

**SRI VENKATESWARA INTERNSHIP PROGRAM FOR RESEARCH IN ACADEMICS (SRI-VIPRA)**



# **SRI-VIPRA**

# **Project Report of 2024: SVP-2411**

"**Comparison of endophytes following the application of fertilizers or pesticides in agricultural** 

**plants**"

# **IQAC**

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## **SRIVIPRA PROJECT 2024**

**Title :** Comparison of endophytes following the application of fertilizers or pesticides in agricultural plants

**Name of Mentor:** Dr. Vartika Mathur **Name of Department:** Zoology **Designation:** Professor



*List of students under the SRIVIPRA Project*



Vartile Vath

**Signature of Mentor**

### **Certificate of Originality**

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVP-2411 titled "**Comparison of endophytes following the application of fertilizers or pesticides in agricultural plants**". The participants have carried out the research project work under my guidance and supervision from 1<sup>st</sup> July, 2024 to 30<sup>th</sup> September, 2024. The work carried out is original and carried out in an offline mode.

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**Signature of Mentor**

#### **Acknowledgements**

This research was conducted under the aegis of SRI-VIPRA by our institution, Sri Venkateswara college, University of Delhi.

Firstly, we are deeply grateful to Prof. Vartika Mathur, for their invaluable guidance, encouragement, and insightful feedback throughout the lab work and report preparation. Their expertise and advice were crucial in the successful execution of the experiments and the development of this report. We also wish to thank Ms. Nishu, Mrs. Kavita Verma, Mr. Rahul, Ms. Surbhi Agarwal and Ms. S. Aneeqa Noor for their collaboration and assistance during the lab sessions. Their teamwork and shared knowledge significantly contributed to the successful outcome of the experiments. We are extremely grateful to them for providing us with their valuable time and unconditional help. We are obliged to work under their mentorship to achieve this endeavour. Special thanks to Mr. Arun Kumar and Mr. Rameshwar Mahto for their support with the lab equipment and resources. Their assistance ensured that the experiments were conducted smoothly and efficiently.

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#### **1. Introduction**

In recent decades, the global agricultural industry has increasingly relied on the use of chemical fertilizers and pesticides to boost crop production and manage pests. As the global population continues to grow, the demand for food has surged, necessitating practices that optimize crop yield to ensure food security (Baweja et al., 2020). Fertilizers and pesticides, in this regard, have become essential tools in modern agriculture, helping farmers to meet the food supply needs efficiently (Sharma and Singhvi, 2017; Timsina, 2018; Pahalvi et al., 2021). However, while these chemical inputs are beneficial in enhancing productivity, they have profound and often overlooked effects on the plant microbiome, especially the endophytes which play a critical role in plant health, survival and growth.

One of the primary concerns with the use of chemical fertilizers and pesticides is their potential to disrupt the delicate balance of the plant microbiome. Fertilizers, while promoting plant growth, may inadvertently alter the environment within the plant tissues, making it less hospitable for certain beneficial endophytes. Pesticides, designed to eliminate harmful pests, may also have unintended effects on non-target microorganisms, including endophytes. These disruptions could result in a reduced diversity of endophytic communities, potentially weakening the plant's ability to resist diseases and cope with environmental stresses. While chemical fertilizers and pesticides undoubtedly offer immediate benefits in terms of increased crop yields and pest control, their long-term impact on the plant microbiome, particularly endophytes, must be carefully considered.

Endophytes are microorganisms, including bacteria and fungi, that reside within the tissues of plants without causing harm (Herlina et al., 2016). Unlike pathogenic microbes, endophytes live in a symbiotic relationship with their host plants, contributing to the plants' well-being by producing bioactive compounds that promote growth and provide resistance against both biotic and abiotic stresses (Strobel & Daisy, 2003; Ludwig-Müller, 2015). These stresses can include drought, disease, extreme temperatures, and nutritional deficiencies. In return for the protective benefits conferred by the endophytes, the host plant offers shelter and nutrients to these microorganisms.

Endophytes are known to produce a diverse range of bioactive compounds, such as antibiotics, antiinflammatory agents, and insecticides, which enable the plants to defend themselves against various external threats (Zhao et al., 2010). The secondary metabolites produced by endophytes are not only crucial for the plant's defence system but are also important for human health (Joseph & Priya, 2011). Despite their importance, the effects of commonly used agricultural chemical fertilizers and pesticides on endophytes are not fully understood and remain an area of active research.

This report presents a comparative analysis of the effects of chemical fertilizers and pesticides on the communities of endophytes in tomato plants (*Solanum lycopersicum*). As one of the most widely consumed vegetables globally, tomatoes are a critical agricultural commodity (Su et al., 2018). The increasing demand for tomatoes, both domestically and internationally, has led to intensified efforts to enhance tomato production through improved agricultural practices (Li et al., 2017; Mariangela et al., 2018). In this experiment, pesticides like Acetamiprid, Propargite and Profenofos are used along with fertilizers like NPK, Urea, DAP, and MOP since these are used extensively in the agriculture practices (Indian Fertilizer Scenario, 2018). While the benefits of fertilizers in optimizing tomato growth are well documented, their impact on endophytes is less understood. Similarly, pesticides, which are applied to protect tomato plants from various pests and diseases, may also influence the population and diversity of endophytic microbes. Given that endophytes contribute to nutrient acquisition, stress tolerance, and disease resistance, any alteration in their composition could have significant implications for plant health and productivity. This report explores the comparative effects of chemical fertilizers and pesticides on the endophytic microbiome of tomato plants, focusing on the changes in endophyte population and their potential impacts on plant growth.

#### **2.OBJECTIVES**

The following were the objective of our study:

- 1. To study the effect of pesticides and chemical fertilizers on plant endophytes
- 2. To determine the effect of chemical fertilizers and pesticides on soil physiochemical properties
- 3. To study the effect of pesticides and chemical fertilizers on the plant growth and development

### **3.METHODOLOY**

### **3.1 Plant**

Tomato seeds (*S. lycopersicum*) var. Pusa Hybrid-4 have been obtained from the Indian Agricultural Research Institute, Pusa, India. Tomato seeds were sown in 10 inches pots, containing soil-sand mixture (3:1) in an insect-free enclosure situated at Sri Venkateswara College (28.5894° N, 77.1681° E), New Delhi, India to ensure that herbivores did not interfere with the study (Figure 1).



**Figure 1.** *S. lycopersicum* was grown in insect-free enclosure at Sri Venkateswara College

#### **3.2 Treatment and Experimental design**

Tomato was grown in pots containing 5 kg soil which were divided into eight treatments (n=12). Chemical fertilizers were added when the plants reached stage 14 according to the BBCH scale (Lancashire et al., [1991\)](javascript:;) and pesticides were sprayed after 10 days of sowing (19 BBCH scale). The concentrations of pesticides and chemical fertilizers are given in the table 1.



**Table 1:** Concentrations of pesticides and chemical fertilizers

#### **3.3 Phenology and growth parameters**

Plants were observed in morning daily for 120 days until they reached maturity (fully ripened fruits) according to the Biologische Bundesantalt, Bundessortenamt and Chemische Industrie (BBCH) scale (Lancashire et al. 1991). The phenology was systematically monitored and recorded every  $5<sup>th</sup>$  day. Upon maturity, shoot and root parts of the plants were carefully harvested, washed with DDW and air dried on a blotting sheet. Their length was measured using meter scale, followed by fresh weight. The samples were then heated at 100  $\degree$ C for 2 d. The plants were again measured for their dry weight using weighing balance (Mettler Toledo, India).

#### **3.4 Soil physiochemical properties**

The physiological properties of rhizosphere's soil such as phosphorus, potassium, nitrogen, temperature, pH, humidity and electrical conductivity were tested using Soil Test Fertilizer and Recommendation Meter (Make: IARI, Pusa, India).

#### **3.5 Isolation and morphological identification of endophytes**

Microbes were isolated using Sharma et al. (2023) on Nutrient agar medium (NAM), Reasoner's 2A agar (R2A) and Potato Dextrose Agar (PDA) medium. Fresh tomato plant leaves of each treatment (n=3)

were taken and pre-washed with double distilled water (DDW) to remove dust particles. The leaves were then sterilized for 1 min in each buffer in the following sequence with increasing alkalinity: 0.15% NaOH, 0.1% Na<sub>2</sub>CO<sub>3</sub>, 0.2% NaClO and 3% NaCl. Then leaves were washed 5 times with 1 mL of DDW for remove epiphytes followed by macerated in 2 mL of 0.9% NaCl. To 1 mL of this solution, 9 mL of DDW was added and this was further serially diluted up to  $10^{-7}$ . The 100  $\mu$ L of the leaf suspension was poured into NAM, R2A and PDA medium. This was followed by incubation at 28 ˚C temperature for 24h (NAM), 24h to 7 days (R2A) and at 25 ˚C for 3 days (PDA). Culture plates were examined for the number of Colony forming units (CFU) and morphologically identified on the basis of form, margin, elevation, colour and texture.

► CFU/ml= Total number of colony on plate\*Dilution Factor

Volume of culture plate











#### **Result:**

#### **1) Phenology and growth parameters**

Propargite and MOP supplementation in soil enhanced the growth of plants as compared to control. They have attained 27 BBCH stage on 30th day after sowing, early than control plants. NPK supplemented plants showed delayed growth as compared to control (Fig. 2). They have attained BBCH stage 24 on  $30<sup>th</sup>$  day of sowing.

In DAP treated plants, the shoot height was reduced as compared to control. Urea supplemented plants showed increased height as compared to control (Fig. 3a). On the other hand, root height was higher in fertilizers and pesticides treated plants as compared to control. Compared to control, Urea and Profenofos treated plants showed significant increase in the root height (Fig. 3b).

The shoot fresh weight was significantly lowered under fertilizers and pesticides treatment as compared to control. Higher decrease was observed in DAP and Acetamiprid treated plants (Fig. 3c). On the other hand, there was increased in shoot dry weight under NPK and Acetamiprid supplemented plants as compared to control. Profenofos, Propargite, DAP, Urea and MOP showed decreased shoot dry weight when compared to control conditions (Fig. 3d). Both fresh and dry weight of root showed significant reduction under fertilizers and pesticides treatment as compared to control. MOP supplemented plants showed much lowered fresh and dry weight of root when compared to control conditions (Fig. 3e, f).







**Figure 3:** (a) Shoot height, (b) Root height, (c) Fresh shoot weight, (d) Dry shoot weight, (e) Fresh root weight and (f) Dry root weight under fertilizers and pesticides supplementation in *S. lycopersicum*

#### **2) Soil Physiochemical properties**

Nitrogen concentration increased under Urea and DAP supplementation in plants as compare to control whereas marginal decrease had been observed in NPK and Propargite treated plants (Fig. 4a). Similarly, phosphorus and potassium were moderately increased after Urea and DAP treatment and decreased after NPK and Propargite treatment as compared to control (Fig. 4b). Potassium levels significantly increased after Urea and DAP treatment and slightly decreased after NPK and Propargite treatment as compared to control (Fig. 4c). Soil pH was relatively lowered in the Urea treated plants exhibited slightly acidic soil conditions as compared to other treatment, while the Acetamiprid showed slightly alkaline pH (Fig. 4d). Electrical conductivity was slightly increased in the DAP and Urea treated plants, while in the Propargite, NPK and MOP, it was slightly decreased as compared to control (Fig. 4f).

Soil humidity was significantly decreased in the Acetamiprid and NPK supplemented plants as compared to control, whereas in the Urea, DAP and MOP, it was slightly increased (Fig. 4e). Soil temperature was significantly increased in all the treatment as compared to control except Profenofos which was similar to control (Fig. 4g).





**Figure 4:** (a) Nitrogen, (b) Phosphorus, (c) Potassium, (d) pH, (e) Electrical conductivity, (f) Humidity and (g) Temperature under fertilizers and pesticides supplementation in *S. lycopersicum*

### **3) CFU and morphological identification of endophytes**

The colony forming units for endophytic colonies were recorded highest in Propargite treated plants as compared to control. Other than this, Profenofos supplemented plants also showed greater CFU count (Fig. 5, 6). The bacterial diversity based on morphological observation was found to be higher under Urea, NPK, MOP and DAP supplemented plants i.e. fertilizers treated plants. On the other, Profenofos supplemented plants showed lowered diversity of endophytes when compared to control (Table 2).



**Figure 5**: Endophyte CFU of *S. lycopersicum* under fertilizers and pesticides supplementation



**Figure 6**: Nutrient agar medium plates showing endophytes under fertilizers and pesticides treatment



**Table 2:** Morphological diversity of endophytes under fertilizers and pesticides supplementation







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**Discussion:** 

Crop productivity is reliant on the ecosystem services provided by indigenous soil physical, chemical properties, and microorganisms (Dignam et al., 2019). Tomato responded significantly various pesticides and fertilizers (Abu-Alrub et al., 2019). The use of chemical fertilizers and pesticides has become a cornerstone of modern agriculture, contributing significantly to the global food supply. However, these inputs have complex effects on the plant microbiome, particularly on endophytic microorganisms that play a crucial role in plant health and productivity. This, in turn, could affect the plant's yield, natural defences, making it more reliant on external chemical interventions.

In this study, DAP-treated plants exhibited reduced shoot height compared to the control, possibly due to phosphorus-induced nutrient imbalances. Conversely, Urea supplementation significantly increased shoot height, likely due to enhanced nitrogen availability, promoting cell division and elongation. Root height was consistently higher in both fertilizer and pesticide-treated plants, with Urea and Profenofos treatments showing the most significant increase. The enhanced root growth in these treatments suggests that nitrogen availability and mild stress responses from pesticides promote root development, improving water and nutrient uptake (Erdinc et al., 2018; Baweja et al., 2020). These findings highlight the differential effects of agrochemicals on shoot and root growth.

The study revealed that shoot fresh weight decreased significantly under fertilizer and pesticide treatments. The reduction in biomass is consistent with previous findings that indicate high doses of fertilizers or pesticides can have phytotoxic effects, inhibiting plant growth and reducing biomass accumulation (Shakir et al., 2016; Jan et al., 2020). The observed decrease in shoot biomass, particularly under DAP and Acetamiprid treatments, may be due to the disruption of hormonal balances or interference with photosynthetic processes (Sharma et al., 2020).

In contrast, root biomass increased in plants treated with fertilizers and pesticides, particularly with Urea and Profenofos. These findings suggest that root systems may exhibit compensatory growth in response to aboveground stress or nutrient imbalances. Previous studies have shown that root systems tend to expand in response to low shoot biomass as plants attempt to access more nutrients from the soil (Fanin et al., 2019). This response may also be related to improved soil structure and nutrient availability in the rhizosphere, particularly in soils treated with fertilizers like Urea, which promotes nitrogen cycling (Ai et al., 2022).

Our study found that Urea and DAP treatments increased nitrogen, phosphorus, and potassium concentrations in plants, which is consistent with the known nutrient-rich composition of these fertilizers. However, the marginal decrease in nutrient concentrations in NPK and Propargite-treated plants contrasts with earlier studies that generally report improved nutrient uptake with balanced NPK fertilization (Xiang et al., 2021). The observed changes in soil pH, with Urea-treated soils exhibiting slightly acidic conditions and Acetamiprid-treated soils showing slightly alkaline conditions, are in line with previous research. Urea is

known to acidify soil as a result of ammonium ion production during nitrification (Goss-Souza et al., 2021). Similarly, pesticides such as Acetamiprid can alter soil pH by affecting microbial activity and organic matter decomposition rates (Nelson,1991; Daneshvar, 2004).

Soil electrical conductivity (EC) was slightly increased under DAP and Urea treatments, which aligns with earlier studies suggesting that high nutrient inputs can increase soil salinity, potentially affecting plant growth (Ai et al., 2022). On the other hand, the reduction in EC observed in Propargite, NPK, and MOP treatments may be due to the adsorption of ions onto soil particles or reduced ion mobility (Fanin et al., 2019). Soil humidity was significantly reduced in Acetamiprid and NPK-treated soils, possibly due to changes in soil structure or the hydrophobic nature of certain pesticide residues (Goss-Souza et al., 2021). In contrast, Urea, DAP, and MOP treatments slightly increased soil moisture, likely due to improved water retention properties associated with organic matter decomposition and microbial activity (Kushwaha et al., 2024). Soil temperature was significantly higher in all treatments except Profenofos, suggesting that fertilizers and pesticides may alter soil thermal dynamics, potentially affecting microbial activity and plant root respiration (Zhang et al., 2020).

Our results indicated a significant increase in bacterial density in soils treated with pesticides, particularly with Propargite and Profenofos and increased in bacterial diversity due to fertilizer application, particularly with NPK and Urea. This increase aligns with the findings of earlier studies, which reported that pesticide application can enhance microbial activity, as soil microorganisms often utilize pesticide residues as carbon and nitrogen sources (Ai et al., 2022). In contrast that fertilizers can foster a more diverse microbial ecosystem by providing essential nutrients for microbial growth. Previous studies demonstrated that the addition of nutrients such as nitrogen, phosphorus and potassium stimulate the proliferation of a wider range of microbial species (Hu et al., 2024). Our findings are consistent with the hypothesis that nutrient-rich environments can enhance microbial diversity but may also shift the microbial community structure in ways that could have long-term implications for soil health.

This study highlights the complex interplay between fertilizers, pesticides, microbial communities, and plant growth. While pesticides significantly increased bacterial density, fertilizers contributed to greater microbial diversity. However, the application of these agrochemicals also had varied effects on plant growth, with some treatments accelerating development while others delayed it. The observed reductions in shoot biomass and increases in root biomass, along with changes in soil physicochemical properties, suggest that both fertilizers and pesticides can have far-reaching impacts on plant physiology and soil health. Future research/studies may explore the long-term consequences of these interactions to optimize agrochemical use for sustainable agriculture.

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