographically situated at 15.510 N latitude, 78.610 E longitude with an altitude of 233.48 meters above the mean sea level in Scarce Rainfall Zone of Andhra Pradesh. The experimental soil was sandy loam in texture with 7.52 pH, 0.42 dsm-1 EC, 0.32 % OC, low available N (175 kg ha- 1), medium in P (18.48 kg ha-1), high in K (580 kg ha-1) and sufficient in Zn status (0.85 ppm). The experiment was laid out in Split plot design with three replications with four main plots and six sub plots total twenty four treatments *viz.,* Control (M1), 50 % RDF (M2) , 75 % RDF (M3) and 100% RDF (M4) as main plots and six humic acid levels to foxtail millet crop comprising of No Humic acid application (S1), 10 kg ha-1 Humic acid as soil application (S2) , 20 kg Humic acid as soil application (S3), 0.2% of foliar application of Humic acid (S4), 10 kg ha-1Humic acid as soil application + 0.2% foliar application of Humic acid (S5) and 20 kg ha-1Humic acid as soil application + 0.2% foliar application of Humic acid (S6) as sub- plot treatments. These treatments were imposed to foxtail millet crop during *kharif* season and bengalgram crop during *rabi* season. The 100% RDF for foxtail millet crop is 40:20:0 kg N, P2O5 and K2O ha-1. P fertiliser was applied as basal dosage and half of the N was applied as basal and other half at 30 DAS. Similarly 50% RDF was also applied. Humic acid was applied as basal at the levels chosen and incorporated as per treatments mentioned.

Bacteria, fungi and actinomycetes were estimated as per the procedures outlined by Kapoor and Paroda (2007). The enumeration of total bacteria in fresh soil samples was carried out by following serial dilution plate count technique (Dhingra and Sinclair, 2000) using nutrient agar medium, the enumeration of total fungi in the fresh soil samples of all treatments was carried out by following the standard serial dilution plate technique using Martins Rose Bengal Agar for fungi (Martin, 1949) and The enumeration of total actinomycetes in the fresh soil samples of all treatments was carried out by following the standard serial dilution plate techinique using Khusters Nutrient Agar medium.

# Results & Discussion:

# Microbial populations Bacterial population:

“Close observation of data presented in table 1 indicated that, combined application of fertilizer treatments and levels of humic acid, have shown significant effect on bacterial populations at all stages of foxtail millet in soil during both the years of study. However, application of different levels of fertilizers and its interaction with different humic acid treatments applied in *kharif* did not significantly influence the soil bacterial populations under foxtail millet during both the years of study.” (Kumar et al 2021)

At harvest stage of foxtail millet, with increasing levels of fertilization from 0 to 100 % RDF, significantly the highest bacterial populations was recorded in 100% RDF (M4**-**37.48x106 CFU g-1 in 2020 and 34.95 x106 CFU g-1 in 2021) which was on par with the application of 75% RDF (M3**-** 33.62x106 CFU g-1in 2020 and 30.73 x106 CFU g-1 in 2021). The lowest bacterial populations were recorded in control (M1**-** 24.83x106 CFU g-1in 2020 and 20.62 x106 CFU g-1 in 2021). Islam and Borthakur (2016) recorded that application of inorganic fertilizers contributed significantly enhancing the microbial growth of total bacterial count and also that the bacterial population was the highest at tillering and lowest at harvest. This might be attributed to increase in the root exudates at tillering leading to more intense microbial activity which gradually decline when the crop attained maturity to harvest stage. The results were in corroboration with those of Colvan *et al.* (2001) and Selvi *et al.* (2003) who attributed it to the release of more root exudates during active growth of the plant. Combined application of organics and fertilizers apparently provided nutrients in balanced proportion which was reflected in terms of increased amounts of microbial biomass.

At harvest stage of foxtail millet, among the sub plots (humic acid levels), the

significantly higher bacterial populations was recorded with the treatment 20 kg ha- 1Humic acid as soil application+ 0.2% foliar application (S6-36.96x106 CFU g-1in 2020 and 33.33 x106 CFU g-1 in 2021), respectively and this treatment was on par with treatment S3. Lower bacterial populations were recorded in S1 (23.44x106 CFU g-1in 2020 and 20.46 x106 CFU g-1 in 2021) at harvest stages respectively. Increase in populations of micro-organisms, especially bacteria might be due to presence of good organic carbon source in the humic acid applied plots which increased the organic carbon in that plot. Increased availability of nutrient source might also have contributed to the increased number of bacteria in humic acid applied plots (Sellamuthuand Govindswamy2003). Humic acid application improves the root growth. Increased root growth might have contributed to increased rhizosphere and hence good proliferation of micro-organisms might have taken place.

# Fungal Population:

Close observation of the data pertaining to the fungal populations at all stages of foxtail millet was presented in the table 2 which revealed that fungal populations in soil differed significantly due to inorganic fertilizer treatments and levels of humic acid, but not by their interaction during both the years of study.

At harvest stage of foxtail millet, with increasing levels of fertilization from 0 to 100 % RDF, significantly highest fungal populations was recorded in 100% RDF (M4**-**10.89x103 CFU g-1 in 2020 and 10.15 x103 CFU g-1 in 2021) which was on par with the application of 75% RDF (M3**-** 9.62x103 CFU g-1 in 2020 and 8.78 x103 CFU g-1 in 2021). The lowest fungal populations were recorded in control (M1**-**5.98x103 CFU g-1 in 2020 and 4.97 x103 CFU g-1 in 2021) at harvest stage of the crop. Increasing level of chemical fertilizer from 0% NPK to 100% NPK registered significant increase in fungal population due to increase in the carbon substrate resulting from higher amount of decomposed organic matter due to increased root biomass. Increase in microbial population in the rhizosphere of foxtail millet in the presence of organics was quite obvious as they acted as a source of energyfor the proliferation of microorganisms in the soil by virtue of their high organic carbon content. Integrated use of organics with chemical fertilizers, ameliorate the soil and improve the productivity of crop, resulting in ecofriendly farming system. Similar findings were reported by Gaganpreet *et al.* (2016) and Verma *et al*. (2022).

At all growth stages of foxtail millet, with increasing the humic acid levels,

the significantly higher fungal populations was recorded with the treatment 20 kg ha-1 Humic acid as soil application+ 0.2% foliar application (S6- 11.15x103 CFU g-1 in 2020 and 10.10 x103 CFU g-1 in 2021) at harvest stage of foxtail millet, respectively and this treatment was on par with application of 20 kg ha-1Humic acid as soil application alone(S3). Lower fungal populations were recorded in control (S1- 5.18x103 CFU g-1 in 2020 and 4.52 x103 CFU g-1 in 2021) at harvest stage of foxtail millet respectively. Presence of more amount of organic carbon combined with good moisture content might have increased the fungal populations in the soil.

# Actinomycetes Population :

Data pertaining to the actinomycetes populations at all stages of foxtail millet was presented in the table 3, which revealed that actinomycetes populations in the soil differed significantly due to inorganic fertilizer treatments and levels of humic acid, but not by their interaction during both the years of study.

With increasing levels of fertilization from 0 to 100 % RDF, at harvest stage of foxtail millet, the significantly the highest actinomycetes populations was recorded in 100% RDF (M4**-** 12.71x104 CFU g-1 in 2020 and 11.85 x104 CFU g-1 in 2021) at harvest stage of foxtail millet, respectively which was on par with the application of 75% RDF (M3**-**11.81x104 CFU g-1in 2020 and 10.80 x104 CFU g-1 in2021).The lowest actinomycetes population was recorded in control (M1**-** 8.64 x104 CFU g-1in 2020 and 7.17 x104 CFU g-1in 2021) at harvest stages of foxtail millet, respectively . Significant increase in bacteria and actinomycetes in 100%NPK treatment as compared to 50%NPK treatment and imbalanced fertilization was observed which could be due to higher root biomass due to higher crop productivity with balanced fertilization which in turn provided greater substrate for source of energy for growth of actinomycetes and bacteria.

Among the humic acid levels, the significantly the highest actinomycetes populations was recorded with the treatment of 20 kg ha-1Humic acid as soil application+ 0.2% foliar application(S6 - 12.15 x104 CFU g-1in 2020 and 10.97 x104 CFU g-1in 2021) at harvest stage of foxtail millet, respectively. Lower actinomycetes populations was recorded in control (S1- 8.63x104 CFU g-1in 2020 and 7.53 x104 CFU g-1in 2021) at harvest stage of foxtail millet, respectively. A significant increase was recorded in actinomycetes population in soil up to a dose of HA @ 20 kg ha-1 combined with 100% NPK and 75% NPK at all the three stages of the crop growth. Increased organic carbon source and availability of sufficiently good amount of nutrients in humic acid applied plots might have contributed to an increase in population of actinomycetes in those plots applied with humic acid. Similar increase of actinomycetes population was reported by Deepa and Govindarajan (2002), Hrselova *et al.* (2007), Mali *et al*. (2015) and Lei *et al*. (2022).

### ****Findings and Conclusion:****

The application of 100% Recommended Dose of Fertilizers (RDF; M4) significantly boosted soil microbial populations—bacteria, fungi, and actinomycetes—across all four seasons, statistically matching the performance of 75% RDF (M3) . Among humic acid treatments, S6 (20 kg ha⁻¹ humic acid in soil + 0.2% foliar spray) recorded the highest microbial counts, statistically equivalent to S3 (20 kg ha⁻¹ soil-only application) . Importantly, there was no significant interaction between RDF and humic acid levels in either year. The integrated soil + foliar application of humic acid (S6) not only amplified microbial populations but also enhanced soil enzyme activity (e.g., urease, dehydrogenase, alkaline and acid phosphatase), nutrient availability, and broader soil fertility . These biological enhancements led to improved growth and yield in foxtail millet and the subsequent chickpea crop. The findings clearly advocate that coupling humic acid application with a reduced fertilizer input (75% RDF) delivers performance on par with full RDF, while promoting more sustainable and cost-effective crop production

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**Table 1.Effect of inorganic fertilizers and humic acid on bacterial population (×106 CFU g-1 soil) at harvest stage of Foxtail millet**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sub Plots (Humic Acid)** | ***Kharif 2020*** | | | | **Mean** | ***Kharif 2021*** | | | | **Mean** |
| **Main Plots (In-Organics)** | | | | **Main Plots (In-Organics)** | | | |
| **M1** | **M2** | **M3** | **M4** | **M1** | **M2** | **M3** | **M4** |
| **S1** | 21.66 | 21.99 | 23.64 | 26.47 | 23.44 | 17.97 | 19.47 | 20.93 | 23.46 | 20.46 |
| **S2** | 23.80 | 24.43 | 34.73 | 37.55 | 30.13 | 19.77 | 22.56 | 32.07 | 34.71 | 27.28 |
| **S3** | 27.43 | 28.41 | 40.68 | 45.95 | **35.62** | 22.78 | 26.24 | 37.57 | 42.47 | **32.26** |
| **S4** | 21.99 | 22.61 | 26.04 | 28.99 | 24.91 | 18.25 | 22.48 | 25.94 | 28.91 | 23.89 |
| **S5** | 24.86 | 25.37 | 34.64 | 38.78 | 30.91 | 20.65 | 22.48 | 30.70 | 34.41 | 27.06 |
| **S6** | 29.26 | 29.44 | 41.97 | 47.17 | **36.96** | 24.30 | 26.09 | 37.20 | 45.74 | **33.33** |
| **Mean** | **24.83** | **25.38** | **33.62** | **37.48** |  | **20.62** | **23.22** | **30.73** | **34.95** |  |
|  | **SEm ±** | | **CD (p=0.05)** | | **CV (%)** | **SEm ±** | | **CD (p=0.05)** | | **CV (%)** |
| **M** | 1.95 | | 5.92 | | 9.3 | 2.11 | | 6.21 | | 8.8 |
| **S** | 1.81 | | 5.28 | | 8.4 | 1.68 | | 4.74 | | 7.9 |
| **M X S** | 0.85 | | NS | |  | 0.60 | | NS | |  |
| **S X M** | 1.04 | | NS | |  | 0.96 | | NS | |  |

**Table 2.Effect of inorganic fertilizers and humic acid on fungal population (×103 CFU g-1 soil) at harvest stage of Foxtail millet**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sub Plots (Humic Acid)** | | ***Kharif 2020*** | | | | | **Mean** | ***Kharif 2021*** | | | | **Mean** |
| **Main Plots (In-Organics)** | | | | | **Main Plots (In-Organics)** | | | |
| **M1** | **M2** | | **M3** | **M4** | **M1** | **M2** | **M3** | **M4** |
| **S1** | | 4.93 | 5.06 | | 5.31 | 5.43 | 5.18 | 4.09 | 4.48 | 4.70 | 4.82 | 4.52 |
| **S2** | | 5.57 | 6.14 | | 8.78 | 10.91 | 7.85 | 4.63 | 5.67 | 8.11 | 10.08 | 7.12 |
| **S3** | | 7.12 | 7.14 | | 13.80 | 15.24 | 10.82 | 5.91 | 6.60 | 12.75 | 14.08 | 9.83 |
| **S4** | | 5.12 | 5.35 | | 6.10 | 6.43 | 5.75 | 4.25 | 5.32 | 6.08 | 6.41 | 5.51 |
| **S5** | | 5.75 | 6.48 | | 9.76 | 11.74 | 8.43 | 4.78 | 5.74 | 8.65 | 10.42 | 7.40 |
| **S6** | | 7.41 | 7.62 | | 13.99 | 15.59 | 11.15 | 6.15 | 6.75 | 12.40 | 15.11 | 10.10 |
| **Mean** | | **5.98** | **6.30** | | **9.62** | **10.89** |  | **4.97** | **5.76** | **8.78** | **10.15** |  |
|  | | **SEm ±** | | | **CD (p=0.05)** | | **CV (%)** | **SEm ±** | | **CD (p=0.05)** | | **CV (%)** |
| **M** | | 0.72 | | | 2.18 | | 8.2 | 0.71 | | 2.20 | | 8.1 |
| **S** | | 0.96 | | | 3.01 | | 7.2 | 0.76 | | 2.14 | | 6.8 |
| **M X S** | | 0.22 | | | NS | |  | 0.34 | | NS | |  |
| **S X M** | | 0.23 | | | NS | |  | 0.28 | | NS | |  |
| **Main Plots** | | **Sub Plots** | | |
| M1-Control | | S1-0kg/ha | | |
| M2-50%RDF | | S2-10kg/ha H.A | | |
| M3-75%RDF | | S3-20Kg/ha H.A | | |
| M4-100%RDF | | S4-0.2% H.A | | |
|  | | S5-10kg/ha H.A+0.2% | | |
|  | | S6-20kg/ha H.A+0.2% | | |

**Table 3.Effect of inorganic fertilizers and humic acid on actinomycetes population (×104 CFU g-1 soil) at harvest of Foxtail millet**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sub Plots (Humic Acid)** | ***Kharif 2020*** | | | | **Mean** | ***Kharif 2021*** | | | | **Mean** | |
| **Main Plots (In-Organics)** | | | | **Main Plots (In-Organics)** | | | |
| **M1** | **M2** | **M3** | **M4** | **M1** | **M2** | **M3** | **M4** |
| **S1** | 8.39 | 8.47 | 8.82 | 8.85 | 8.63 | 6.96 | 7.50 | 7.81 | 7.85 | 7.53 | |
| **S2** | 8.55 | 8.57 | 11.87 | 12.94 | 10.48 | 7.10 | 7.92 | 10.96 | 11.96 | 9.49 | |
| **S3** | 8.72 | 8.99 | 13.80 | 15.24 | **11.69** | 7.24 | 8.30 | 12.75 | 14.08 | **10.59** | |
| **S4** | 8.42 | 8.57 | 9.64 | 9.86 | 9.12 | 6.98 | 8.52 | 9.60 | 9.83 | 8.73 | |
| **S5** | 8.75 | 9.21 | 12.76 | 13.81 | 11.13 | 7.27 | 8.16 | 11.30 | 12.25 | 9.75 | |
| **S6** | | 8.98 | 10.06 | 14.00 | 15.59 | **12.15** | 7.46 | 8.91 | 12.40 | 15.11 | **10.97** |
| **Mean** | | 8.64 | 8.98 | **11.81** | **12.71** |  | 7.17 | 8.22 | 10.80 | 11.85 |  |
|  | | **SEm ±** | | **CD (p=0.05)** | | **CV (%)** | **SEm ±** | | **CD (p=0.05)** | | **CV (%)** |
| **M** | | 0.45 | | 1.42 | | 8.1 | 0.52 | | 1.61 | | 7.5 |
| **S** | | 0.57 | | 1.61 | | 7.2 | 0.45 | | 1.28 | | 6.8 |
| **M X S** | | 0.13 | | NS | |  | 0.15 | | NS | |  |
| **S X M** | | 0.22 | | NS | |  | 0.23 | | NS | |  |