The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
20(1): S.I (1), 41-45, 2025 www.thebioscan.com

EFFECT OF pH AND TEMPERATURE ON GROWTH OF *Fusarium oxysporum* f.sp.*vasinfectum*

R. ANNADURAI[1], M. KAMARAJ[1*] and B. BALAGURU[1]

[1]Research Scholar, Department of Botany, Jamal Mohamed College (A), Tiruchirappalli, Tamil Nadu, India

Affiliated to Bharathidasan University, Tiruchirrappalli-620 024, Tamil Nadu, India

E.mail - *kamarajjmc@gmail.com*

Corresponding Author: **Dr. Kamaraj. M,**

E-mail: kamarajjmc@gmail.com

Mobile: +91 9384503406.

DOI: https://doi.org/10.63001/tbs.2025.v20.i01.S.I(1).pp41-45

KEYWORDS
Fusarium oxysporum, pH, temperature, isolation, specific medium, Growth

Received on:
12-01-2025

Accepted on:
10-02-2025

Published on:
18-03-2025

ABSTRACT

The current analysis aids in understanding the effect of pH and temperature in growth of *Fusarium oxysporum* f. sp. *vasinfectum* (FOV). This study also focuses on incidences of *Fusarium* wilt disease in six places of Tamil Nadu. The isolation of the fungus was done using three different media including potato dextrose agar media, *Fusarium* specific medium and Czapek's (Dox) broth. The result imparted that the fungus has higher disease incidences in thiruchencodu region 20.50%) and significant effect on the selected parameters. it emphasis that wilt disease of cotton is endemic and causes destruction to the crop. It gives us a reason to adapt to resistant varieties and integrated pest management strategies to efficiently produce disease free cotton to support the economy of our country.

INTRODUCTION

In India, four million farmers cultivate fiber plants across 7.4 million hectares of land as per ICAR report. India leads world in cotton (*Gossypium* species) production. Production grew rapidly after Bt cotton hybrids were introduced in 2002. We grow over a 3rd of global cotton and a quarter of vegetables. Increasing acreage drove most of the production advances, but our productivity remains below half a tonne. India's textile sector accounts for "2.3% of GDP and 13% of industrial production" (Desouza Blaise, 2021). To increase productivity, certain industrial technologies must be adopted. Over 65% of India's textile industry's basic resources come from cotton, making it an indispensable raw material. More over 35 million people in India are directly or indirectly employed by the textile sector, making it a significant economic driver for the nation. More than 1,500 mills, 4,500,000 handlooms, 1,750,000 authority looms, and countless processing, garment, and hosiery factories are all part of it (Sankaranarayanan et al., 2011).

The Malvaceae family includes the cotton plant. Wendel and Gro ver (2015) estimate that there are 52 species in this genus. Seven of these species are diploid, whereas other forty-five are tetraploid. Worldwide "*G. hirsutum* L., *G. barbadense* L., *G. arboreum* L. and *G. herbaceum* L" are most common varieties..The most common species cultivated for its natural fiber is upland cotton, scientifically known as *Gossypium hirsutum* L. (Wendel and Grover, 2015).

India's cotton sector, while resilient, has significant challenges such as invasions of pests, dearth of water, shifts in climate, market price fluctuations, and soil degradation, with environmental issues like excessive water consumption, pesticide application, soil depletion, and diversity in BT cotton. Sustainable approaches such as Nestled Pest Management, irrigation via drip, organic cotton cultivation, crop rotation, and cover crops constitute the challenges of cotton production.

Numerous fungal diseases pose threat for cotton cultivators, including "Verticillium wilt (*Verticillium dahliae*), damping-off (*Rhizoctonia* spp. and *Pythium* spp.), and Ascochyta blight

(*Ascochyta*). Gossypii, black root rot (*Thielaviopsis basicola*)", and numerous foliar spots. *Fusarium* wilt is a significant worry for cultivators due to its propensity to inflict substantial economic losses and its longevity in agricultural fields (Cianchetta & Davis, 2015).

FOV is a soil-borne fungus, causes *Fusarium* wilt, a significant disease of *Gossypium hirsutum* (upland cotton), leading to wilting, vascular discoloration, and yield losses. It manifests wilting of leaves followed by yellowing and eventual death. Internal symptoms include brown marks of the xylem vessels (vascular tissue) when the stems are cut. *Fusarium* wilt can cause severe losses in cotton production, with initial disease losses reported as high as 90%.

To mitigate crop loss, employing biocontrol agents is a viable approach. Nonetheless, sustainability of these to meticulously evaluated, specifically arid regions where soils lack organic matter. Consequently, utilizing a biocontrol agent analogous to the pathogen may be feasible. Antagonistic but non-harmful *Fusarium* serves as an appropriate substitute (Minuto et al., 2006). The pursuit of secure alternative management strategies is essential due to the adverse impacts of fungal toxicants, which encompass phytotoxicity, health risks, environmental contamination, disease resistance, and elevated expenses. Currently, the design of eradicating soil-borne plant diseases such as *Fusarium* via biological management may be crucial for sustainable agriculture (Pandey et al., 2014).

The treatment of difficult diseases typically employs a combination of naturally existing nematodes, organic amendments, biological control, and induced resistance, as evidenced by prior research. Furthermore, it impedes the activity of pathogenic microorganisms through enzymatic mechanisms. Utilizing "*Bacillus amyloliquefaciens, Bacillus subtilis, and Paenibacillus polymyxa*", is deemed safe in animal and environmental contexts (Zhao et al., 2015). They produce antagonistic activities against many bacterial and fungal diseases and can persist in the plant for enhanced protection

Organic matter strongly influences plant health, soil composition, biological properties, and disease prevention. It is among the most often incorporated and compositionally varied soil additives, either as organic amendments or through exudation and plant root decomposition. Furthermore, inputs, temperature, minerals, organisms, and management influence amount and composition of organic matter in soil. Distinguishing between the suppression mechanisms induced by biological substances and extraneous or ambiguous factors may prove difficult. This complicates the identification of regular patterns, particularly when coupled with the data's volatility and the limited analytical characterization of organic components. Reviews seeking to discern unifying trends by correlating the characteristics of organic materials with disease suppression have achieved inconsistent success. Nonetheless, a range of interrelated environmental factors often leads inhibition of *Fusarium* wilt associated with organic matter (Baum et al., 2015).

Earlier literature impacts of *Fusarium* sps. suggest that the organism is destructive for all kinds of crops. According to Nirmaladevi (2016), fusariosis produced by sub sp. *lycopersici* can result in yield setbacks of "30 to 40% and in certain cases, up to 80%", depending on the weather situation that promote the fungus's growth. McGovern, (2015) shown that *Fusarium oxysporum*, which infected tomato wilt, poses a major danger to tomato production worldwide and results in significant financial losses in both greenhouse and field settings. Furthermore, the wilt disease in chickpeas has several control methods, although control is challenging due to the pathogen's soil borne origins. Through the widespread use of resistant cultivars, disease races were able to overcome host resistance and likely attempt a larger occurrence globally. So, current report aims to isolate and understand the effect of various parameters that influence the severity of wilt disease in cotton.

MATERIALS AND METHODS

A study of *Fusarium* wilt disease incidence in Tamil Nadu's

In 2022 and 2023, a roving field survey was carried out to determine the prevalence of wilt disease in Tamil Nadu State's key cotton-growing regions. For the purpose of determining frequency the region where cotton is traditionally produced is chosen. For the survey, five districts were chosen. In each field, 100 plants were chosen at random for the survey. The quantity of wilted plants was recorded, and the average wilt frequency was represented as a percentage.

To calculate the percent disease incidence (PDI), the following formula was used:

$$PDI = \frac{\text{Number of plants observed}}{\text{Number of diseased plants}} \times 100$$

To isolate pathogen, rhizosphere soil and infected plants exhibiting the characteristic signs of wilt caused by infection with FOV were gathered. In the corresponding study fields, additional data on the kind of soil used to grow the crop, cotton variety planted, and the existence of nematodes were also noted. The modified Baermann's funnel method (Schindler, 1961) and Cobb's sieve and decanting method (Cobb, 1918) were used to determine the presence of nematodes in the disease complex.

Isolation of Pathogens

Pathogen was isolated using the methodology by Ainsworth, 1961using potato dextrose agar media, *Fusarium* specific medium (Nash and Snyder, 1962) and Czapek's (Dox) broth (Ainsworth, 1961)

Consequence of nutrient media on growth characteristics of Foc

Research on the development properties of the foe were conducted using three different media types: liquid (PDB, COB, & FSM-Liquid) and solid (PDA, CDA, & FSM). To make PDB medium, 24 grams of powder (Make -Himedia Lab.) were added to 1000 millilitres of sterile distilled water (OW). Per litre of OW, COB medium was made by adding 2 g of sodium nitrate, 1 gram of dipotassium hydrogen phosphate, 0.5 grams of magnesium sulphate, 0.5 grams of potassium chloride, 0.01 grammes of ferrous sulphate, and 30 grams of sucrose. Peptone (15.0g), KH_2P0_4 (1.0g), MgS04.7H20 (0.5g), Pentachloronitrobenzene (1.0g), Streptomycin (0.3g) and distilled water were added to create FSM-Liquid (Nash and Snyder, 1962). In a 250 ml conical flask, 100 ml of each of three liquid media mentioned above was added. To make PDA, 39.0 grams of powder (Make Himedia) were added to 1000 millilitres of distilled water. Using the above-described procedure, CDA and FSM were made, and 20 grams of agar per litre was added. The previously prepared medium was sealed in an autoclaved for 15 min at 121 °C and 15 p.s.i. of pressurize to sterilize them. Petri plates were filled with solid medium following sterilization. A single 5mm piece of 7-day-old Foc was used to inoculate conical flasks and Petri plates, and they were then incubated at 25±2°C. Radial diameter and biomass data were recorded for a maximum of 14 days. The aforementioned experiment was carried out three times.

***Fusarium oxysporum* f. sp. *vasinfectum* isolation**

The pathogen FOV was discovered after examining infected roots of displaying the classic indications of wilt using tissue segment technique (Ran gaswami, 1972). After being thoroughly cleansed with tap water, the diseased stems and roots were sliced into little pieces. The pieces were surface sterilized into a 1% $NaOCl_2$ solution for 30 seconds before being put on a sterile Petri plate with PDA. After that, they were repeatedly washed in deionised water to eliminate any last traces of sodium hypochlorite. Room temperature was used to incubate the pteri plates a period of five to seven days. Following the transfer of hyphal points developing from contaminated pieces to subculture slants, the fungi was purify with the "hyphal tip" procedure (Rangaswami, 1972) and stored. The isolates were designated from Fov1 to Fov 6.

Effect of pH on growth characteristic of Foc

Three liquid (PDB, COB, and FSM-L) and three solid (PDA, CDA, and FSM) media were created. With the use of solutions containing hydrochloric acid (HCl) and sodium hydroxide (NaOH), the pH of the aforementioned medium was kept up to date at 3, 5, 7, 9, and 11. Within a 250 ml conical flask, 100 ml of freshly made liquid media were distributed. Following sterilisation, solid medium were added to Petri plates. A single 5mm chunk of 7-day-old Fov was used to immunize conical flasks and petri plates, which were then incubated at 25±2°C. Radial diameter and biomass data were recorded for a maximum of 14 days. The aforementioned experiment was carried out 3 times

Effect of temperature on growth of Foc

Within a 250 ml conical flask, 100 ml of freshly made liquid media were distributed. Following sterilization, solid medium were added to Petri plates. A single 5 mm chunk of 7-day-old Fov was used to immunize conical flasks and Petri plates. The samples were then incubated at 15, 20, 25, 30, 35, 40, and 45 degrees Celsius. Radial distance and biomass data were observed for a maximum of 2 weeks. The aforementioned experiment was repeated thrice.

RESULT AND DISCUSSION

Biological management of *Fusarium* wilt in Cotton (*Gossypium herbaceum* L.) *Trichoderma* was thoroughly investigated in the current work. The following information presents the findings on the causative organism, biological control agents, and their impacts on the host plant Cotton.

Symptoms of the Disease

Fusarium wilt damages cotton plant roots and causes damage at all stages of development. *Fusarium* wilt, on the other hand, often affects young plants with 2-10 nodes or seedlings. External symptoms include "leaf edge yellowing and wilting, stunting", defoliation, and mortality. The most common internal indication is vascular darkening, which manifests as persistent, dark brown stains in the stem or tap roots.

Separation and Recognition of the causal Organism

Isolation of FOV was obtained from wilt infected cotton tissues of black, red and sandy loam soil region in Tamil Nadu (Table 1) along with their disease incidences. The identification of fungal colony was white cottony mycelium with light pink color bottom on potato dextrose agar.

Table 1. List of *Fusarium oxysporum* obtained from wilt infected cotton tissues and their diseases incidences

Isolates	Locations	District	Soil type	Disease incidence (%)	Presence of nematode
FO - 1	Thuraiyur	Trichy	Red	19.24	+
FO - 2	Perugamani	Trichy	Black	17.91	-
FO - 3	Veppathattai	Perambalur	Sandy loam	20.28	-
FO - 4	Thiruchencodu	Namakkal	Sandy loam	20.50	+
FO - 5	Vellakoil	Erode	Black	17.97	-
FO - 6	Peryanayakkanpalayam	Coimbatore	Black	19.21	-

"+" indicates Presence; "-" indicates Absence of nematodes

On 15 DAS old seedlings, a pathogenicity test of the tentatively identified cotton wilt pathogen demonstrated vein clearing signs of cotyledonary leaves as the first symptom. The seedlings also had darker hypocotyls and roots, which was indicative of wilt infection. The pathogen was reisolated using Komada's agar medium and matched the initial isolation. *Fusarium oxysporum* f.sp.*vasinfectum* was recognized as the pathogen based on its physical characteristics and pathogenicity to cotton.

Prevalence of *Fusarium* wilt in Tamil Nadu districts

Endemic characters of the wilt disease incidence were highlighted by the data reported in Table 2 of the study conducted in key cotton-growing regions of Tamilnadu. Among the different location survey for cotton wilt incidence, Thiruchencodu indicated the maximum incidence of the disease (20.50%) followed by Veppathattai with 20.28 per cent. The other locations viz., Thuriyur (19.24%), Peryanayakkanpalayam (19.21%) had moderate range while the minimum wilt incidence of 17.97 and 17.91 per cent was recorded in Vellakoil and Perugamani. Regarding soil type, wilt was more common in sandy loams (20.50 and 20.28%) than red soil (19.24%) and black soil (19.21, 17.91 and 17.97%). The soil and root samples gathered for the study were used to determine if nematodes were present. Generally speaking, only two cotton fields had the minimal amount of nematode population (*Meloidogyne incognita*) discovered in them Thuraiyur and Thiruchencodu location.

Isolation and Characteristics of Pathogen

The colony properties of isolate *Fusarium oxysporum* f.sp.*vasinfectum* were each locations examined on Potato Dextrose Agar (PDA) medium. Isolates varied in their mycelium type, color created on medium, growth pattern, and number of days needed to cover a 90 mm Petri plate, among other morphological characteristics. In contrast to *chlamydospores* that grow in chains, the spores formed in tri-cells and were kidney-shaped, oval, tapering, and septate. Earlier civilizations' mycelium was floccose, brittle, and either abundant or sparse. The isolates Fov4 was noted the highest mycelial development (78.52 mm), whereas it was the lowest (65.80 mm) in the instance of Fov5. These were the same as the *F. oxysporum* reference strain (Table 2).

Table 2. Population characteristics of *Fusarium oxysporum* f.sp.*vasinfectum* isolate on PDA Petri plates.

Fusarium species	Characteristics of Colonies	Mycelium colour	Mycelial growth (mm) diameter
FO – 1	Discreet, thread-like growth that is thin, fluffy, and spreads irregularly in the periphery	Pale white	75.13
FO – 2	Thick, fluffy cotton growth with a faint, thread-like spreading around the edges	Pinkish white, margins compact	71.52
FO – 3	Abundant, fluffy cotton growth with regular margin	White purple	70.64

FO - 4	Discreet, thread-like growth that is thin, fluffy, and spreads irregularly in the periphery	Orange Red	78.52
FO - 5	Thick, fluffy cotton growth with a faint, thread-like spreading around the edges	Pale white	65.80
FO - 6	Discreet, thread-like growth that is thin, fluffy, and spreads irregularly in the periphery	Pale white	69.81

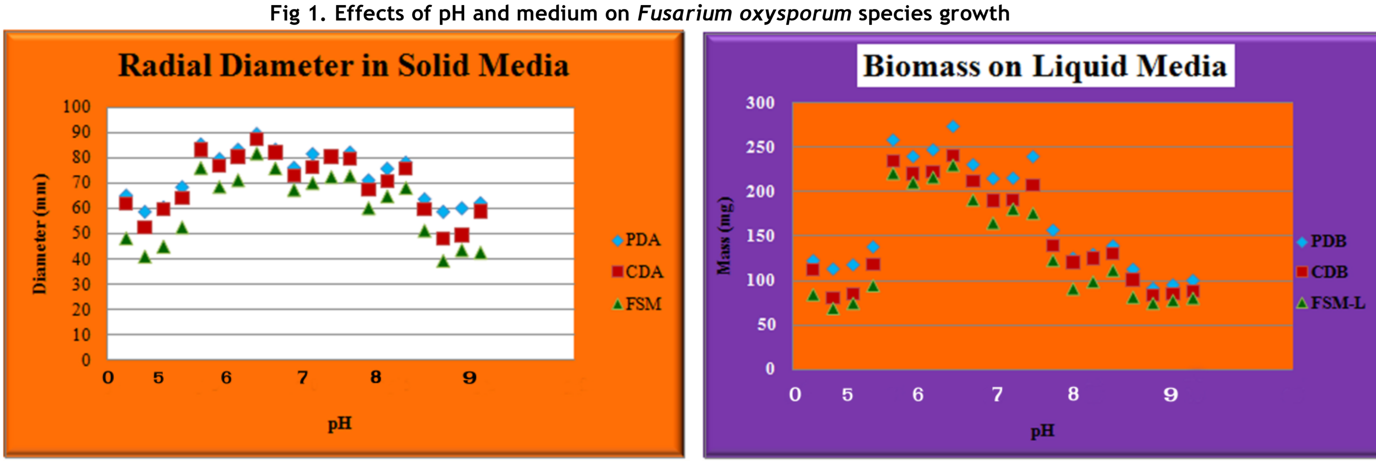
Effects of varying medium and pH on the development of *Fusarium oxysporum*

Six isolates of *Fusarium oxysporum* showed significantly higher growth on pH - 6 followed by pH - 7, 8, 5 and 9. Biomass on all liquid media and radial diameters on all solid media was maximum at pH - 6. Minimum biomass and radial diameter was obtained at pH - 9 for all the isolates. At acidic pH (pH - 6) The maximal biomass and radial diameter generated by isolate FO-4 were considerably different from those of the other isolates (Fig 1). Isolates FO-4 was able to tolerate low pH while as isolate FO - 1 was able to tolerate high range of pH. On all the solid and liquid media, maximum biomass was recorded at pH-6 for all the isolate which declined as the pH was increased or decreased.

Impact of media and temperature on *Fusarium oxysporum* growth

Growth of all the *Fusarium* species Biomass (mg) on Liquid Media and Radial Diameter(mm) on solid Media was recorded maximum on 25°C followed by 30°C, 20°c and 35°C. While, the *Fusarium* isolates did not show any mycelial growth at 40°C. In liquid medium at 35°C all the pathogen showed very less mycelial growth (biomass) as compared to lower temperatures. Like that, at 35°C maximum growth in the biomass was recorded in PDB medium, followed by CDA and FSM. However, no radial growth was observed on solid medium. At temperature 20°C and 25°C Compared to the other isolates, FO-1 generated more biomass. On the other hand, when FO-4 was grown on biomass (mg), it generated significantly more biomass on Liquid Media and Radial Diameter (mm) on solid Media at 30°C. Isolate FO-4 was more sensitive to high temperature while as isolate FO-1 was sensitive to lower temperature. The ideal temperature for all isolates to grow was 25°C and PDA and PDB medium respectively, followed by CDA and COB. Pathogen growth was more supported by liquid media showed Fig 2.

Fig 1. Effects of pH and medium on *Fusarium oxysporum* species growth



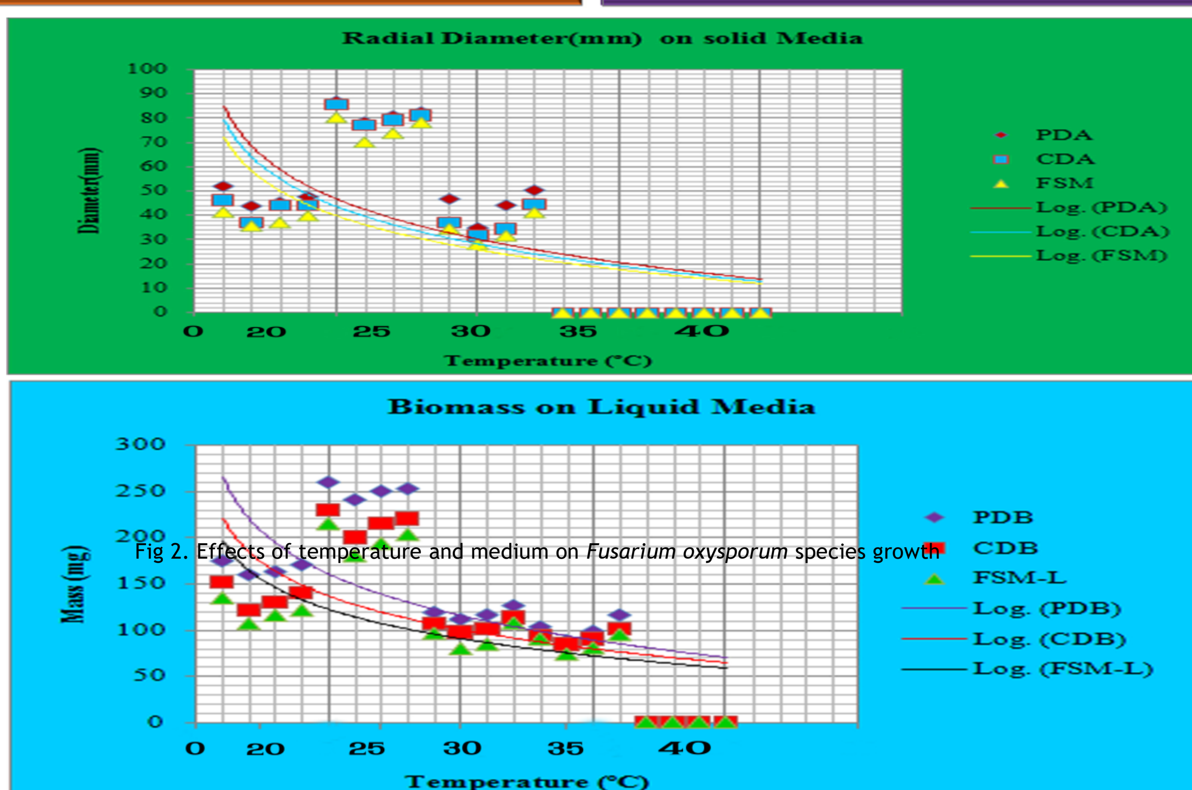


Fig 2. Effects of temperature and medium on *Fusarium oxysporum* species growth

Thiruchencodu exhibited the highest prevalence of the illness (20.50%) among the many locations examined for cotton wilt incidence, succeeded by Veppathattai (20.28%). The remaining localities, namely Thuraiyur (19.24%) and Peryanayakkanpalayam (19.21%), exhibited intermediate disease incidence, whilst the lowest wilt incidence of 17.97% and 17.91% was seen in Vellakoil and Perugamani, respectively. Wilt incidence was greater in sandy loam (20.50 and 20.28%) compared to red soil (19.24%) and black soil (19.21, 17.91, and 17.97%). Prior researchers have observed similar disparities in the wilt disease of cotton, which has been found to impact all four cultivated cotton cultivars (Davis et al., 2006). Furthermore, Smith et al., (2001) indicated that the fungus exhibited a substantial population and had infected all ten cotton fields in California.

CONCLUSION

The result of the endemic character of wilt disease incidence was established by the study conducted in main cotton-growing areas of Tamilnadu. Among the several areas where cotton wilt incidence was surveyed, Thiruchencodu had maximum incidence of the disease Veppathattai tagged along. The other locations had moderate incidence while Vellakoil and Perugamani marked lowest of all. All the isolates of wilt pathogen *F. oxysporum* f. sp. *vasinfectum* produced chlamydospores that grow in chains; the spores formed in tri-cells and were kidney-shaped, oval, tapering, and septate. From the current investigation, it is evident that *Fusarium* depends upon pH and temperature. As pH increases biomass and radial diameter decreases similarly temperature for isolation of *Fusarium*. So a suitable isolation protocol concerning all physical and environmental parameters, with and proper control of biological agents would help in restraining the production of wilt disease in cotton. Thus, enhances the productivity and economy of a region influencing countries with agricultural background.

REFERENCES

- Ainsworth, G.C. 1961. Dictionary of Fungi. Commonwealth Mycological Institute, Kew, Surrey, England, pp. 547.
- Baum, C. Eichler-Lobermann, B. Hrynkiewicz, K. 2015. Impact of organic amendments on the suppression of Fusarium wilt. In: Meghvansi, M.K., Varma, A. (Eds.), Organic Amendments and Soil Suppressiveness in Plant Disease Management. Springer International Publishing, Cham, pp. 353–362.
- Cianchetta, A.N. and Davis, R.M. 2015. *Fusarium* wilt of cotton: Management strategies. Crop Protection, 73, 40–44. doi:10.1016/j.cropro.2015.01.014
- Davis, R. M. Colyer, P. D. Rothrock, C. S and Kochman, J. K. 2006. Fusarium wilt of cotton:population diversity and implications for management. Plant Disease., 90:692–703.
- Desouza Blaise. 2021. Cotton (Gossypium species) production systems of India: Historical perspective, achievements and challenges. Indian Journal of Agronomy, 66: 119-128.
- McGovern, R.J. 2015. Management of tomato diseases caused by *Fusarium oxysporum*. Crop Prot 73:78–92.
- Minuto A, Spadaro D, Garabaldi A, and Gullino, M.L. 2006. Control of soil borne pathogens of tomato using a commercial formulation of Streptomyces griseoviridis and solarization. Crop Prot., 25: 468-475.
- Nash, S.M. and Snyder, W.C. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology., 52: 567-572.
- Nirmaladevi, D. Venkataramana, M. Srivastava, R. Uppalapati, S. and Gupta, V. 2016. Molecular phylogeny, pathogenicity and toxigenicity of *Fusarium oxysporum* f. sp. lycopersici. Scientific reports. 6:213-267.
- Pandey, M. Simon, S. and Neelam. 2014. Effect of organic soil amendment and soil solarization on wilt of chickpea. Int. J Bot. Res., 4(3):61-66.
- Rangaswami, G.. 1972. Diseases of crop plants in India. Prentice Hall of India Pvt. Ltd., New Delhi, pp. 520.
- Sankaranarayanan K, Nalayini P, Sabesh M, Usha Rani S, Nachane, R.P, and Gopalakrishnan N. 2011. Low Cost Drip–Cost Effective and Precision Irrigation Tool in Bt Cotton. Technical Bulletin No.1/2011, Published by Central Institute for Cotton Research, Regional Station, Coimbatore 641 003.
- 13.Smith, S.N. DeVay, J.E. Hsieh, W.H and Lee, H.J. 2001. Soil-borne populations of *Fusarium oxysporum* f. sp. *vasinfectum*, a cotton wilt fungus in California fields. Mycologia., 93: 737-743.
- 14.Wendel, J. and Grover, C. In: Fang, C. Percy, R.G. editors. 2015. Taxonomy and evolution of the cotton genus, *Gossypium*. Guilford Road, USA. pp. 25–44.
- 15.Zhao, L. Xu, Y. Lai, X.H. Shan, C. Deng, Z. and Ji, Y. 2015. Screening and characterization of endophytic *Bacillus* and *Paenibacillus* strains from medicinal plant *Lonicera japonica* for use as potential plant growth promoters. Braz. J. Microbiol., 46, 977–989.