Review Article

Studies on morphological and molecular traits of tuberose diseases and its chemical and biological control

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ABSTRACT

Tuberose is a popular flower crops with a unique place in loose flower as well as cut flower category. It is a key component used in the creation of premium perfumes. Tuberose productivity is reducing due to lot of pest and disease attacks. Tuberose flower is affected by many fungal, bacterial and viral diseases. It affects the growth and lead to losses in the flower yield of the tuberose. Common diseases of tuberose are stem rot, blossom blight, peduncle blight, *Alternaria* leaf spot, flower blight, *Sclerotium* wilt, flower bud rot and anthracnose. Chemical compounds have been used to control plant diseases at large scale, they do not increase the yield of the crops and instead it protects the crops from the harmful foreign invasion. The development of safer and environmentally feasible plant disease control has become a top priority. In this regard, biological control becomes an urgently needs for modern agriculture. The bio-agents aid in both disease management and crop yield enhancement. Therefore, the use of bio-agents for management of tuberose diseases is the intense research area in recent years.

Keywords: *Tuberose, Diseases, Morphology, molecular character, Bio-control agents, Chemical Management*

INTRODUCTION

India's floral industry is one of the growing profitability and export potential. Among the flower crops, tuberose (*Polianthes tuberose* Linn.) is a member of the *Asparagus* family and is commonly known as lily in the Indian market. It originated in Mexico. It is commercially grown for the cut and loose flower trades are used to make garlands, bouquets, and floral decorations for bridal makeup. The long flower spikes are excellent for table decoration. The flowers emit a delightful fragrance. Tuberose represents sensuality and is used in aromatherapy for its ability to open the heart and calm the nerves, restoring joy, peace, and harmony. Tuberose flowers have long been used in perfumery as a source of essential oils and aroma compounds. Tuberose

blooms are used for extraction of their highly prized natural flower oil. Tuberose oil is used in high-value perfumes and cosmetic products. Furthermore, fragrant flowers are added along with stimulants or sedatives to the favorite beverage prepared from chocolate and served either cold or hot as desired. Tuberose bulbs contain an alkaloid-lycorine, which causes vomiting. The bulbs are rubbed with turmeric and butter and applied as a paste over the red pimples of infants. Dried tuberose bulbs in powdered form are used as a remedy for gonorrhea. In Java, the flowers are eaten along with the juices of the vegetables. In India, Maharashtra, West Bengal, Tamil Nadu, Karnataka, Andhra Pradesh apart from Rajasthan, Gujarat, Punjab, UP and Assam are major states of single flowered type of tuberose (White) cultivation. Tuberose exported from Karnataka and Tamil Nadu alone accounts for 70% of the absolutes produced in India. In Tamil Nadu, it is one of the largest producers of tuberose in India. The districts of Madurai, Dindigul, Theni, Dharmapuri and Tirunelveli are well-known for tuberose farming due to the favorable climate and soil conditions. According to Ministry of Agriculture and Farmers Welfare, Govt. of India 2022-23 the area under tuberose cultivation in India was 21.75 lakh hectares and production of loose flower was 93.74 lakh metric tonne and cut flower was 172.45 lakh metric tonne. The area under tuberose cultivation in Tamil Nadu was 5.96 lakh hectares and production of loose flower was 53.76 lakh metric tonne. Tuberose market value is losing due to lot of disease attacks.

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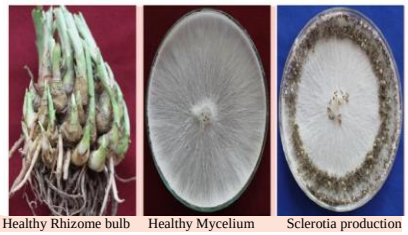

Fig .1. **Common Diseases of Tuberose**

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We going to see about the diseases of tuberose are Stem rot (*Sclerotium rolfsii*), Peduncle blight (*Lasiodiplodia theobromae*), Flower blight (*Botrytis cinerea*), *Alternaria* leaf spot (*Alternaria polianthi*), Leaf blight (*Botrytis elliptica*), *Sclerotium* wilt (*Sclerotium rolfsii*), Flower bud rot (*Erwinia* spp.), Anthracnose (*Colletotrichum truncatum*) and Blossom blight (*Fusarium equiseti*) (Jahagirdar *et al*., 2018; Zaman 2019; Verma *et al*., 2020). These diseases may lead to a crop loss even up to 75%. Management of tuberose diseases can be done by various techniques are cultural method, physical method, resistant varieties, chemical and biological control. In Integrated Disease Management (IDM) approaches fungicides are used as a last line of defense. Generally, for large scale management of diseases chemical control is used. Application of fungicides is mostly dependent upon the infection pressure on the plant and the effectiveness of the fungicide to control the disease. Across the globe, fungicides use the same active ingredients to combat a comparable range of fungal pathogens. As an alternative to fungicides, biological control agents have grown in favor in recent years. Weindling identified *Trichoderma* species as biocontrol agents (BCAs) for the first time in the early 1930s. *Pseudomonas* and *Bacillus* species have been employed in the biological treatment of tuberose diseases. Biocontrol agents are crucial, particularly when growing tuberose organically. The following section of this review is to address the morphological and molecular traits of tuberose diseases, as well as the chemical and biological approaches to their management.

INFECTION AND PATHOGENICITY

STEM ROT (*Sclerotium rolfsii*)

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1. Infection and pathogenicity (heading)
1.1. Stem rot (sub heading)

Stem rot causes significant losses in tuberose production, which can reach 50–60% in extreme circumstances. The estimated range of losses in Tamil Nadu is about 20–40%. It causes sucker rot, stem rot, basal stem rot, rhizome rot and as well as premature death of affected plants. In case of more sever conditions; affected plant does not produce flowering shoots. Fluffy mycelium and sclerotia production were used to identify the pathogen, *S. rolfsii*. The pathogen produces dormant resting bodies (sclerotia). These resting bodies survive in soil for many years which germinate under favourable condition and infect the plants.

Morphological characteristics: Boopathi (2023) conducted an *in vitro* investigation on the morphological and physiological characteristics of *S. rolfsii* in tuberose. He used eight isolates, numbered SR1 through SR8, concentrating on various *S. rolfsii* isolates that were cultivated and had mycelium development that ranged from 85.30 mm to 90.00 mm at the maximum.

Variations were observed in the growth pattern from fluffy to profusely thick, colony morphology and hyphae colour from white to dull white colour. Sclerotia size ranges from 1.06 - 1.69 mm; sclerotia shape were observed in round, irregular, and spherical; sclerotial weight 70 - 180 mg /weight of 100 sclerotial bodies; sclerotial production duration of 9 - 15 days; and arrangements from the periphery to all over petri plates of all eight isolates grown were concluded from his work.

Molecular characterization: Kokub *et al*. (2007) stated that this investigation among ten random decamer primers (A-01 to A-10) used to amplify the DNA of eight fungal strains of *Sclerotium rolfsii*. Out of ten primers, nine of them were able to identify polymorphism, and one (A-10) produced a monomorphic pattern. With an average of 11.6 bands produced per primer, 116 bands were produced in total. 64 (55.17%) of the 10 amplified products were polymorphic. The fungal strain D4 was able to amplify up to the maximum number of bands (91), which was followed by strains D2 and D5 with 85 bands while the fungal strains D9 and D6 respectively amplified the minimum number of bands, 79 and 81. In terms of genetic similarity, the strains D5 and D7 (94.44%) and D3 and D8 (83.52%) were the most similar, while the strains D2 and D7 (81.69% similar) were the most distant. Fungal strains of *S. rolfsii* could be categorized into two groups according to a cluster analysis (dendogram) created with Nei & Li's coefficients. Cluster II included six strains, namely D2, D4, D5, D6, D7, and D9, with similarity ranging from 83.75 to 94.44%. Cluster I which included D3 and D8, demonstrated similarity of 83.52%. Eighty-six percent of the two groups were similar when they joined. According to the dendrogram, strains D5, D7, and D4 shared 88.70% genetic similarity, while strains D5, D7, D4, and D9 shared 91.17%. In group I, the isolates with the highest genetic similarity were D5 and D7 (94.44%). Additional information from the dandogram showed that while D5, D7, D4, D9, D6, and D2 were 83.75% similar, D5, D7, D4, D9, D6, and D6 were 89.56% similar. All strains were 81.26% similar to one another overall, with only 18.74% of them differing from one another. Additionally, strains D5 and D7 were found to be 94.44% similar, while strains D3 and D8 were found to be 83.52% similar based on RAPD analysis. The results of this experiment validated the effectiveness of the RAPD-PCR method for identifying and estimating the genetic similarities and differences between the fungal strains that were gathered for this investigation. Consequently, it was discovered that RAPD analysis was a useful DNA marker system for determining the genetic diversity and relatedness between various strains (Malik *et al*., 2007;

Asif *et al*., 2005). Other researchers have also examined genetic variation among *S. rolfsii* isolates obtained from various geographic locations using RAPD-PCR analysis. Using random amplified polymorphic DNA (RAPD) polymerase chain reaction (PCR), Panja & Sun (1997) compared 128 isolates of *S. rolfsii* from 36 host species and 23 geographic regions. The results showed that many isolates from the same host belong to the same mycelial compatibility group (MCG). Almeida *et al*. (2001) examined the variation between 30 isolates of *S. rolfsii* from various hosts and geographical areas of Brazil by analyzing genomic DNA using the random amplified polymorphic DNA (RAPD–PCR) technique. These methods verified that the quantity, size, and positioning of sclerotia on the surface of media varied significantly between isolates.

Chemical management: In 2018, Divya Bharathi and Narayanaswamy worked on integrated management of *Sclerotium rolfsii*. They reported that carbendazim + press mud + trichomonazole recoded a lower incidence (10.66%), while hexaconazole @ 0.1% among fungicides recorded the lowest disease incidence of 19.33%. Only applying *Trichoderma viride* to the soil resulted in a lower disease incidence rate (28.00%). Neem cake and Press mud both exhibited minimal disease incidence (32.66% and 33.33%, respectively) among the organic amendments. Hexaconazole at 0.1%, Vitavax power at 0.1%, and carbendazim at 0.1% were comparable fungicide treatments, but they were substantially better than the untreated control group. Hexaconazole had the lowest incidence at 0.1% (19.33%), while Captan had the highest disease incidence at 0.2% (26.66%). These findings are consistent with Vanitha and Suresh's (2002) theory that triazole fungicides impede the fungus's ability to synthesize ergosterol.

Biocontrol management: The biocontrol effectiveness of native *Pseudomonas fluorescens* against *Sclerotium rolfsii* in tuberose was studied by Sankara Reddy *et al*. (2021). Ten isolates, Pf1–Pf10, were used in this study to examine the *in vitro* effectiveness of *P. fluorescens* against *S. rolfsii* using the dual culture method. Six treatments, T1–T6, were used to examine the impact of *P. fluorescens* culture filtrate on *S. rolfsii* mycelia growth using the poison food technique. By using the dual culture method, all native *P. fluorescens* isolates were found to significantly inhibit *S. rolfsii* mycelial growth. Pf7 exhibited the highest growth inhibition of 75.94%, followed by Pf9 (74.41%), Pf5 (73.00%), and Pf6 (55.58%), which showed the lowest growth inhibition of the pathogen. According to Manjunatha *et al*. (2012), using the dual plate method, *P. fluorescens* demonstrated the greatest inhibition of *S. rolfsii* mycelial growth. As per O'Sullivan and O'Gara (1992), a key mechanism underlying the biological activity of

Pseudomonas spp., which are well-known for producing broad-spectrum antibiotics like 2, 4-diacetylphloroglucinol, has been demonstrated. *Pseudomonas* species also produce siderophores and HCN, which contribute to their antifungal activity. The findings of the *in vitro* experiments carried out to determine the impact of *P. fluorescens* on culture filtrate on the mycelial dry weight and growth of *S. rolfsii* using the poison food technique. When the concentration of *P. fluorescens* culture filtrates increased, the mycelial growth of *S. rolfsii* decreased. This reduction was greatest when *P. fluorescens* culture filtrates contained 39.63, 29.31, 11.32, and 0.00 mm at 10, 20, 30, and 40% of the culture filtrate, respectively, compared to the control's maximum growth of 90 mm. In the case of the liquid medium assay, the same patterns were observed. While the flasks inoculated with *S. rolfsii* alone (control) recorded the maximum mycelial dry weight (430.15 mg), the flasks inoculated with the pathogen and amended with *P. fluorescens* culture filtrate showed a significant reduction in the mycelial dry weight. In 40% of the culture filtrate of *P. fluorescens*, the minimum mycelial dry weight (1.65 mg) of *S. rolfsii* was found. This value was comparable to that of *P. fluorescens* at 40% and carbendazim at 0.1 percent (3.89 and 1.65 mg, respectively). In a similar vein, Chanutsa *et al*. (2014) reported that *S. rolfsii* growth was totally inhibited by the isolates culture filtrate of three bacteria. The decrease in pathogen growth may be explained by the antifungal metabolites that *P. fluorescens* produces. According to O'Dowling and O'Gara (1994), *P. fluorescens* was known to produce a variety of low-molecular weight metabolites, some of which had the potential to be antifungal agents. According to a number of studies (Velazhahan *et al*., 1999; Meena *et al*., 2001), *P. fluorescens* antagonistic potential against different soil-borne plant pathogens was correlated with the production of lytic enzymes. Pseudomonads cell-free extracts efficiently stopped *R. solani* from growing (Saxena *et al*., 1995). According to Revathy and Muthusamy (2003), *P. fluorescens* culture filtrate was the most successful in preventing *S. rolfsii* from growing mycelially. The mycelial growth of *Sclerotium rolfsii* was inhibited by the culture filtrate of *Pseudomonas fluorescens* isolates I7 (Venkatesh, 2013) under *in vitro* conditions. Khosla and Gupta (2005) found that in stem rot-affected tuberose plants, soil drenching with *T. viride* alone resulted in a lower disease incidence (28.00%) compared to the untreated check (94.66%). Islam (2005) discovered that the stem rot-causing *S. rolfsii* was effectively antagonistic to an isolate of *T. harzianum*. Vitavax-200 was found to be the most effective chemical to saturate soil with in order to control the growth of *S. rolfsii* and *T. harzianum* isolate R1. In both pot experiments and

field conditions, Vitavax-200 was found to be the most effective chemical to control foot and tuber rot of tuberose caused by *S. rolfsii*.

Fig.2 **A. *Alternaria* Leaf spot; B. Pure culture; C. Conidia**

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ALTERNARIA LEAF SPOT (*Alternaria polianthi*)

In tuberose, *Alternaria polianthi*, the cause of *Alternaria* leaf spot, is a significant fungal disease (Mariappan *et al*., 1977). The occurrence of leaf spot in tuberose was initially documented in the Indian state of Tamil Nadu (Muthukumar *et al*., 2007) and later in the Coimbatore locality (Mariappan *et al*., 1977). There have also been reports of another kind of *Phoma* spp. related leaf blight/tip burn (Punja *et al*., 2016). Because of the frequent high rainfall and humid weather, the disease is common in both single- and double-type tuberose varieties. Brown spots on the tip or margin of leaves, measuring 10–30 cm in length and 4-5 cm in diameter, are a sign of the *A. polianthi* disease. These spots gradually become round to oval in shape. Later, spots coalesce and develop typical blight symptom.

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Morphological characteristics: *Alternaria polianthi* isolate's growth characteristics on PDA medium showed that the culture started out looking grayish white before changing to dark grey or black. The mycelium has a fluffy, cottony texture and a somewhat rounded edge. There is no evidence of zonation. The mycelia measured between 8.5 and 9 cm in diameter on the third and fourth days. According to the differences in the growth characterization of the *A. polianthi* isolates, the majorities of the cultures had regular or irregular growth patterns and were gray or brown in color with light or dark brown pigmentation (Loganathan *et al*., 2016). Five *A. polianthi* isolates showed notable differences in their cultural traits, pigmentation, and daily growth rate, according to Zaman *et al*. (2019). Various isolates of the pathogen produced colonies that ranged in color from white to dark black, irregular to circular, smooth to rough, and with or without concentric zonation. As according to Singh *et al*. (2020), the mycelia in the culture grew from grey to brown at first before turning dark black. The fungus's septate hyphae were found to be dark brown to black in color.

Chemical management: In order to manage *Alternaria* leaf spot on tuberose, Mazumder *et al*. (2016) evaluated six chemicals: Mancozeb 75% WP @0.2%, Chlorothalonil 75% WP @0.2%,

Tricyclazole 75 WP @0.1%, Iprodione 25% + Carbendazim 25% WP @0.1%, Difenconazole 25 EC @0.1%, and Azoxystrobin 23 SC @0.1%. The most successful treatment was four sprays of Azoxystrobin (0.1%) spaced 15 days apart, beginning at the onset of the disease. This led to the lowest disease incidence (10.98 PDI) when compared to the control group (34.39 PDI) and a cost-to-benefit ratio of 6.87. Nonetheless, when applied at a 0.1% concentration, the combo fungicide of Iprodion (25%) + Carbendazim (25%), followed by Difenconazole, proved to be effective, according to the economic analysis. The greatest B:C ratios for these treatments were 7.11 and 6.90, corresponding to 69.98% and 71.47% disease reduction, respectively. According to Kakade *et al*. (2016), tuberose receives less protection; however, because of changes in the climate, *Alternaria polianthi*, which causes leaf blight disease, is becoming a major threat in the state of Maharashtra, accounting for 15-20% of losses in tuberose yield and quality. It was discovered that six applications of Iprodine + Carbendazim @ 0.1%, spaced ten days apart, beginning with the first spray at the onset of the disease, were beneficial in improving the control of leaf blight and boosting tuberose yield and financial returns. The effectiveness of Iprodione (25%) and Carbendazim (25%) as well as Difenconazole (0.1%) in reducing tuberose leaf spot was reported by Sharma and Bhattacharjee (2002). The most effective combination fungicide, according to their economic analysis, was one that sprayed a mixture of 25% Iprodione, 25% Carbendazim, and 0.1% Difenconazole. This combination registered higher benefit-cost ratios of 7.11 and 6.90, with respective percentages of disease control of 69.98 and 71.47.

Biocontrol management: The management of leaf spot by biocontrol agents (*Trichoderma asperellum* and *Bacillus subtilis*) was reported by Mahalakshmi *et al*. (2023). Using the dual plate method, *T. asperellum* and *B. subtilis* were evaluated *in vitro* against the tuberose *Alternaria* leaf spot pathogen, *A. polianthi*. The growth of pathogen was significantly reduced in all of the isolates. For *T. asperellum* and *B. subtilis*, the mean value from the three replications showed growth inhibition of 49.34 and 37.45% over control, respectively. Ten native antagonistic bacteria were tested *in vivo* by Moges (2012) to see if they could suppress the pathogen. He detailed the effectiveness of five antagonists that showed promise, with higher percent disease control (38.16–43.79%) and zone of inhibition (ZOI) (38 mm and above). Ramanujam *et al*. (2015) assessed the bacterial antagonists *in vitro*, in a glasshouse, and in the field against the tuberose leaf spot pathogen *A. polianthi*. *P. fluorescens* was the isolate that tested best, with a 62% reduction in leaf spot incidence and a 37% increase in flower yield over

control. In this work, the effectiveness of *B. subtilis* was evaluated *in vitro* against *A. polianthi*. It demonstrated a notable slowdown in the pathogen's growth. The average of the three replications' growth inhibition over the control was 37.45%.

PEDUNCLE BLIGHT (*Lasiodiplodia theobromae*)

Fig .3A Peduncle dieback **B. Darkbrown, flask-shaped, Ostiolate pycnidia**

On Polianthes in Cuba, *Lasiodiplodia theobromae* was documented by Arnold (1986). The fungus affects both monocot and dicot plants, resulting in a variety of symptoms such as dieback and shoot blight. The *Lasiodiplodia theobromae* pathogen, which causes tuberose peduncle blight, was described by Punithalingam in 1980. There are many different types of hosts for this fungus in the tropics and subtropics. Durgadevi and Sankaralingam (2012) reported observations of pedicle blight in Madurai and Dindugal district of Tamil Nadu, with an incidence of up to 43%. The infection was found to be exhibit blossom blight followed by peduncle blight starting from the tip, and leaf blight at the tips. It caused all of the flower buds to disappear. Over the infected spike, several pycnidia were seen. Peduncle blight, a previously unidentified illness, was discovered to be a significant hindrance to the cultivation of tuberose. Although *L. theobromae* is a common pathogen, its manifestation on tuberose represents a novel finding. Confounding symptoms caused by the fungus included leaf blight at the tips, peduncle blight, and blossom blight.

Morphological characteristics: The prevalence and characterization of *L. theobromae*, an emerging tuberose disease in Tamil Nadu, were reported by Durgadevi *et al*. (2017). Of the seven isolates of *L. theobromae*, namely I1–I7, no symptoms were seen until the fourth day. Five days after inoculation (DAI) was when the symptoms appeared, and the lesion length grew over time. When the fungal inoculum was applied to the pin-pricked bud as opposed to the one without the lesion length increased. In pinprick buds, the lesion length at seven DAI measured 570 nm, whereas in non-pinprick buds, it measured only 300 nm. Out of the seven isolates, the colony type ranged from fluffy to dense, with colors ranging from dull white to black. After 48 hours, the mycelium growth rate was observed to be between 64.30 mm and 90.00 mm

Molecular characteristics: The confusion caused by morphological characteristic overlap is minimized by molecular techniques (Pavlic *et al*., 2004; Burgess *et al*., 2006). Using DNA sequence data (amplification of rDNA ITS 1 and ITS 4) derived from fungal species, the genus *Botryosphaeria* was distinguished (Slippers *et al*., 2004; Begoude *et al*., 2010). Identification and description of the fungus were most successfully achieved through ITS-rDNA region amplification. It was determined by comparing the sequence with the NCBI database that the fungus was *L. theobromae*. The *L. theobromae* isolate from *H. cannabinus* grown in a tropical climate was confirmed by the sequence data. This study demonstrated the utility of ITS rDNA sequence information in the identification of *Botryosphaeriaceous* species. There are cryptic species that cannot be distinguished based only on their ITS-rDNA sequence data, despite the region of the genome's general phylogenetic utility (De Wet *et al*., 2003; Slippers *et al*., 2004).

Chemical management: Banik *et al*. (1998) discovered that at 400 ppm, carbendazim completely stopped *L. theobromae* linear growth, which was then followed by thiophanate-methyl at 450 ppm. Thiophanate-methyl (0.1%) and benlate (0.1%) were shown to be able to completely inhibit *L. theobromae* growth *in vitro*, according to Shelar *et al*. (1997). Similar to this, Mahmood *et al*. (2002) found that *L. theobromae* colony growth was totally inhibited by Benlate at 100 ppm and Topsin-M (Thiophanate-methyl) at 50 ppm. Thiophanate-methyl inhibited *L. theobromae* mycelial growth and conidial germination, according to Li *et al*. (1995).

Biocontrol management: Durgadevi *et al*. conducted research on biocontrol agents for the management of tuberose peduncle blight (2014). The study employed a dual culture technique to test three isolates of *Fluroescent pseudomonads* (Pf1, Fp7, and Pf2), *Bacillus* spp. (Bs10, Bs1, and Bs2), and *Trichoderma* spp. (Tv1, Tv2, and Tv3) against *L. theobromae* (Dennis and Webster, 1971). Treatment T7 (Tv1+Pf1+Bs10), one of the biocontrol agents, had a 30.50% disease incidence, followed by treatment T6 (Pf1+Bs10), which had a 31.60% incidence. Both treatments were comparable. With the least amount of disease reduction (54.44%), bulb treatment and foliar spray with T1 (Tv1) recorded 42.00% disease incidence. Although the growth of *L. theobromae* was inhibited by all three of the isolates of *Bacillus* spp., Bs10 was found to be more effective. Previous researchers have reported that *B. subtilis* strains inhibit *B. theobromae* (Okingbo, 2005; Swain and Ray, 2009; Swain *et al*., 2008). It was discovered that the three *P. fluorescens* isolates had differing antagonistic activities against *L. theobromae*, with two of the isolates, Pf1 and Fp7, exhibiting highly inhibitory effects on the pathogen. The most

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successful bacterial biocontrol agent was *P. fluorescens* isolate Pf1, which was able to inhibit *B. theobromae* growth by up to 84.8% (Govindaiah *et al*., 2003; Sharma *et al*., 2009). The management of fruit rot caused by *B. theobromae* has made use of *T. harzianum* and *T. atroviridae* (Kexiang *et al*., 2002). Pramod *et al.* (2007) reported that B. *theobromae*-induced post-harvest rot of papaya was effectively prevented by *Trichoderma* spp.

BOTRYTIS BLIGHT (*Botrytis cinerea*) **a) Infection on leaves b) severe infection c) typical rotting flower**

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A polyphagus necrotrophic fungal pathogen is called *Botrytis*. Numerous species that vary in their biology, ecology, morphology, and host range have been identified within the highly diverse genus *Botrytis* (Elad *et al*., 2004). *Botrytis* blight is the newly emerging disease of tuberose affecting foliar parts of the plant and in severe stages; it affects the floral parts and causing them to rot. *Magnoporthe oryzae* is the most significant fungal pathogen, with *Botrytis cinerea* ranking second in terms of damage caused (Dean *et al*., 2012). It can live on more than 200 different types of plants. Crops growing in temperate to subtropical regions are susceptible to infection. It establishes its position as one of the most significant and harmful post-harvest pathogens. It harms fruits, vegetables, and decorative crops while they are being transported as well as in the field or stored (Jarvis, 1977).

Fig.4 Symptoms of Botrytis blight

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Morphological characteristics: Every isolate was identified as belonging to *B. cinerea* based on morphological characteristics (Hennebert, 1973). The first characterization of *Botrytis cinerea* infecting tuberose in India was reported by Priyadharshini *et al*. (2019). They take four isolates, Bc1 through Bc4, from two districts of Tamil Nadu viz., Erode and Coimbatore. Microscopic observations showed that, with the exception of the Bc1 isolate, all four isolates showed substantially different mycelial patterns and growth characteristics. Full plates were covered in 5 to 10 days, and the colonies ranged in color from dull white to brown. The mycelium was fluffy to irregular warty type. The observation on sclerotial production showed that the sclerotia were formed when the fully grown cultures were exposed to stress conditions and were incubated at a temperature of approximately 35°C for five to six days. Each plate had a minimum of eight and a

maximum of forty numbers of sclerotia from the four isolates, with the prominent black sclerotia formed at the center and the irregular sclerotia formed randomly throughout. From the cultures of all four isolates that were seven days old, histopathological characteristics were also noted. According to Ahmed *et al*. (2007), conidia of *Botrytis cinerea* were found in clusters at the terminal end, giving the appearance of grape bunches. Each conidium was hyaline, shaped like an oval, ellipsoid, or globose, with a hilum at the tip. These traits were consistent with *Botrytis cinerea* microscopic observations. Where the pathogen produced alternately branching conidiophores that produced oval-shaped conidia that resembled grape bunches.

Molecular characteristics: PCR molecular technique was used to confirm the identities of the isolates. Several species-specific primers have been employed in the detection of *Botrytis cinerea* (Rigotti *et al*., 2002). One of the key primers used in the identification of *B. cinerea* was created by Rigotti *et al*. (2002) and is specific to this species in particular. All 30 isolates as well as the reference isolate had an amplified single band measuring 0.7 kb, which is unique to *B. cinerea*. Not a single band in the negative control was amplified. *B. cinerea* isolates can be quickly and precisely identified using a molecular technique that uses primers specific to each species.

Chemical management: Verma *et al*. (2020) state that the disease can be effectively controlled by spraying the plants with carbendazim at a rate of 2 g/l of water. There should be a 15-day gap between treatments. Additionally, the plant can be sprayed with greeno (0.5%) or ammonical copper (2%) to control the disease. There should be a 15-day gap between treatments. Spraying with carbendazim at a rate of 2 g/l or hexaconazole at a rate of 1 ml/l can be recommended as soon as the disease manifests, with a 15-day interval. This was stated by Jahagirdar *et al*. (2018). Removing contaminated flower buds will aid in the inoculum reduction process. Another way to control them is to apply Greeno or ammonical copper spray.

Biocontrol management: Orozco-Mosqueda *et al*. (2023) state that different PGPB metabolites, like *Pseudomonas* spp., can have different effects on the early or intermediate stages of the life cycle of *B. cinerea* as well as the infection process. For instance, by generating substances that influence the development of aberrant germ structures and lessen the extension and morphology of the germ tube, *P. antimicrobica* prevents the germination of conidia in *B. cinerea*. Additional research has shown that antifungal substances in *P. fluorescens* strain QBA5 supernatant can seriously harm the plasma membrane of *B. cinerea* conidia. Similarly, some strains of

Pseudomonas spp. produce lipopolysaccharides that play a crucial role in regulating the growth of the *B. cinerea* mycelium by causing damage to the hyphae and altering their morphology.

BLOSSOM BLIGHT (*Fusarium equiseti*)

Fig .5 Blossom blight symptoms **_F. equiseti_ (white colony)**

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There have been reports of *F. equiseti*-caused tuberose blossom blight (Roy, 1984). On petals, light brown lesions appear; these quickly turn dark and cause the tissue to dry out. The blighted flowers fall off the stalk. There was also an infection on the flower stalk, which caused it to collapse. Taiwan has reported cases of tuberose (*Polianthes tuberosa*) blossom blight. There haven't been any systematic studies done on Bangladesh's tuberose disease problem up to this point but they recently done vegetative growth and molecular identification of *Fusarium equiseti* in thankudi plant by Akter *et al*. (2022). Diseases that are spread by the soil, like *Fusarium* spp., can seriously harm greenhouses, orchards, and field crops. After 10 to 12 days of inoculation, the first signs of the disease appeared in the case of seedlings inoculated with *Fusarium equiseti*. It has been known to cause wilt in cauliflower in China and wilt in cumin in some parts of India.

Morphological management: In 2021, Hami *et al*. In the instance of *Fusarium equiseti*, pure culture produced white colonies that, after 10 days of incubation at 25 ± 1 °C, later turned peach orange at the agar base with a growth of 90 mm. The mycelium was smooth, branched, septate, cylindrical, and measured 3.25–4.80 µm in width, according to microscopic observations. Conidiophores were septate, simple, cylindrical, short, and measured 72.50–106.30 × 3.00–4.5.0 µm. The oval, hyaline, 0–1 septate microconidia had a size range of 12.00–16.00 × 3.20–4.50 µm. The curved macroconidia had a prominent foot cell, a tapered and elongated apical cell, were hyaline, two to five septate, and measured 24.00–40.00 × 4.00–6.00 µm in size. Clamydospores were nearly spherical, hyaline, produced intercalary, singly or in chains, and had a diameter of 5.00–11.00 µm.

Biocontrol management: Rahman *et al*. (2012) attempted to manage *Fusarium equiseti* using a variety of techniques in a lab setting, such as fungicides, organic amendments, and *Trichoderma harzianum*. The four chosen fungicides are Aimcozim, Cupravit, Dithane M-45, and Newban have been tested *in vitro* at three distinct concentrations are 100, 200, and 400 ppm to see if they

can stop *F. equiseti* radial growth. Aimcozim significantly outperformed other fungicides in terms of results, completely inhibiting the radial growth. In terms of hyphal growth inhibition of *F. equiseti*, Dithane M-45 and Cupravit demonstrated moderate inhibition of 75.00-77.94%, but were significantly better than Newben. The study examined the impact of organic amendments on the hyphal growth of *F. equiseti* isolate F15. Mustard oil cake (*Brassica napus*) at the highest concentration of 3% resulted in the maximum 59.07% inhibition of the hyphal growth of *F. equiseti*, which is significantly better than all other amendments. At a 3% concentration, til (Sesame indicum) oil cake showed a significant 56.18% radial growth. At all concentrations, soybean (Glycine max) oil cake was shown to be the least effective in stopping the radial growth of *F. equiseti*. The most effective *T. harzianum* isolates were determined to be T6, T1, T2, T6, T9, T11, T14, and T18. These isolates demonstrated the highest level of 71.69% inhibition of colony growth against the test pathogen. The isolates T16 and T10 had the lowest radial growth inhibition (50.91%). *Trichoderma* sp. was discovered to be one of their highly promising biocontrol agents against *Fusarium* sp., which causes tuberose blossom blight.

ANTHRACNOSE (*Colletotrichum truncatum*)

Fig .6 Anthracnose on leaf and peduncle **Colony of *C. truncatum* in PDA medium**

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Due to its extensive host range in warmer and more humid regions of the world, the genus *Colletotrichum* is well-known as a significant plant pathogenic fungus (Ford *et al*., 2004; Shenoy *et al*., 2007; Damm *et al*., 2009; Diao *et al*., 2014; He *et al*., 2016; De-Silva *et al*., 2017). According to Hyde *et al*. (2009), it causes anthracnose disease in a variety of fruits, vegetables, and other crops. Three Taluks (Nanjangud, Thirumakoodalu Narasipura, and Kollegala) of two districts Mysore and Chamarajanagar recorded cases of anthracnose disease on tuberose leaves and peduncles. It spans roughly 12 hectares between October 2015 and March 2016. From each district, ten fields were chosen at random, and the percentage of infected plants among every 100 plants observed was used to calculate the disease incidence (Mahadevakumar *et al*., 2017). On afflicted leaves and peduncles, anthracnose disease was identified by sunken, circular lesions containing masses of black acervuli, or spores. Necrotic spots were the first signs of the disease, and these eventually grew larger and merged to form larger concentric rings. It was also noted

that acervuli were developing on the leaves and peduncles throughout the necrotized area. The lesions had a diameter of 2-3 cm. Early-stage infections result in total defoliation. In severely damaged plants, the inflorescence never emerged, causing peduncles to topple and inhibiting full flower production. In 12 ha of tuberose fields studied in Karnataka, the disease incidence ranged from 18 to 27%. Thirumakoodalu Narasipura had the highest incidence (27%) followed by Nanjangud (22%) and Kollegal (18%) had the lowest disease incidence.

Morphological characteristics: The first report of *Colletotrichum truncatum* associated with anthracnose disease on tuberose in India was done by Mahadevakumar *et al*. in 2019. Morphological traits are the primary means of identifying different species of *Colletotrichum* (Sutton, 1992). Recently, molecular identification tools have been used to identify certain *Colletotrichum* species that are known to have overlapping conidial and colony characteristics (Sherriff, 1995; Hyde *et al*., 2009; Cannon *et al*., 2012; He *et al*., 2016). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and ß-tubulin genes are employed in this study in addition to ITS-rDNA to identify *C. truncatum* and bolster the pathogen's morpho-cultural identity. From leaves and peduncles affected by anthracnose, a total of seven isolates were obtained. The color of the fungus colony turned from gray to dark gray. After seven days, the colony had grown at a rate of 4.2 ± 0.4 mm/day (3.5–4.9), with an average colony diameter of 47.2 ± 3.8 mm. The mycelia of fungi were septate, branched, and hyaline. Pale brown to dark brown (73–165 µm) setae with two to six tapering septa were present. On a long conidiogenous cell, conidia were born. The conidium long central section had parallel walls and a slight curvature. Conidia are hyaline, single-celled, falcate to nearly straight, with a noticeable clear area in the middle of the highly granular cytoplasm. They end abruptly at the round and truncate base (17.6-21.6 µm×2.57–3.31 µm).

Molecular characteristics: Andrus and Moore were identified *Colletotrichum truncatum* (Schwein.) as the fungal pathogen based on micro-morphological and cultural characteristics (Sutton, 1992; He *et al*., 2016). There was evidence of successful amplification of the predicted amplicon sizes of 290 bp (GAPDH), 450 bp (ß-tubulin), and 600 bp (ITS). 100% similarity with the reference sequence of *C. truncatum* (ITS-AJ301945, KC460308, JX971160; GAPDHKC109579, KC109580, KC109581; and ß-tubulin-KC109459, KC109460) was found by nBLAST sequence analysis. Accession numbers were obtained and the sequences were deposited in GenBank. The fungal isolates from the current study also shared a common clade of

C. truncatum, which was represented by reference sequences from GenBank, according to phylogenetic analysis of concatenated sequences of ITS + GAPDH + ß-tubulin. This finding confirmed the identity of the isolated pathogen as *C. truncatum* based on morphological, cultural, and molecular sequence analysis. After eighteen days following inoculation, pathogenicity tests on healthy tuberose plants elicited classic anthracnose disease symptoms. On the inoculated plants, fusiform, grayish brown lesions appeared. Smaller necrotic spots appeared on leaves and peduncles, which eventually combined to form larger areas. On PDA medium, the associated fungal pathogen was reliably isolated from challenge-inoculated plants, and its identity was verified by micro-morphological traits.

Flower bud rot (*Erwinia* spp.)

Fig . 7 **Symptoms of flower bud rot**

The prime suspect is *Erwinia* spp. for causing flower bud rot in tuberose. Brown water soaked lesions on the buds are indicative of the early symptoms. The increased severity causes brown necrotic discoloration of the peduncles and dry rotting of the buds. According to Trujillo (1968), *Erwinia* sp. is responsible for a bacterial rot of flower buds that happens in warm, humid weather. The unidentified insect vector linked to this bacterial blight lays eggs on the flowers when they are still in the bud stage. Usually, the soft rot bacteria start to rot the tissues as soon as the eggs hatch, allowing the insect larvae to grow. According to Mandal *et al*. (2018), *Erwinia* sp. is the bacterium that causes flower bud rot. The symptoms included brown necrotic discoloration on the peduncles and dry rotting of the buds. The application of Streptomycin (0.01%) spray can help manage the illness.

Management: Jahagirdar *et al*. (2018) stated that it is best to remove and destroy diseased plants. A spray containing 0.5 g/l of streptomycin and 2.5 g/l of copper oxychloride can be used to measure the disease. Depending on how severe the illness is more sprays may be applied.

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CONCLUSION:

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In conclusion, the review on diseases of tuberose and its chemical and biocontrol management highlights the importance of understanding and addressing the challenges posed by these diseases. The analysis of various diseases affecting tuberose, such as Stem rot, Blossom blight, Peduncle blight, *Alternaria* leaf spot, Flower blight, *Sclerotium* wilt, Flower bud rot and Anthracnose has demonstrated the detrimental impact they can have on the crop's growth and yield. Through the analysis of various research studies and expert opinions, it is evident that a combination of chemical and biocontrol measures is necessary for effective management. While chemical control methods offer immediate results, they come with their own set of limitations and environmental concerns. To combat these diseases, growers have employed chemical measures such as fungicides are carbendazim, trichomonazole, hexaconazole, vitavax, captan, triazole, mancozeb, chlorothalonil, tricyclazole, iprodione, carbendazim, difenconazole, azoxystrobin, thiophanate-methyl, benlate, greeno, ammonical copper, aimcozim, cupravit, dithane M-45, streptomycin and copper oxychloride for reduces the diseases of tuberose. On the other hand, biocontrol measures are *Trichoderma viride*, *T. harzianum*, *T. asperellum*, *T. atroviridae*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *P. antimicrobica* & *Fluroescent pseudomonads* provide a sustainable and eco-friendly approach, albeit with a longer-term perspective. Therefore, a strategic integration of both approaches can ensure optimal disease management in tuberose cultivation, leading to improved yields and plant health. By adopting a holistic approach, farmers and researchers can strive towards sustainable, environmentally friendly solutions that ensure the health and productivity of tuberose crops. Overall, this review provides valuable insights for the agricultural community in tackling tuberose diseases and preserving its economic significance.

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Overall paper is very nice, good for publishing, little bit mistakes otherwise paper is so good.

Commented [Rg14]: