RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume 59 Issue 8: 1224-1231 (August 2025)

Root Rot Disease on Tomato Seedlings Caused by *Pythium ultimum* and the Effect of Nutrient Elements N, P and K on Disease Incidence

Khalaf Alhussaen[1]

10.18805/IJARe.AF-919

ABSTRACT

Background: Nutrients are important factors that plants need, which also affect the plant's sensitivity to plant diseases. These nutrients may increase the plant's sensitivity to disease or may increase the plant's resistance to these diseases.

Methods: This research was designed to determine the effect of the nutrient's nitrogen (N), phosphorus (P) and potassium (K) on the incidence of root diseases affecting tomato seedling caused by *Pythium ultimum*. *Pythium ultimum* were isolated and identified through a combination of morphological characteristics and DNA sequencing.

Result: The results of this research showed that nutrient elements (N, P and K) play a major role in increasing the tomato plants ability to resist root diseases caused by *Pythium ultimum*. Different concentrations of N, P and K showed low root disease incident on tomato seedlings caused by *Pythium ultimum*. The best concentrations of N, P and K were 20% each and with a lower percentage 10%. Nutrient elements are very important in the plant's ability to resist diseases, but within specific quantities for each element.

Key words: DNA sequencing, Morphological characteristics, Nitrogen, Phosphorus, Potassium, *Pythium ultimum*.

INTRODUCTION

Plants, like all living organisms, are influenced by their environment, including the elements or chemical elements that are essential for their growth and development. The presence and availability of specific elements can have a significant impact on plant health and susceptibility to diseases (El-Sonbaty, 2021).

Plants require a wide range of elements for their basic physiological functions, including macronutrients such as nitrogen (N), phosphorus (P) and potassium (K), as well as micronutrients like iron (Fe), zinc (Zn) and copper (Cu) (Gómez-Trejo *et al.,* 2021; Krithika *et al.,* 2024). The availability of these elements in the soil plays a crucial role in shaping a plant's ability to defend itself against diseases.

Nutrient imbalances can make plants more vulnerable to diseases. For instance, excessive nitrogen fertilization can promote the growth of lush, succulent foliage, making plants more attractive to herbivorous insects and providing a fertile breeding ground for certain fungal pathogens (Wiser and Blom, 2016). Conversely, nutrient deficiencies can weaken plants, reducing their resistance to diseases (Agrios, 2005; Choudhury *et al.,* 2018).

Micronutrients, though required in smaller quantities, are essential for various enzymatic processes within plants. Insufficient micronutrient levels can lead to a weakened plant immune system, making it easier for pathogens to invade (Kah *et al.,* 2019). On the other hand, excessive micronutrient levels can be toxic to plants, causing stress and susceptibility to diseases (Langridge, 2022).

The pH level of the soil can affect the availability of essential elements to plants. Some pathogens thrive in specific pH ranges, so soil pH can indirectly influence disease susceptibility (Lichuan, 2022). For example, clubroot disease in cruciferous plants is more common in acidic soils (Chia *et al.,* 2014).

Some trace elements like selenium (Se) and silicon (Si) have been found to enhance a plant's resistance to diseases. Selenium, in particular, can stimulate the production of enzymes that detoxify harmful compounds produced by pathogens (Bhardwaj *et al.,* 2022; Naglaa *et al.,* 2023).

Iron is a critical element for plant growth, but it can also be a point of competition between plants and pathogens. Some plants employ strategies to limit the availability of iron to pathogens as a defense mechanism (Yi *et al.,* 2021).

Pythium is a genus of water mold-like oomycete pathogens that can cause destructive diseases in plants, particularly in agricultural and horticultural settings. The availability and balance of plant nutrients can influence the severity and incidence of *Pythium* infections in various ways.

[1]Department of Biology, College of Haqel, University of Tabuk, Saudi Arabia.

Corresponding Author: Khalaf Alhussaen, Department of Biology, College of Haqel, University of Tabuk, Saudi Arabia.
Email: khalhussaen@ut.edu.sa
ORCID: https://orcid.org/0000-0002-8561-0240

How to cite this article: Alhussaen, K. (2025). Root Rot Disease on Tomato Seedlings Caused by *Pythium ultimum* and the Effect of Nutrient Elements N, P and K on Disease Incidence. *Indian Journal of Agricultural Research.* **59(8):** 1224-1231. doi: 10.18805/IJARe.AF-919.

Submitted: 05-11-2024 **Accepted:** 15-02-2025 **Online:** 05-05-2025

Excessive nitrogen in the soil or growing medium can stimulate rapid vegetative growth in plants. This lush growth can make plants more susceptible to *Pythium* infections because the pathogen thrives in conditions of high moisture and humidity (Wiser and Blom, 2016). Additionally, excess nitrogen can reduce a plant's ability to defend itself against pathogens.

Adequate phosphorus is important for plant development and root growth. Balanced phosphorus levels can help plants establish stronger root systems, which can enhance their resistance to *Pythium* by improving overall plant health (Malhotra *et al.,* 2018).

Potassium plays a crucial role in regulating plant water balance and overall stress tolerance. A well-balanced potassium supply can help plants better withstand the water stress associated with *Pythium*-infected roots (Gómez-Trejo *et al.,* 2021).

Soil or growing medium pH can also affect *Pythium*. *Pythium* tends to be more active in acidic conditions (low pH), so maintaining a proper pH level that is closer to neutral can help reduce the risk of *Pythium* infections (Alhussaen, 2012).

The overall health and condition of plant roots are crucial in resisting *Pythium* infections. Nutrient imbalances or deficiencies can weaken roots and make them more susceptible to *Pythium* attack.

It's important to note that while nutrient management can influence a plant's susceptibility to *Pythium*, it is just one of many factors that affect disease development. Other factors, such as environmental conditions (temperature, moisture), crop management practices and the presence of other pathogens, also play important roles in *Pythium* disease dynamics.

To effectively manage *Pythium* infections, it is essential to adopt integrated disease management strategies that include practices like proper irrigation management, crop rotation, using disease-resistant plant varieties and applying fungicides when necessary. Additionally, maintaining a balanced nutrient program tailored to the specific needs of the crop can contribute to overall plant health and reduce susceptibility to *Pythium* and other diseases.

The Jordan Valley is considered one of the most fertile areas and its climate is suitable for winter cultivation of crops that do not like cold, the most important of which is tomatoes.

This research was designed to examine the effect of major nutrients N, P and K on plant resistance to root diseases caused by *Pythium* sp. on tomato plants grown in Jordan Vally.

MATERIALS AND METHODS

Isolate recovery

Pythium species were isolated from diseased roots of tomato seedlings growing from Jordan Valley on March 2022. Small bits measuring about 5 mm were surface sterilize with 70% ethanol then placed on potato carrot agar (PCA) and incubated in the dark at 25±1°C according to (Van der Plaats-Niterink, 1981). Pure culture of the fungus was obtained by hyphal tip isolation method. The experiments were conducted in Biology department in Yarmouk University in Jordan on 2022.

Morphological characterization of *Pythium* sp. isolates

Morphological characterization was conducted on PCA medium microscopically (Olympus CX41RF, Olympus Optical, Philippines) to perform preliminary identifications. The observed microscopic traits encompassed sexual structures, appressoria and hyphal swellings. Subsequently, slides were fabricated from these cultures, stained with lacto-phenol cotton blue following the method outlined by Parija and Prabhakar (1995) and subjected to microscopic examination. Measurements of hyphal diameters, oogonia, oospores and oospore wall thicknesses were determined using eyepiece and stage micrometers. Thirty to fifty measurements were taken for each of these structures. Additional characteristics under scrutiny included the number, shape and arrangement of antheridia, as well as the size and shape of oogonial projections (spines). Moreover, the shape and abundance of appressoria formed at the points of contact with the Petri dish were investigated. These scrutinized morphological features were then compared to those documented in Van der Plaats-Niterink's (1981).

To induce sporangia and zoospores, 2- to 4-day-old cultures growing on PCA were used. Small rectangular agar culture pieces (20 mm square) were aseptically transferred into sterile plastic Petri dishes and submerged in 20 mL of sterile distilled water. The dishes were initially incubated at 4°C for 1-3 hours and subsequently maintained at room temperature (25±1°C). During the first three hours, the water was replaced every hour. Sporangial development was monitored through light microscopy at each water change. In cases where no zoospores were observed within the first 24 hours, the cultures were left at room temperature (25±1°C) for an additional 5 days and re-examined periodically. If no zoospore production occurred during this extended period, they were categorized accordingly in accordance with the taxonomic keys used.

Effect of temperatures

Pythium isolates were introduced into the center of a 90 mm diameter Petri dish containing 20 mL of PCA. This inoculum source was derived from a 5 mm diameter plug extracted from the periphery of actively growing 3-day-old PCA cultures incubated at 25±1°C in complete darkness.

To assess the impact of temperature on its growth, the isolate was subjected to various temperature conditions, ranging from 5 to 40°C. Five replicate dishes were prepared and maintained at each temperature setting. The diameters of the resulting colonies were measured at both 24 and 48 hours along two perpendicular axes. To quantify the colony's growth over a 24-hour span, the mean

colony diameters at 24 and 48 hours were used to calculate the expansion in millimeters.

Effect of pH levels

Pythium isolates were introduced into the center of a 90 mm diameter Petri dish containing 20 mL of PCA. This inoculum source was derived from a 5 mm diameter plug extracted from the periphery of actively growing 3-day-old PCA cultures incubated at 25±1°C in complete darkness.

To assess the impact of pH on its growth, the isolate was subjected to various pH levels (ranging from 4 to 9) were established by the addition of either 0.1 N sodium hydroxide or 0.1 N hydrochloric acid. Small 5 mm diameter discs, obtained from the periphery of a fungal culture, were used as inoculants. These inoculated discs were then placed in a dark environment and maintained at a temperature of 25±1°C for a period of 3 days. The study incorporated three replicates for each pH treatment. To quantify the colony's growth, the mean colony diameters at 3 days were used to calculate the expansion in millimeters.

Molecular Methods

The DNA from *Pythium ultimum* mycelium was extracted and was amplified by PCR. The method of Paul (2000) was used to examine the Internal Transcribed Spacer (ITS1) region of the ribosomal nuclear DNA (rnDNA). Tools of the BLAST search http://blast.ncbi.nlm.nih.gov; it was used to analyze the sequences and the sequences was amplified by ClustalW tools http://www.ebi.ac.uk; to examine the phylogram of the isolate and its relative.

Furthermore, to create a phylogenetic tree (phylogram) that illustrates the relationships between the isolated strain and its relatives, the ClustalW software was employed. The phylogram construction was completed based on the sequence data and this analysis aimed to visualize the evolutionary connections between the isolate and its taxonomic relatives.

Fertilized elements

The effect of Nitrogen (N), Phosphate (P) and Potassium (K) on the root rot disease caused by *Pythium ultimum* were examined. Tomato seedlings were planted in pots contain 1:1 peat moss and perlite. Three pots were used for each element (N, P and K) for each percentage of fertilizer elements (5%, 10%, 20% and 50%) and three pots were used as control (without elements tested) (three replicates where used).

Plants were kept for three weeks from the time of inoculation to harvest in greenhouse and irrigated three times a week with the right solution to keep the fertilizer element in the pot at the same level. Plants were assessed at 7, 14 and 21 days.

Inoculum preparation

Agar cultures from two PCA plates of *Pythium ultimum* were finely blended in 500 mL of sterile distilled water using three two-second pulses, creating an inoculation solution for the plants. Each plant received 20 mL of the macerated agar solution as an application.

Data analysis

A completely randomized design (CRD) was employed for all treatments, with each treatment having 3 replicates. The average growth area for each treatment was determined by employing a colony counter on Petri dishes. To identify significant differences ($p \leq 0.05$) among the treatment means, a General Linear Model (GLM) analysis of variance (ANOVA) was conducted using SPSS Version 16.0.

RESULTS AND DISCUSSION

Isolate recovery

Several fungal isolates were recovered from tomato roots, all displaying morphological similarity and were identified as *Pythium ultimum* through a combination of morphological characteristics and DNA sequencing.

Morphological characterization

The colonies cultured on PCA exhibited a radiate pattern. The hyphae had a width of up to 10 µm. Sporangia were mostly absent and zoospores were infrequently produced through short discharge tubes at 5°C. Hyphal swellings were globose, occurring both intercalary and sometimes at the terminal ends, with a diameter of 17-23 (av. 20.4) µm. The oogonia were primarily terminal but occasionally intercalary, smooth-walled and globose, measuring 21-25 µm in diameter, with an average of 22.6 µm. Antheridia were either solitary or 1 (-3) per oogonium, sac-like in appearance, predominantly originating immediately below the oogonium. Occasionally, they were hypogynous, or 2-3 antheridia were present and in such cases, they could be either monoclinous or diclinous, often with a straight configuration. Oospores were solitary, plerotic and globose, measuring 18-22 µm in diameter, with an average of 20 µm and their walls were frequently 2 µm or more in thickness.

Effect of temperatures

Regarding temperature tolerance, the cardinal temperatures for this isolate were a minimum of 5°C, an optimum range of 25-30°C and a maximum of 35°C. The daily growth rate on potato-carrot agar at 25°C was 4 cm (Table 1).

There were significant differences between the colony growth at different temperatures tested after 24 h and 48 h ($p \leq 0.05$). However, there were no significant differences in colony growth at temperatures 5°C of 4°C and 8°C after 24 h growth and between 5°C and 40°C ($p \leq 0.05$) and between 6 and 7 (Table 1). Moreover, there were no significant differences in colony growth at temperatures 25°C and 30°C after 48 h growth.

Effect of pH levels

The optimum pH level of the isolate of *Pythium* ultimum tested was 6 to 7. Isolate tested grew very well at pH level of 5. However, slightly growth was appeared at pH levels of 4 and 8. Moreover, there were no growth at pH levels of 3 and 9.

There were significant differences between the colony growth at different pH levels ($p \leq 0.05$). However, there were no significant differences between in colony growth at pH levels of 4 and 8 and between 6 and 7 (Table 2, Fig 1).

Molecular identification based on ITS region of rDNA

ITS1 sequence (760 bp) of one representative isolate of *Pythium ultimum* was found to be 99% identity with *Pythium ultimum* (Alhussaen *et al*., 2011). The closest relatives of our isolate, *Pythium ultimum* are shown in Table 3.

Effect of fertilizer elements (N, P and K) on root disease

Nitrogen

In testing the effect of nitrogen on the root diseases severity on tomato seedlings, it was found that the lowest disease rate was when 20% nitrogen was added at all growth periods (Table 4, Fig 2). When the nitrogen element was added at concentrations of 5% and 10%, the disease rate was apparent at rates of 20% and 17% after seven days of growth respectively and 22% and 18% after 14 days of growth respectively. After 21 days of growth, the disease rate was 25% and 20%.

When 50% of nitrogen was added, it was found that the disease rate was significantly high in all growth periods (63%, 85% and 90%) (Table 4, Fig 2).

However, when there were no nitrogen added the percentage of the disease severity was high at all growth periods (50%, 70% and 75%) (Table 4, Fig 2).

In testing the effect of phosphor on the root diseases severity on tomato seedlings, it was found that all percentages add (5%, 10% 20 and 50%) showed low disease rate at all growth period tested compare with the seedlings without adding any phosphor (Table 5). However, at 50% per cent of phosphor after 21 days of growth showed 33% of disease severity.

When testing the effect of potassium on root diseases on tomato seedlings, it was found that all concentrations used in this experiment showed a reduction in the rate of root disease on tomato seedlings compared to seedlings

Table 1: Mean colony growth (cm^2) of *Pythium ultimum* isolates from tomato roots grown on PCA media with temperature ranging from 5 to 40°C at 24 and 48 hours after inoculation and incubation in the dark at 25±1°C.

Temperature (°C)	Growth (cm^2)	
	24 h	48 h
5	0.3[a]	1.2[a]
10	1.1[a]	2.4[b]
15	2.0[b]	3.1[b]
20	2.5[b]	5.1[c]
25	5.6[c]	9.0[d]
30	5.3[c]	8.6[d]
35	2.8[b]	5.3[c]
40	0.8[a]	1.6[a]

Within a column, means followed by the same letter are not significantly different from each other at $p \leq 0.05$.

Table 2: Mean colony growth (cm^2) of *Pythium ultimum* isolates from tomato roots grown on PCA media with different pH levels (3 to 9) 3 days incubation in the dark at 25±1°C.

pH level	Growth (cm^2)
3	0.0[a]
4	1.1[b]
5	5.7[c]
6	7.5[d]
7	7.1[d]
8	1.4[b]
9	0.0[a]

Within a column, means followed by the same letter are not significantly different from each other at $p \leq 0.05$.

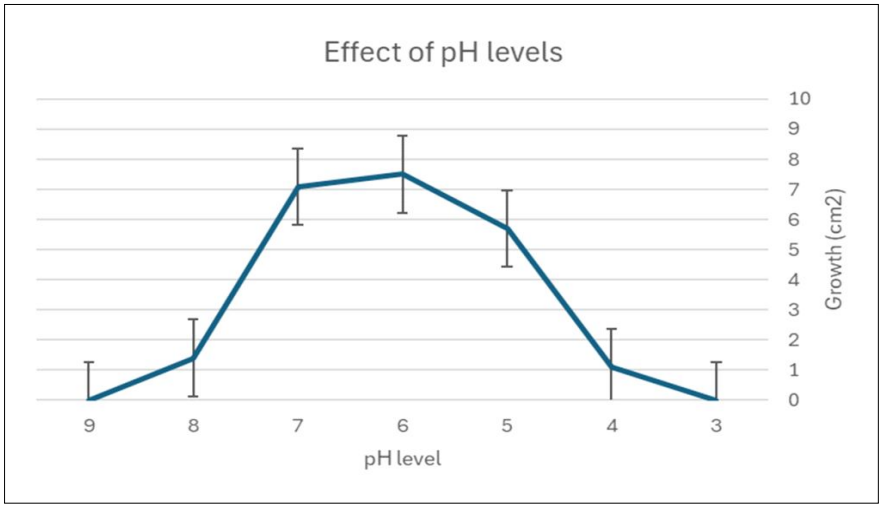


Fig 1: Mean colony growth (cm^2) of *Pythium ultimum* isolates from tomato roots grown on PCA media with different pH levels (3 to 9) 3 days incubation in the dark at 25±1°C.

to which no potassium was added (Table 6, Fig 3). The best concentration of potassium that reduced root disease was 20% and 50%.

In this investigation, *Pythium ultimum* obtained from tomato seedlings cultivated in the Jordan Valley (Jordan) was identified through a combination of morphological and physical traits, according to the Van der Plaats-Niterink (1981) key alongside molecular methods relying on ITS1 sequence analysis.

Accurate identification of plant pathogens plays a crucial role in the development of effective disease control or management strategies. Incorrect identification may result in the implementation of ineffective control measures. Traditionally, fungi, especially Oomycetes, have been identified based on morphological characteristics. However, this approach is challenging due to the tendency of many species to exhibit overlapping features that are difficult to distinguish (Agrios, 2005). Morphological similarities are often found across various species groups and intraspecific morphological variations are commonly observed among different field isolates (Van Os, 2003).

In more recent times, molecular techniques utilizing various DNA methods have emerged for species identification and elucidating inter-species relationships (Levesque and de Cock, 2004; Drenth *et al.,* 2006; Mar Htun *et al.,* 2021). The objective is to reevaluate identifications and relationships previously determined through morphological methods. The sequences of the Internal Transcribed Spacer (ITS1) region of the ribosomal DNA (rDNA) were acquired to identify the *Pythium* isolate at the species level. Comparative analysis revealed a 99% identity to *Pythium ultimum* in the Gene-Bank database (Table 3). This outcome substantiates the identification achieved through morphological and physical characterization.

The results of this research showed that nutrient elements (N, P and K) play a major role in increasing the tomato plants ability to resist root diseases caused by *Pythium ultimum*. Different concentrations of N, P and K showed low root disease incident on tomato seedlings caused by *Pythium ultimum*. The best concentrations of N, P and K were 20% each and with a lower percentage 10%.

Nitrogen is one of the elements that effect the sensitivity of the plant, so it must be given in measured amounts. The results in this research showed that increasing the concentration of N will lead to increasing the sensitivity of tomato seedlings to root diseases (Table 4; Fig 2). Moreover, not providing nitrogen to plants also leads to an increase in the incidence of root diseases. Nitrogen assimilation is intricately linked to vital physiological functions, including photosynthesis, photorespiration, respiration and the tricarboxylic acid cycle, among various others (Sun *et al.,* 2020). Various researchers found that the low N reduced plant diseases in general and root rot disease in particular as well as high concentration increase the incidence of diseases (Howard *et al.,* 1994; Huber and Graham, 1999; Harrison and Shew, 2001; Celar, 2003; Singh, 2015).

All concentrations of P used in this research showed a reduction in the rate of root disease on tomato seedlings compared to seedlings to which no potassium was applied (Table 5). The best concentration of potassium that reduced root disease was 20% and 50%. The impact of P on disease occurrence and severity varies, contingent upon the specific crop and pathogen involved. Similar results were found by Singh (2015) when he reported that adequate phosphorus fertilization can notably mitigate the severity and occurrence of soil-borne ailments like *Pythium* root

Table 3: The closest relative of *Pythium* sp. isolated from diseased roots of tomato seedlings based on BLAST search of ITS1 sequences.

Organism	Identity (%)	Sequence ID or Reference
Pythium ultimum	99%	Khalaf Alhussaen *et al.,* 2011
Pythium ultimum	99%	LT670911.1
Pythium ultimum	99%	JN695787.1
Globisporangium ultimum	99%	MT457768.1

Table 4: Effect of different nitrogen concentrations (0% as a control and 5%, 10%, 20%, 50%) treatments on tomato seedlings at period of (7, 14 and 21 days).

Plant age	Without N	5% N	10% N	20% N	50% N
7 Days	50%	20%	17%	7%	63%
14 Days	70%	22%	18%	10%	85%
21 Days	75%	25%	20%	12%	90%

Table 5: Effect of different phosphor concentrations (0% as a control and 5%, 10%, 20%, 50%) treatments on tomato seedlings at period of (7, 14 and 21 days).

Plant age	Without P	5% P	10% P	20% P	50% P
7 Days	48%	12%	5%	5%	10%
14 Days	73%	18%	8%	7%	15%
21 Days	80%	22%	12%	11%	33%

rot in wheat or common potato scab induced by *Streptomyces* spp. Additionally, P application can suppress diseases such as downy mildew, blue mold and blight. Moreover, the application of P fertilization can exert a noteworthy impact and nearly eradicate economic losses stemming from *Pythium* root rot (Huber, 1980).

The results of this research found that all concentrations of K used in this experiment showed a reduction in the rate of root disease on tomato seedlings compared to seedlings to which no potassium was added (Table, 6; Fig 3) and the best concentration of potassium that reduced root disease was 20% and 50%. Huber and

Table 6: Effect of different potassium concentrations (0% as a control and 5%, 10%, 20%, 50%) treatments on tomato seedlings at period of (7, 14 and 21 days).

Plant age	Without K	5% K	10% K	20% K	50% K
7 Days	50%	30	24	18	17
14 Days	76%	35	30	29	26
21 Days	83%	42	39	32	31

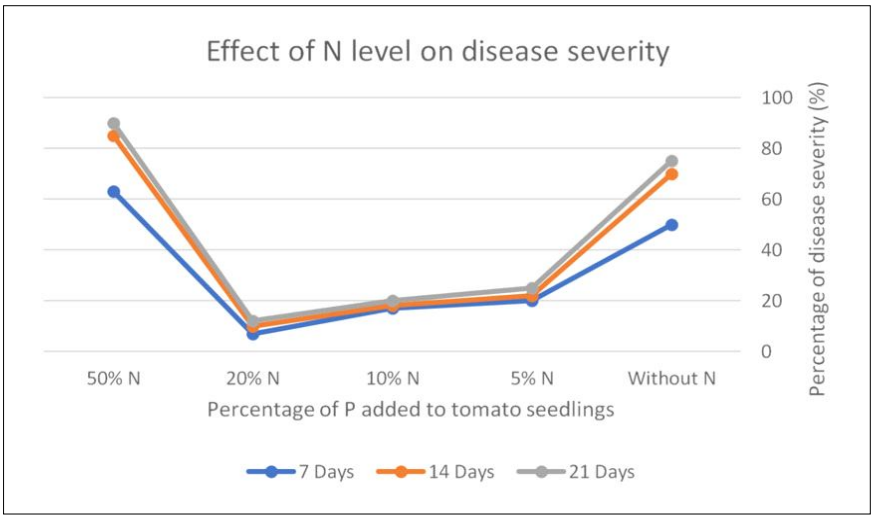


Fig 2: Effect of different nitrogen concentrations (0% as a control and 5%, 10%, 20%, 50%) treatments on tomato seedlings at period of (7, 14 and 21 days).

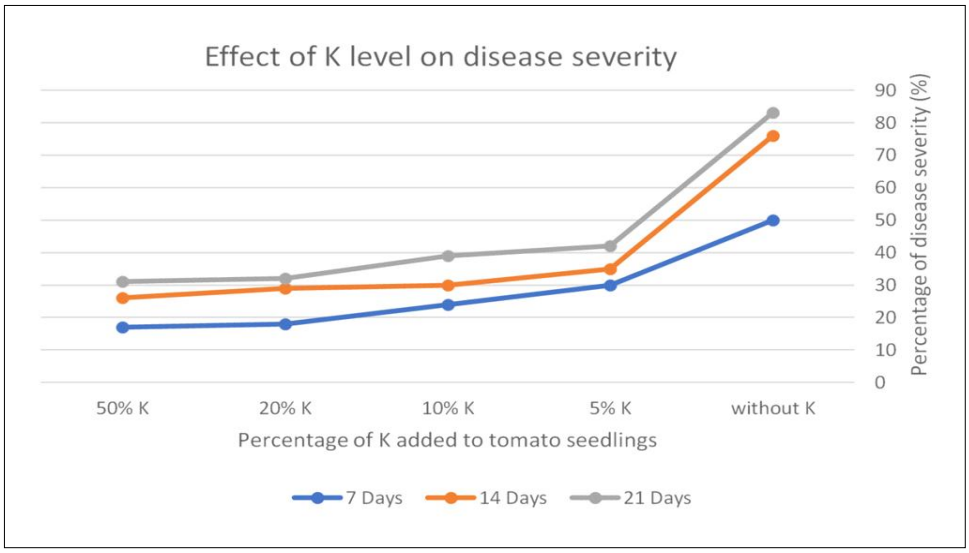


Fig 3: Effect of different potassium concentrations (0% as a control and 5%, 10%, 20%, 50%) treatments on tomato seedlings at period of (7, 14 and 21 days).

Graham (1999) reported that Potassium diminishes the vulnerability of host plants, reaching an optimal level conducive to growth. Beyond this threshold, elevating the supply of potassium and its concentration in plants does not lead to additional resistance gains. The heightened susceptibility of potassium-deficient plants to parasitic diseases is attributed to the metabolic roles of potassium in plant physiology.

This study explored the role of nutrient elements (N, P and K) in enhancing resistance to root diseases caused by *Pythium ultimum* in tomato seedlings. It found that moderate concentrations (20% each) of nitrogen, phosphorus and potassium were effective in reducing root disease incidence. Excessive nitrogen increased plant sensitivity to diseases, highlighting the importance of balanced nutrient application. Phosphorus and potassium similarly showed beneficial effects in reducing disease severity, with optimal concentrations identified for each nutrient.

The findings from this investigation on *Pythium ultimum* and nutrient management in tomato seedlings have several potential economic and environmental implications. The study highlights specific concentrations of nitrogen (N), phosphorus (P) and potassium (K) that enhance resistance to root diseases. Farmers can optimize their fertilizer applications based on these findings, potentially reducing costs associated with excessive fertilizer use while maximizing crop health. Effective disease management through balanced nutrient application can potentially reduce the need for chemical pesticides and fungicides, promoting more sustainable agricultural practices.

CONCLUSION

In conclusion, Nutrient elements are very important in the plant's ability to resist diseases, but within specific quantities for each element. This research suggest that nutrient elements (N, P and K) play a major role in increasing the tomato plants ability to resist root diseases caused by *Pythium ultimum* Different concentrations of N, P and K showed low root disease incident on tomato seedlings caused by Pythium ultimum. The best concentrations of N, P and K were 20% each and with a lower percentage 10%.

Conflict and interest

All authors declared that there is no conflict of interest.

REFERENCES

Agrios, G.N. (2005). Plant pathology. (Academic Press: San Diego, USA).

Alhussaen, K.M. (2012). Effect of soil acidity on diseases caused by *Pythium ultimum* and *Fusarium oxysporum* on tomato plants. *Journal of Biological Sciences.* **12(7):** 416-420.

Alhussaen, K., Hussein, E., Al-Batayneh, K., Al-Khatib, M., Al-Khateeb, W., Jacob, H.J., Shatnawi, M., Khashroum, A. and Hegaz, Y.M. (2011) Identification and controlling *Pythium* sp. infecting tomato seedlings cultivated in jordan valley using garlic extract. *Asian Journal of Plant Pathology.* **5(2):** 84-92.

Bhardwaj, H., Singh, C., Nayyar, S., Sodhi, S., Jindal, R. (2022). Effect of Vitamin E and Selenium (Se) supplementation on biochemical parameters and expression of metallothionein (MT-2) in heavy metals exposed buffaloes. *Indian Journal of Animal Research.* **56(3):** 281-289. doi: 10.18805/IJAR.B-4465.

Celar Franci (2003). Competition for ammonium and nitrate forms of nitrogen between some phytopathogenic and antagonistic soil fungi. *Biological Control.* **28(1):** 19-24.

Chai, A.L., Xie, X.W., Shi, Y.X. and Li, B.J. (2014). Research status of clubroot (*Plasmodiophora brassicae*) on cruciferous crops in China. *Canadian Journal of Plant Pathology.* **36:** 142-153. https://doi.org/10.1080/07060661.2013.868829.

Choudhury, D., Dobhal, P., Srivastava, S., Saha, S., Kundu, S. (2018). Role of botanical plant extracts to control plant pathogens. *Indian Journal of Agricultural Research.* **52(4):** 341-346. doi: 10.18805/IJARe.A-5005.

Drenth, A., Wagels, G., Smith, B., Sendall, B., O'Dwyer, C., Irvine, G., Irwin, J.A.G. (2006). Development of a DNA-based method for detection and identification of *Phytophthora* species. *Australasian Plant Pathology.* **35:** 147-159.

El-Sonbaty, A. (2021). Effect of foliar application of N P K nanoparticle fertilization and soil addition of mineral N P K fertilization as combined treatments on soil fertility and wheat plant performance. *Journal of Soil Sciences and Agricultural Engineering, Mansoura Univ.* **12(8):** 567-572.

Gómez-Trejo, L.F., Hernández-Acosta, E. and Peralta-Sánchez, M.G. (2021). N, P, K nutrition differentially affects the incidence and severity of the attack of pests and diseases in plants. *Agro Productividad.* **14(05)**. https://doi.org/10.32854/agrop.v14i05.2050.

Harrison, U.J., Shew, H.D. (2001). Effects of soil pH and nitrogen fertility on the population dynamics of *Thielaviopsis basicola*. *Plant and Soil.* **228:** 147-155. https://doi.org/10.1023/A:1004845715885.

Huber, D.M. (1980). The Role of Mineral Nutrition in Defense. In: Horsfall JG, Cowling EB (eds) Plant Disease, an Advanced Treatise, vol V, How Plants Defend Themselves. Academic, New York. 381-406.

Huber, D.M., Graham, R.D. (1999). The Role of Nutrition in Crop Resistance and Tolerance to Diseases. In: Mineral Nutrition of Crops: Fundamental Mechanisms and Implications. Rengel Z (ed). The Haworth Press, London. pp 169-204.

Kah, M., Tufenkji, N. and White, J.C. (2019). Nano-enabled strategies to enhance crop nutrition and protection. Nat. Nanotechnol. 14: 532-540.

Krithika, C., Santhi, R., Maragatham, S., Devi, R.P. and Vijayalakshmi, D. (2024). Effect of integrated plant nutrient management based on soil test crop response on primary plant nutrients uptake and quality parameters of blackgram on alfisols in western zone of Tamil Nadu, India. *Indian Journal of Agricultural Research.* doi: 10.18805/IJARe.A-6175.

Langridge, P. (2022). Micronutrient toxicity and deficiency. In Wheat improvement: Food security in a changing climate Springer International Publishing. (pp. 433-449).

Levesque, C.A., De Cock, W.A. (2004) Molecular phylogeny and taxonomy of the genus *Pythium*. *Mycological Research.* **108:** 1363-1383.

Lichuan, Qiu. (2022). Research progress on the effects of soil acidity and alkalinity on plant growth. *Open Journal of Applied Sciences.* doi: 10.4236/ojapps.2022.126071.

Malhotra, H., Vandana, Sharma, S., Pandey, R. (2018). Phosphorus Nutrition: Plant Growth in Response to Deficiency and Excess. In: Plant Nutrients and Abiotic Stress Tolerance. [Hasanuzzaman, M., Fujita, M., Oku, H., Nahar, K., Hawrylak-Nowak, B. (eds)] Springer, Singapore. https://doi.org/10.1007/978-981-10-9044-8_7.

Mar Htun, Z., Laikul, A., Pathomsakulwong, W., Yurayart, C., Lohnoo, T., Yingyong, W., Kumsang, Y., Payattikul, P., Sae-Chew, P., Rujirawat, T. *et al.* (2021). Identification and biotyping of *Pythium insidiosum* isolated from urban and rural areas of Thailand by Multiplex PCR, DNA Barcode and Proteomic Analyses. *Journal of Fungi.* **7(4):** 242. https://doi.org/10.3390/jof7040242.

Naglaa, T., Salem, H., Yousry, A.B., Tamer, E., El-Ramady, H. and Svein, S. (2023). Nanofungicides with selenium and silicon Can boost the growth and yield of common bean (*Phaseolus vulgaris* L.) and control alternaria leaf spot disease Microorganisms. **11(3):** 728. https://doi.org/10.3390/microorganisms11030728.

Parija, S.C. and Prabhakar, P.K. (1995). Evaluation of lacto-phenol cotton blue for wet mount preparation of feces. *Journal of Clinical Microbiology.* **33(4):** 1019-1021.

Paul B (2000) ITS1 region of the rDNA of *Pythium* megacarpum sp. nov., its taxonomy and its comparison with related species. *FEMS Microbiology Letters.* **186:** 229-233.

Singh, D.P. (2015). Plant Nutrition in the Management of Plant Diseases with Particular Reference to Wheat. In: Recent Advances in the Diagnosis and Management of Plant Diseases, [L.P. Awasthi (Ed.)], New Delhi, India: Springer. (pp. 273-284). doi: 10.1007/978-81-322-2571-320.

Sun, Y., Wang, M., Mur, L.A.J., Shen, Q. and Guo, S. (2020). Unravelling the roles of nitrogen nutrition in plant disease defenses. *International Journal of Molecular Sciences.* **21(2):** 572. doi: 10.3390/ijms21020572.

van der Plaats-Niterink, J. (1981). Monograph of The Genus *Pythium*. Studies in Mycology.Central-bureau voor Schimmecultures, Baarn, Utrecht, The Netherlands. 21: 1-242.

van Os, G.J. (2003). Ecology and control of *Pythium* root rot in flower bulb culture. (Uitgeverij Eigen Beheer: Amsterdam)

Wiser, L. and Blom, T. (2016). The effect of nitrogen and phosphorus ratios and electrical conductivity on plant growth. American *Journal of Plant Sciences.* **7:** 1590-1599. doi: 10.4236/ajps.2016.712150.

Yi, L., Kong, D., Hui-Lan, W., Hong-Qing, L. (2021). Iron in plant-pathogen interactions. *Journal of Experimental Botany.* **72(6):** 2114-2124. https://doi.org/10.1093/jxb/eraa516.