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RESEARCH ARTICLE

HOST-PATHOGEN INTERACTIONS OF TOMATO AND *FUSARIUM OXYSPORUM* F.SP. *LYCOPERSICI* PATHOGEN CAUSING WILT AND ITS CONTROL THROUGH BOTANICAL EXTRACT

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ABSTRACT

Fusarium oxysporum f. sp. *lycopersici* (Fol) is a soil-borne plant pathogen that causes wilt in tomato (*Lycopersicon esculentum* L.*)* and also incurs economic losses threatening tomato industry worldwide. The interaction between tomato and Fol has become a model system for the study of the histological basis of disease resistance and susceptibility in plants. Therefore, in the present study the host pathogen interactions of Fol on tomato during the wilt infestation and *in vitro* antifungal assay of *Cissus quadrangularis* in minimizing the pathogen growth was studied to generate remedies to prevent the disease for the benefit of farming community. During the study it was observed that 80% concentration of *Cissus quadrangularis* plant extract has significantly suppressed the fungal colony growth (16.2mm) followed by the 60% concentration of the plant extract (13.1mm). However, lower concentration of plant extracts (20 & 40%) has exhibited their inability in suppressing the fungal growth. The *in vivo* studies on fungal spore suspension inoculation to normal seedling of T1 (no plant extract treatment) and treated with plant extract (T2) and T3 of resistant (INDEX-300) seedlings showed minimal host pathogen interaction compared to normal seeds (T1) indicating that plant extract has induced resistance in T2 & T3 seedling to the Fol inoculation. The above results clearly indicated that plant extract of *Cissus quadrangularis* can be used as biological control agent (botanical extra) for suppressing and minimizing the tomato wilt disease.

Key words: Botanical extract, *Cissus quadrangularis, Fusarium oxysporum* f. sp. *lycopersici* (Fol), Tomato wilt disease.

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INTRODUCTION

Fusarium oxysporum f. sp. *lycopersici* (Fol) is a soil borne pathogen causes wilt in tomato and pathogen occurs throughout the tomato-growing worldwide areas. Tomato being important commercial vegetable crop rich in vitamins A, B & C grown throughout the world. In India it occupies 0.54 million hectares with a production of 7.60 mil. Tons (Asha *et al.,* 2011; Reis *et al.,* 2005). It can be grown in three season's *viz*. winter, summer & autumn. Seed is the most important input for any crop production. Many diseases and disorders can affect tomatoes during the growing season. *Fusarium oxysporum* f. sp. *lycopersici* is a highly destructive pathogen of both greenhouse and field grown tomatoes in warm vegetable production areas. The disease caused by this fungus is characterized by wilted plants with yellowed leaves and minimal (30 to 40% yield loss) or absent crop yield (Kirankumar *et al*., 2008).

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Fol is a soil-borne plant pathogen that causes wilt in tomato (*Lycopersicon esculentum* L.) and also incurs economic losses threatening tomato industry worldwide. The interaction between tomato and Fol has become a model system for the study of the histological basis of disease resistance and susceptibility in plants. The fungus colonizes the vascular bundle and clog water flow and nutrient movement, leading to wilt, and ultimately causes plant death. Successful plant infection and tissue colonization by *Fol* is an active process that involves a variety of cell wall degrading enzymes (CWDE), regulation of nutrient metabolism, and secretion of effectors to suppress and/ or overcome the physical basal defence in tomato plants (Essarioui *et al.,* 2016). In several cases, losses in tomato production can reach 80% (Huang and Lindhout, 1997). In general, Fusarium wilts first appear as slight vein clearing on the portion of the younger leaves, followed by epinasty (downward drooping) of the older leaves (Fig. 4). At the seedling stage, plants infected by *F.oxysporum* may wilt and die soon after symptoms appear. In older plants, vein clearing and leaf epinasty are often followed by stunting, yellowing of the lower leaves, formation of adventitious roots,

wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves, and finally death of the entire plant (Agrios, 1998). All strains of F. oxysporum are saprophytic and able to grow and survive for long periods on organic matter in soil and in the rhizosphere of many plant species (Garrett, 1970). Fusarium wilt pathogens show a high level of host specificity based on the plant species and plant cultivars they can infect (Armstrong and Armstrong, 1981). Jones (1991) noticed that Fol is the causal agent of a severe wilt disease in tomato whereas *F. oxysporum* f.sp. *radicis-lycopersici* (Forl) causes crown and root rot . Management of seed-borne and soil-borne diseases such as wilt caused by *Fusarium* species has always been problematic. Soil solarisation/ disinfection, crop rotation and mixed cropping are the best ways of eliminating soil borne pathogens. Seed treatment with synthetic fungicides considerably reduces the wilt incidence in tomato. However, their use is costly as well as causes environmental pollution.

The use of resistant varieties is one of the most effective alternative approaches to control the wilt disease, but due to breakdown of resistance in the face of high pathogenic variability in the pathogen population, the usefulness of many cultivars is restricted to only a few years (Singh, 2005). Though number of studies on mechanism of plant infection, penetration and tissue colonization by Fol is narrated but still detailed studies on these aspects are needed (Essarioui *et al.,* 2016). Therefore there is a need to develop alternative strategies to provide durable resistance over a broad geographic area. In this context, biocontrol and botanicals is an eco-friendly way of managing *Fusarium* wilt in tomato which offers an alternative to fungicides. Several biocontrol agents such as *Pseudomonas* sp and plant growth promoting rhizobacteria (PGPR) are extensively used for controlling the soil and foliar plant pathogenic microorganisms, Several workers attempted controlling tomato wilt through the use of biological, chemical as well as botanical extracts. *Cissus quadrangularis* L. is commonly known as "*Asthisamhari*" in ayurveda is a succulent plant of family Vitaceae and found hotter part of the India. Historically the plant has its own importance in Indian Ayurveda and used as medicinal plant for various ailments and purposes (Shah *et al.,* 2011). However, use of *Cissus quadrangularis* L. plant extract in controlling tomato wilt is limited. Hence through this experiment an attempt was made to assess the said plant extract impact on tomato wilt control.

MATERIALS AND METHODS

Samples of diseased tomato plants were collected from Bilikere region, Hunsur taluk, and Mysore district. Infected plant material was surface sterilized with 0.1% $HgCl_2$ for 30 sec, washed thrice with sterile distilled water. Seeds of Tomato both local (Bilikere region, Hunsur taluk, Mysore district, Karnataka) and Resistant varieties-INDEX 300 was collected from local market of Mysore

i). Isolation, identification and maintenance of pathogen: Isolation of pathogen was carried out by cutting the fragments, surface sterilized with 3% NaOCl for 3 minutes followed by washing with sterile distilled water and transferred on potato dextrose agar media (PDA) plates. The plates were incubated at 25°C at room temperature for seven days in invert position followed by the standard procedures (Mathur and Kongsdal, 2003). The pathogen was purified by regular transferring of the active growth zone of the mycelia into fresh PDA plates which were stored at 4°C as stock cultures for further studies. The isolation, identification and confirmation of *Fusarium oxysporum f. sp. lycopersici* based on the examination under different magnifications of a stereomicroscope on the basis of cultural and morphological characteristics (Burgess, 1981; Snyder and Hausen, 1940).

ii). Pathogenesis test for isolated fungi: Pathogenesis test of the isolate was conducted according to Jasnic *et al*. (2005). Pathogenecity of the isolated pathogen was sowing of tomato plants in artificially infected soil mixture made of sterilized soil and fungi suspensions. Fungal suspensions was prepared by pouring 50ml autoclaved water in Petri plates containing 10days old *Fusarium* culture, stirring the culture with sterilized glass rod. Conidial concentration was measured by haemocytometer and standardised with $1x10^6$ spores/ml. Control plants were sown in sterilized soil pots supplementing with sterile distilled water. Plants were incubated at 22-25ºC for 14 days.

iii). Preparation of plant extract: *Cissus quadrangularis* was collected from medicinal garden of Botany Department, University of Mysore. Collected material allowed to air drying for 7-8days. The air-dried medicinal plant of *Cissus quadrangularis* L was weighed 5g and added with 50 ml of distilled water and macerated with mortar and pestle under

Fig. 1: Isolation and identification of *Fusarium oxysporum* from infected tomato plant

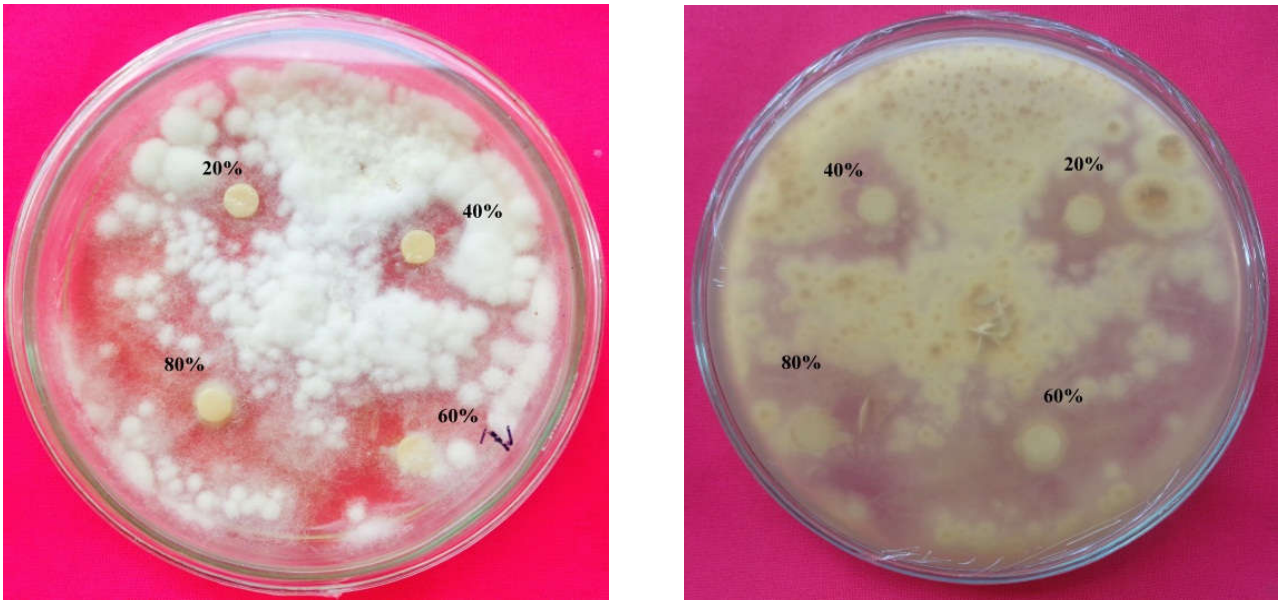

Fig. 2. Antifungal impact of *Cissus quadrangularis* plant extract on *F. oxysporum*

aseptic condition and the extract biomass was incubated for overnight (24 hrs). After incubation the plant extract was filtered using muslin cloth followed by filtration method of Whatman No.1 filter paper. The filtrate was subjected to centrifugation at 10,000 RPM for 5 min and the supernatant was sterilized and stored at 4ºC as stock solution. (Rivill Acevedo and Soriano-Garcia, 2007; Ashwani Tapwal *et al*. 2011).

iv). Antifungal activity assay of botanical extract by using disc diffusion technique: The sterilized Petri dished were plated with 20ml PDA agar media and allowed for solidification. On the solidified agar plates the *Fusarium oxysporum* spore suspension of 1ml of spore suspension ($1x10^6$spore/ml) was inoculated to the plates by spreading with L shaped glass rod. The *Fusarium* spore culture inoculated plates were placed with plant extract loaded discs of different concentrations 20%, 40%, 60% and 80% were placed and replicated plates were incubated at room temperature at 27 ºC in invert positions for 6-7 days. After the completion of incubation period the plate were observed for the antifungal effect of plant extract and the inhibition zones of various concentrations of plant extract were measured by using measuring ruler .

v). In vivo studies on host pathogen interaction studies: Seeds of tomato *viz.* local (T0), local treated (T1-soaked with *C. quadrangularis* extract for 24hrs) and resistant INDEX-300 (T2) were surface-sterilised with 3.8% NaOCl for 5 min, rinsed four times in sterile double-distilled water and sown in trays containing steam-sterilised sand and coco peath (70:30ratio). Trays were maintained in an air conditioned glasshouse at 23–28°C, 60–70% relative humidity for 8-10 days (i.e. first leaf unfolded) after seeding. The seedlings were removed by gently washing roots with tap water and subjected for further histo-pathological studies (Hochmuth and Hochmuth 2003; Amini *et al.*, 2010). Seedlings of T0, T1 & T2 washed with DW followed by surface sterilized with 0.1% of NaOCl for 3minutes and again were rinsed in DW. These seedlings were soaked in spore suspension (2.5×10^6 spores/ml) of *Fusarium oxysporum* for 2, 4, 6, 12, 16, 24, 48 & 72hrs, respectively. The soaked seedlings were macerated in 3% NaOH and fixed on slides at 60ºC and the same were thoroughly washed in running water gently for 30 min to remove NaOH and were stained with 0.2% warm cotton blue for two hours (Sharada, *et al.*, 1996). The stained slides were subjected for microscopic observation of fungal interactions.

RESULTS AND DISCUSSION

The perusal of the results shows that the effect of different concentrations *Cissus quadrangularis* plant extracts i.e. 20, 40, 60 & 80% on *F. oxysporum* revealed that, the extract has shown marked level of inhibition of colony growth basing on the level of concentration. It was noticed that higher concentrations of botanical plant extract (80%) has shown significantly suppressed the fungal growth (16.2mm) followed by the 60% (13.1mm). Whereas lower concentrations 20 & 40% of plant extract shown least impact on the suppression of pathogenic fungal colony growth, similar results were observed by Anil Kumar and Raj Kumar, 2015 with *Cissus quadrangular* as plant extract on *F. oxysporum*. The management of *Fusarium oxysporum* wilt diseases of tomato is mainly based on the chemical pesticides, crop rotation and the use of pathogen resistant varieties. Application of chemical fungicides was a conventional method to control diseases caused by fungal pathogens.

A number of antifungal compounds of diverse skeletal patterns have been found in the plants. These compounds belong mainly to six broad chemical groups, such as phenolics, phenolic acids, coumarins, pyrones, falvonoids, Inflavonoids, steroids, steroidal alkaloids and other miscellaneous compounds (Mitra *et al.,* 1984). Though *Cissus quadrangularis* a botanical plant extract was unable to completely inhibit the pathogens but they could be used in the combination with the fungicides as IPM strategy to minimize the use of fungicides. The efforts made through this investigation could be an important step towards the possibilities of using plant products as pesticides in the plant disease control. Tomato seedlings of local (T0), Local treated (T1) and Resistant INDEX-300 (T2) subjected to the soaking of fungal spore suspension (10^6spores/ml) in different intervals of durations (i.e., 2, 4, 8, 16, 24, 48 and 72 hours). Observations on electron microscopy of ultra-thin sections revealed that, no spore attachment and germination was noticed before 2, 4 & 8 hours of incubation.

Table 2. Inhibition of Fol in varied concentrations of *Cissus quadrangularis* plant extract

Replicated plates	Concentrations of *Cissus quadrangularis plant* extract.			
	20%	40%	60%	80%
	F. oxysporum zone of inhibition (mm)			
1	11	13	18	13
2	09	09	10	12
3	12	11	16	18
4	12	12	14	18
5	08	11	13	14
6	10	14	14	16
7	08	09	11	14
8	11	13	09	17
9	10	11	13	21
10	12	14	13	19
Average	10.3	11.7	13.1	16.2

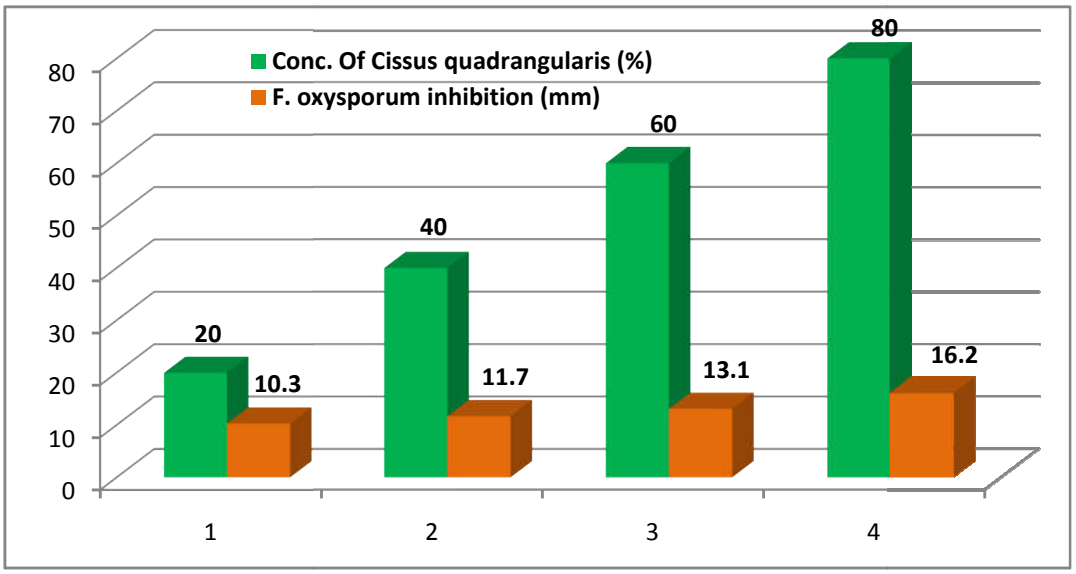

Fig. 3. Inhibition of *F. oxysporum* under varied concentrations of *Cissus quadrangularis*

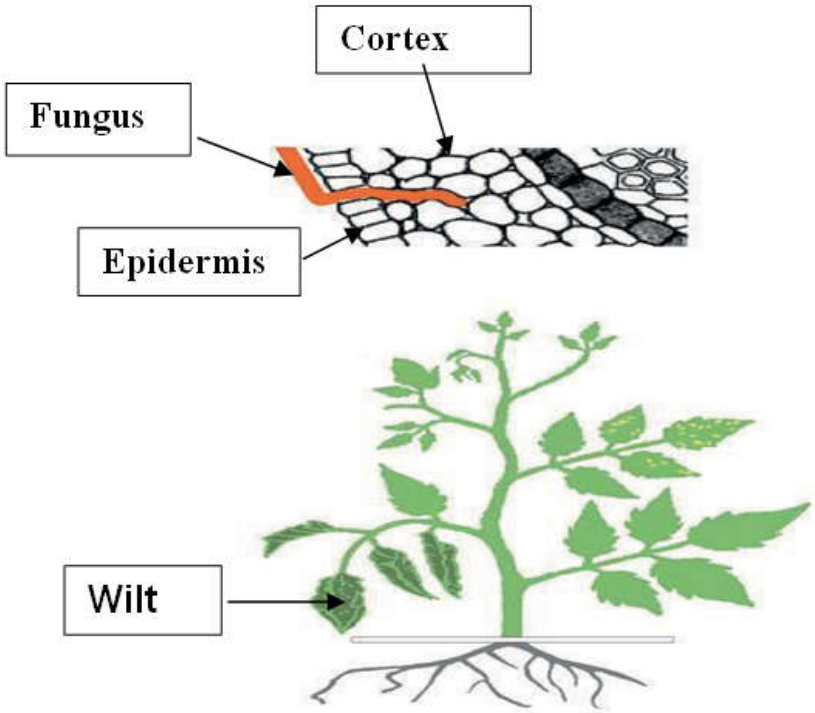

Figure 4. Infection by *Fusarium oxysporum f. sp. lycopersici.* penetration through the root cortex. Source from Catanzariti and Jones (2010)

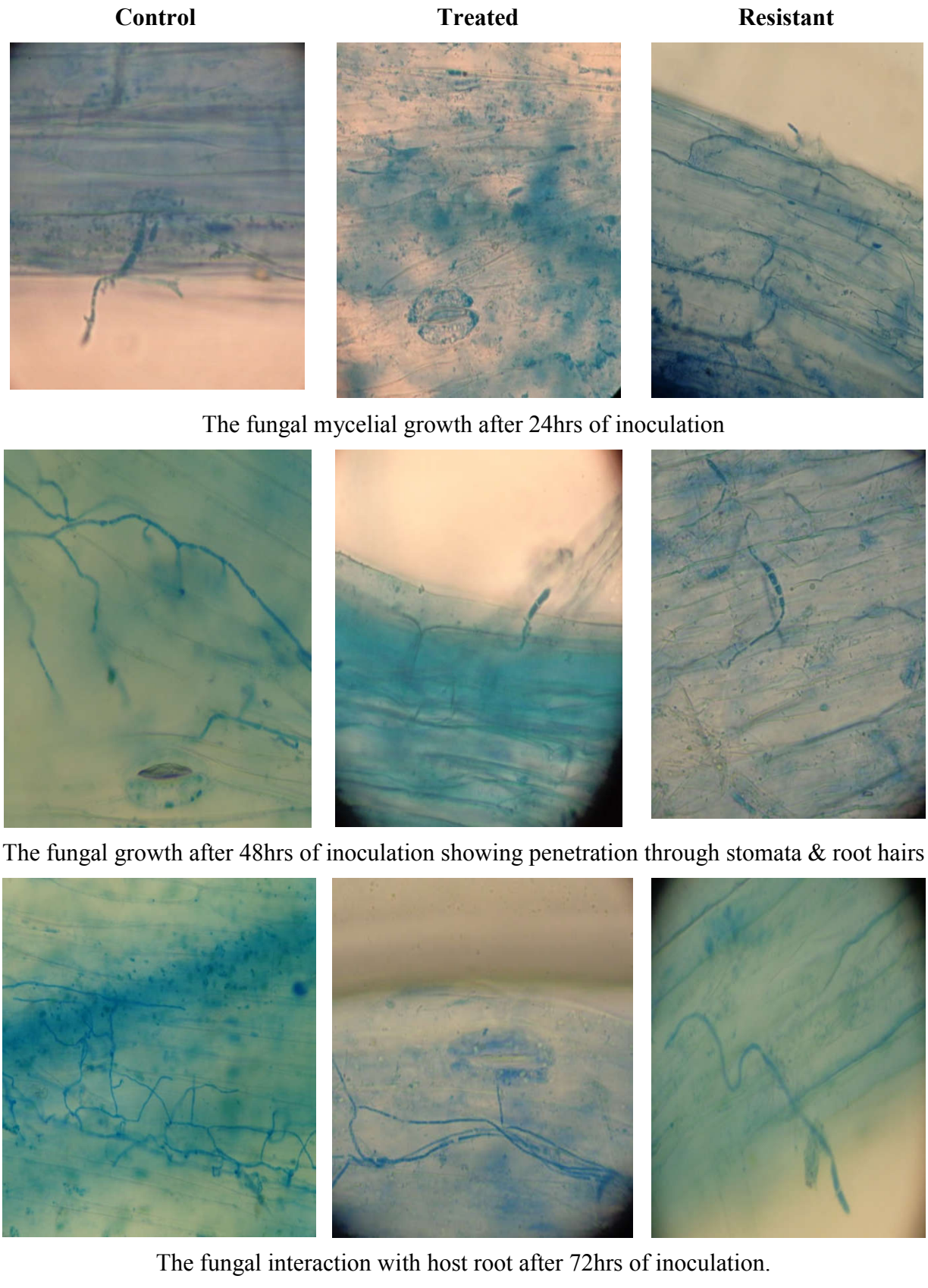

Fig. 5. Infection by *Fusarium oxysporum* f. sp. *lycopersici.* penetration through the root cortex in different intervals of inoculation

However, the initiation and attachment of fungal spores to the external wall of epidermal cells of root hair zone was noticed after 16hrs of incubation in local seedlings (T0) but not in T1 & T2. However, significant level of spore germination and development of fungal hyphae was noticed after 48hrs of incubation in T0 and T1 seedlings whereas the same not occurred in T2.

Prolific spore germination and fungal hyphal formation was observed 72 hrs of incubation in local seedlings (T0) however the same was limited in treated seed lings (T1) where as no or limited followed T1, whereas the same was minimum level was observed (Fig. 4 & 5). This indicates that the spore germination and fungal hyphae formation is predominant after 48hrs of incubation in the seedlings. The spore germination and fungal

hyphae development was found prolific after 72hrs of incubation of seedlings. These results are in agreement with those of Beswetherick & Bishop (1993), who described random growth of several fungal species on the tomato root surface. Colonization pattern in the differentiated part of the root after penetration of the pathogen into an epidermal cell, the adjacent hypodermal cells reacted intensively. These ' sensitive cells' showed an aggregated cytoplasm with an electron-dense layer attached to the plasmalemma and formed 'buckles' with thick walls that tended to entrap the fungus . (Beswetherick & Bishop, 1993). Olivain and Alabouvette (1998) observed that attachment of hyphae to the external wall of epidermal cells in the root-hair zone was noticed after 17 hrs after inoculation only. There was no specialized penetration structure, but the penetrating hyphae appeared constricted and often formed a wall on a level with the penetration point.

CONCLUSION

Based on the above experimental studies and results obtained it can be conclude that *Fusarium oxysporum* f. sp. *lycopersici* is pathogenic to tomato causing wilt, root and stem rot in seedling. Plant extract of *Cissus quadrangularis* shown potential impact in suppressing the fungal organisms indicating that it can be used as source of natural fungicidal material. Though the selected concentrations of the plant extract was unable to completely inhibit the pathogen growth but it may become effective in combination with other plant extracts as IPM strategy to minimize the use of fungicides as pronounced by Tapwal *et al*. (2011). However, the present study has generated a ray of hope in using the *Cissus quadrangularis* plant extract as an effective biocontrol antifungal component. The findings of the present investigation could be an important step towards the possibilities of using natural plant product as pesticides in the plant disease control. The histological study concluded that the pathogen starts its infection cycle in the root of seedlings 16 hours and prolific formation of fungal hyphae takes place after 72 hours. Therefore, the moment the plant wilt symptoms noticed it is wise to impart the treatment with plant extract (*Cissus quadrangularis*) in an effective concentration may suppress the infection level and prevent the further damage to plants due to wilt.

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REFERENCES

Agrios, G. N. 2005. *Plant pathology* (5th Ed.). USA and UK: *Elsevier Academic.*

Amini J. 2009. Physiological race of *Fusariumoxysporum* f. sp. *lycopersici* in Kurdistan province of Iran and reaction of some tomato cultivars to race 1 of pathogen. *Plant Pathol. J.* (8): 68–73.

Anil Kumar and Raj Kumar H.G. 2015. *In vitro* antifungal activity of some plant extracts against *Fusarium oxysporum* f. sp. *lycopersici*. *Asian J. Plant Sci and Res.* 5(1): 22-27.

Armstrong, G.M., Armstrong, J.K. 1981. Formaespeciales and races of *Fusarium oxysporum* causing wilt diseases. *In*: *Fusarium*: disease, biology, and taxonomy (Eds.). Nelson. P.E., Toussoun, T.A., Cook, R.J. University Park, PA, USA: *State University Press*, pp. 391-399.

Asha, B.B., Chandra Nayaka, S., Udayashankara, A.C., Srinivas and Niranjana, S.R. 2011. Biological control of *F. oxysporum* f. sp. *lycopersici* causing wilt of tomato by *Pseudomonas flurosence*. *Int. J. of Microbiol. Res.* (2): 79-84.

Ashwani Tapwal., Nisha., Shipra Garg., Nandini Gautam and Rajesh Kumar. 2011. *In vitro* antifungal potency of plant extract against five phytopathogens. *Braz. Arch. Biol.Technol*. 54(6): 1093-1098.

Beswetherick J.T, Bishop CD. 1993. An ultrastructural study of tomato root inoculated with pathogenic and non-pathogenic necrotrophic fungi and a saprophytic fungus. *Plant Pathology*. 42: 577–588

Burgess, L.W. 1981. General Ecology of the Fusaria. *In*: *Fusarium: diseases, biology and taxonomy* (Eds.) Nelson, P.E., Toussoun, T. A. and Cook, R.J. University Park, PA, USA: The Pennsylvania State University Press. Pp. 225-235.

Catanzariti A.M and David A. J. (2010). Effectors proteins of extracellular fungal plant pathogens that trigger host resistance. *Funct. Plant Biol.*37: 901-906.

Essarioui, F., Mokrini, M and Afechtal. 2006. Molecular interactions between tomato and its wilt pathogen *Fusarium oxysporum* f. sp. *Lycopersici, Rev. Mar. Sci. Agron. Vét.* (2016) 4 (1):66-74.

Huang C.C. and Lindhout P. (1997) Screening for resistance in wild Lycopersicon species to *Fusarium oxysporum* f. sp. *lycopersici* race 1 and race 2. *Euphytica*.(93): 145–153.

Jasnic, S.M., Vidic, M.B., Bagi, F.F and Djordjevic, V.B. 2005. Pathogenecity of *Fusarium* species in soybean. *Matica Srpska*. *J. for Natural Sci*., 109: 113-121.

Jones, J. P. (1991). Fusarium wilt. In J. B. Jones, R. E. Stall, &T. A. Zitter (Eds.) Compendium of tomato diseases. *St.Paul, Minnesota*: APS.

Kirankumar R., Jagadeesh K. S., Krishnaraj P. U and Patil, M.S. 2008. Enhanced growth promotion of tomato and nutrient uptake by plant growth promoting rhizo-bacterail isolates in presence of tobacco mosaic virus pathogen. *Karnataka J. of Agric. Sci*., 21(2): 309-311.

Mathur SB, Kongsadal O .2003. Common laboratory seed helath testing methods for detecting fungi. *International seed testing Assosiiation, Bassersdorf, Switzerland*, p.427.

Mitra, S. R., Choudhuri, A and Adityachaudhury, N. 1984. Production of antifungal compounds by higher plants- a review of recent researches. *Plant Physiol. Biochemi.,* 11: 53-77.

Olivain C, Alabouvette C. 1997. Colonization of tomato root by a non-pathogenic strain of Fusariumoxysporum. *New Phytologist*.(137): 481–494

Rivillas-Acevedo LA Soriano-García M. 2007. Isolation and biochemical characterization of an antifungal peptide from Amaranthus hypochondriacus seeds. *J Agric Food Chem.* 55(25):10156-61.

Rivillas-Acevedo, L and Soriano-García, M, 2007. Antifungal activity of a protean extract from *Amaranthus hypochondriacus* seeds. *J. of Mexican Chem. Soc*., 51: 136-140.

Sharada, M.S, Shetty, A.S and Shetty, H.S.1995. Infection processes of *Sclerospora graminicola* on *Pennisetum glaucum* lines resistant and susceptible to downy mildew, *Mycol. Res*. 99 (3): 317-322

Singh R. S. 2005. *Plant diseases.* Oxford and IBH publication Co. Pvt. Ltd. New Delhi: Pp. 470-476.

Snyder WC and Hausen HN, 1940.The Species concept in Fusarium . *Ann. J. Bot*. (27):64-67.

Unnati M. Shah, Patel SM, Patel PH, Hingorani L, Jadhav RB. 2010. Development and Validation of a Simple Isocratic HPLC Method for Simultaneous Estimation of Phytosterols in *Cissus quadrangularis. Indian J Pharm Sci*; 72(6).

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