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Analysis of thermal and drought tolerance conferred by Endophytes in tomato

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Abstract

Tomato (*Solanum lycopersicum* L.) belongs to Solanaceae family. It is a valuable source of several minerals and vitamins. The world food security is highly disturbed by the unpredictable drought and heat stresses which will upsurge in frequency and brutality in the near future leading to a probable decline in global food production. Improvement in drought tolerant varieties through plant breeding is a promising and effective approach but it is an expensive and also a long drawn procedure. Endophytic fungi, residing in the root and shoot tissues can play pivotal role in this regard. Hence the present study was carried out to evaluate the potential of fungal endophytes in tomato crop against thermal and drought stress. The experiment was executed in research area of Department of Plant Pathology, University of Agriculture Faisalabad during 2014-2015. Eight varieties of tomato were sown under Randomized Complete Block Design (RCBD) in pots and later were transplanted to field. Endophytic fungus was isolated from healthy leaves, stems and roots of *Acacia nilotica*and *Convulvulusarvensis*and identified on the basis of morphological characters of the fungal culture and spores. Inoculation was done by immersing the tomato seedlings in fungal suspension of known concentration. Drought stress was given to the plants by delaying the irrigation and heat stress was accomplished by the bright sunshine. The endophytic fungi was re-isolated and identified from the inoculated plants to confirm the colonization of the fungus in the plant tissues. Data regarding shoot length, leaf size, fruit size and weight was collected and subjected to analysis of variance and statistical difference between mean values or percentages was determined by the Student's t-test.o

Keywords: endophytes, drought, stress, tomato

1. Introduction

Tomato (*SolanumlycopersicumL.*) belongs to the family Solanaceaealong with other important crops of economic value such as potato, eggplant and pepper. Tomato was first classified by Miller (1754) [21]. as *Lycoper siconesculentum*but later it was renamed as *Solanumlycopersicum* (Peralta *et al*., 2008) [25]. It is the most important grown fresh market vegetable worldwide with more than 5 million hectares harvested in China, United States of America, India, Turkey and Egypt. In Pakistan average yield is 11.05 tonnes per hectare which is quite low, total area under cultivation is 44.46 thousand hectares with is 491.4 thousand tonne sproduction (GOP, 2013) [13]. Environmental stress is the principal cause of crop losses worldwide as reduction in average production of most vegetable crops by more than 50%. The brutality of environmental stress imposed on vegetable crops depends on climatic changes. Moreover increasing temperatures, flooding, drought and salinity are the major restrictive factors in increasing and sustaining vegetable productivity (Bray *et al*., 2000) [8]. The hazardous effect of environmental stresses on crop plants depends on the length and severity of the stress and plant developmental stage (Bray, 2002) [7]. Among the natural disasters, drought is of paramount importance because of its severe damages (Sheffield *et al*., 2009) [30]. Current stresses on water resources is due to the global warming and climate change as a result of which many semi-arid areas suffer a decline in water resources. The world food security is highly disturbed by the unpredictable drought which also serves as a catalyst of the great famines of the past (CGIAR, 2003) [10]. Being succulent in nature vegetables generally contains more than 90% water (AVRDC, 1990) [3]. Thus water scarce circumstances significantly influence the quality and production of vegetables. A drop of soil water content, turgor loss and reduced leaf water potential, closing of stomata and lessen cell enlargement and growth rate are the typical symptoms induced by drought stress. Brutal water stress may cause reduction of photosynthesis, disturbance of metabolism and consequently results in death of the plant (Jaimez*et al*., 2000; Bhardwaj and Yadav, 2012) [6]. This upsurge in the temperature is due to global warming which possess harmful effects on the growth of plants. Plants prepare their food and energy through photosynthesis which they utilize for their growth and metabolism. Optimal growth and development of most plants were achieved at 30-35 °C depending on crop species (Prasad *et al*., 2008). On the other hand, high temperature have negative impacts on membrane fluidity, crucial protein and amino acids structures as well as osmolyte and metabolite ratio in plants (Wahid *et al*., 2007 [34]; Zinn*et al*., 2010), thus upsetting the complete function of cellular apparatus (Jaakola and Hohtola, 2010 [15]; Khan *et al*., 2011) [17]. Host-plant growth is significantly influenced by the existence of endophytic fungi present in the root tissues as they have direct influence on the mineral concentration, hormonal balance of plants and composition of materials excreted from the root and plant defense against abiotic and biotic stresses. Earlier scientific research has demonstrated that existence of endophytic fungi in the root tissues can considerably upsurge plant growth and biomass (Hamilton *et al*., 2010) [14]. The present study was conducted to evaluate the most effective endophytic fungi in enhancing the resistance against thermal and drought stress and the yield parameters.

2. Materials and Methods

2.1 Seed collection of different tomato cultivars

The seed of different cultivars of tomato were collected from National Agriculture Research Centre (NARC) and Ayub Agriculture Research Institute (AARI) Faisalabad.

2.2 Establishment of tomato nursery for the evaluation of fungal endophytes

The experiment was carried out in research area of Department of Plant Pathology, University of Agriculture Faisalabad during 2014-2015. Eight varieties of tomato were sown under randomized complete block design (RCBD) in pots with five replications. After emergence of seedlings, nursery was transplanted to field.

2.3 Collection and Isolation of fungal endophytes

Healthy leaves, stems and roots were sampled from the deserts plants like Junglikikar (*Acacia nilotica*) and Bathu (*Convulvulusarvensis*) which were collected from various locations of Thal Desert of District Layyah. Samples were randomly collected from three to five healthy plants per site. The plant were placed in zip-lock bags, stored in a refrigerator and were used for isolation of endophytic fungi within 72 hours after sampling. Plants were washed until the soil was removed, then surface sterilized in 1 % (v/v) sodium hypochlorite solution for 3 min (Rodriguez *et al*., 2008b). Using aseptic technique, plants were cut into sections of 2-3 cm lengths from the roots, leaves and stems separately, were placed on 10 % PDA in petri dishes and incubated for 5-7 days at 28 °C to allow for the emergence of fungi. Fungal identification methods were based on the morphology of the fungal culture and the characteristics of the spores (Barnett and Hunter, 1987).

2.4 Preparation of endophytic fungal media for inoculating tomato seedlings

Spores of endophytic fungi were harvested in distilled water by rubbing the surface of a sporulating pure culture with a sterile bent glass rod. Spore density was estimated using a hemo cytometer and compound microscope (200x total magnification). The spore suspension was diluted in distilled water to prepare 10^5 spore mL^{-1}. The tomato seedlings were inoculated with endophytic fungi by making the seedlings immersed in 15-20 mL of spore solutions in petridish for 30 minutes.

2.5 Abiotic stress assay

Drought stress was given to the plants by delaying the irrigation and heat stress was accomplished by the bright sunshine.

2.6 Re-isolation of beneficial fungal endophytes from inoculated tomato plants

The endophytic fungi was re-isolated and identified from the inoculated plants to confirm the colonization of the fungus in the plant tissues. Data regarding shoot length, leaf size, fruit size and weight was determined using simple scale, leaf area meter, vernier caliper and digital weight balance respectively.

2.7 Statistical analysis

Recorded data was subjected to analysis of variance and statistical difference between mean values or percentages were determined by the Student's t-test. Differences were considered significant when probability was less than 0.05 (Steel and Torrie, 1960) [31].

3. Results

3.1 Fresh shoot weight under heat stress

Significant differences were observed in the fresh shoot weight of all tomato varieties when treated with endophytic fungi as compared to the untreated check (Fig. 1). Analysis of variance table for fresh shoot weight depicted that all treatments were highly significant in enhancing fresh shoot weight of tomato varieties and also differs significantly among themselves. Graph depicted that all the tested fungi show better result for fresh shoot weight in all varieties except Pakit, in which best result was observed in control treatment (48.43). Overall Colletotrichum show best results in varieties Nageena (50.52), Naqeeb (47.72), Money maker (48.42), Raja plus (51.62) and Red andoo (50.92). In variety Pakit maximum fresh shoot weight (48.43) was observed in control treatment which clearly indicated that all the tested endophytic fungi have a negative interaction with this variety. Curvularia showed satisfactory results than Fusarium in all the tested 8 varieties.

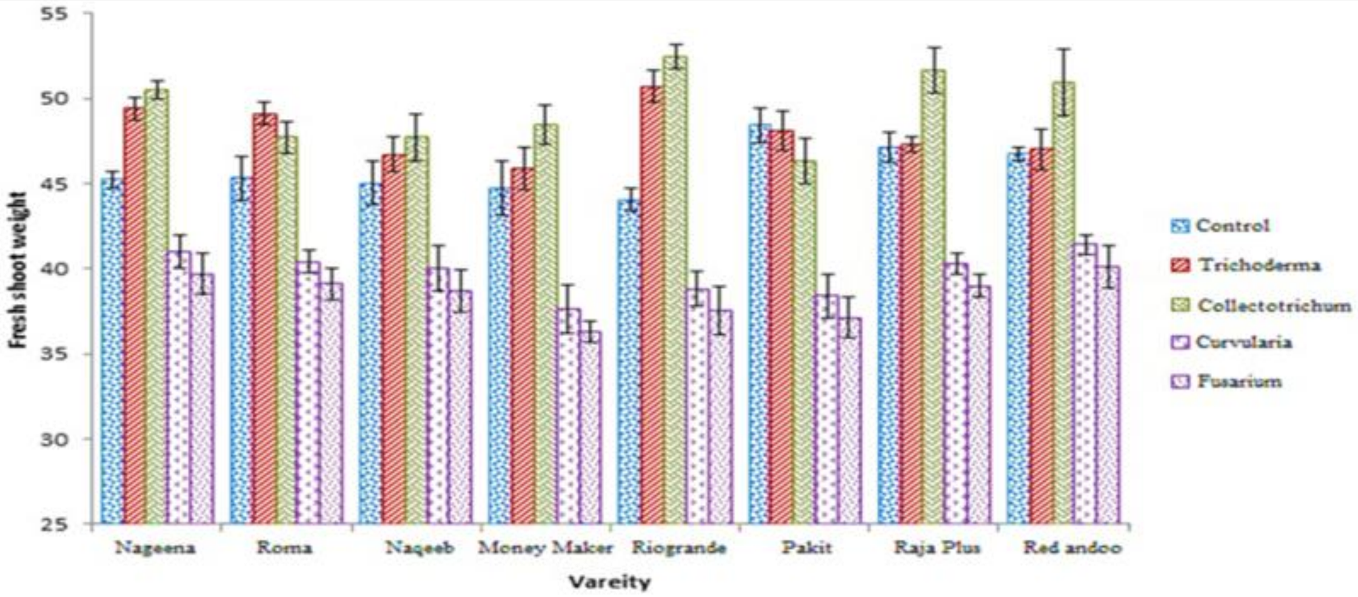

Fig 1: Fresh shoot weight under heat stress

3.2 Fresh root weight under heat stress:
Significant differences were observed in the fresh root weight of all tomato varieties when treated with endophytic fungi as compared to check (Fig. 2). Analysis of variance table for fresh root weight illustrated that all treatments were highly significant in enhancing fresh root weight of tomato and also differs significantly among themselves. Graph showed that in variety Nageena, maximum fresh root weight 16.14 was observed in control treatment followed by trichoderma (15.35), colletotrichum (14.75), curvularia (13.55) and Fusarium (11.10). In Roma, colletotrichum showed maximum results as 15.25, while trichoderma and control treatment were statisticaly at par with mean values of 14.75 and 14.74 respectively. curvularia was better than fusarium in variety Roma. In varieties Naqeeb, Riogrande and Renan dootrichoderma gave best results with mean values of 16.65, 18.45 and 17.65 respectively. In varietiy Money maker, Pakit and Raja plus, maximum fresh root weight was observed in control with values of 16.84, 18.44 and 17.74 respectively which depicted negative interaction of all tested fungi with these varieties.

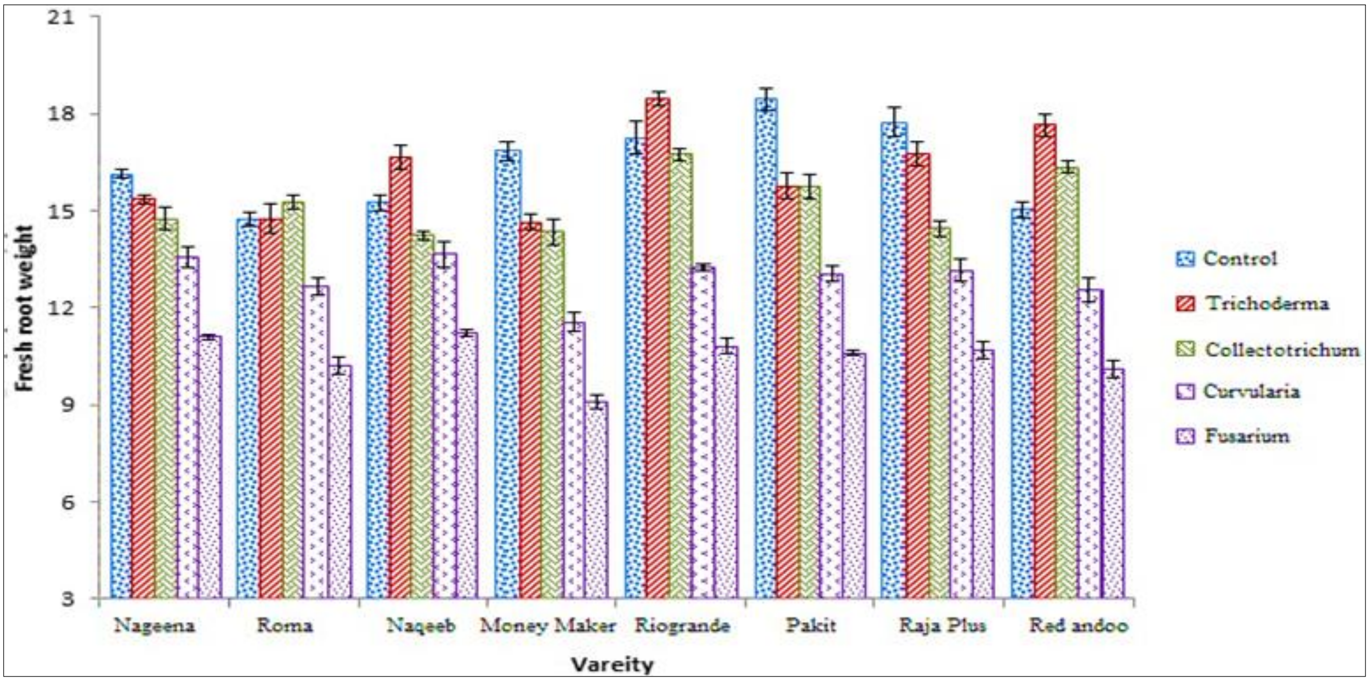

Fig 2: Fresh root weight under heat stress

3.3 Yield (kg/10 plants) under heat stress:
Analysis of variance table for yield (kg/10 plants) showed that all treatments were highly significant in enhancing fresh root weight of tomato varieties and also differs significantly (Fig. 3). The maximum yield was observed in Nageena, Roma, Money maker and Red andoo as 23.17, 22.87, 25.17 and 23.30 respectively was obtained from plots treated with colletotrichum followed by trichoderma and control treatment. In variety Naqeeb, colletotrichum and control treatment were statisticaly at par with mean values of 22.77 and 22.65 respectively. In variety Riogrande, maximum yield 26.74 was obtained from plots treated with

trichoderma. However in variety Pakit and Raja plus, maximum yield i.e. 27.65 and 24.25 respectively were obtained from untreated check. Overall Curvularia showed better yield than fusarium in all tested varieties.

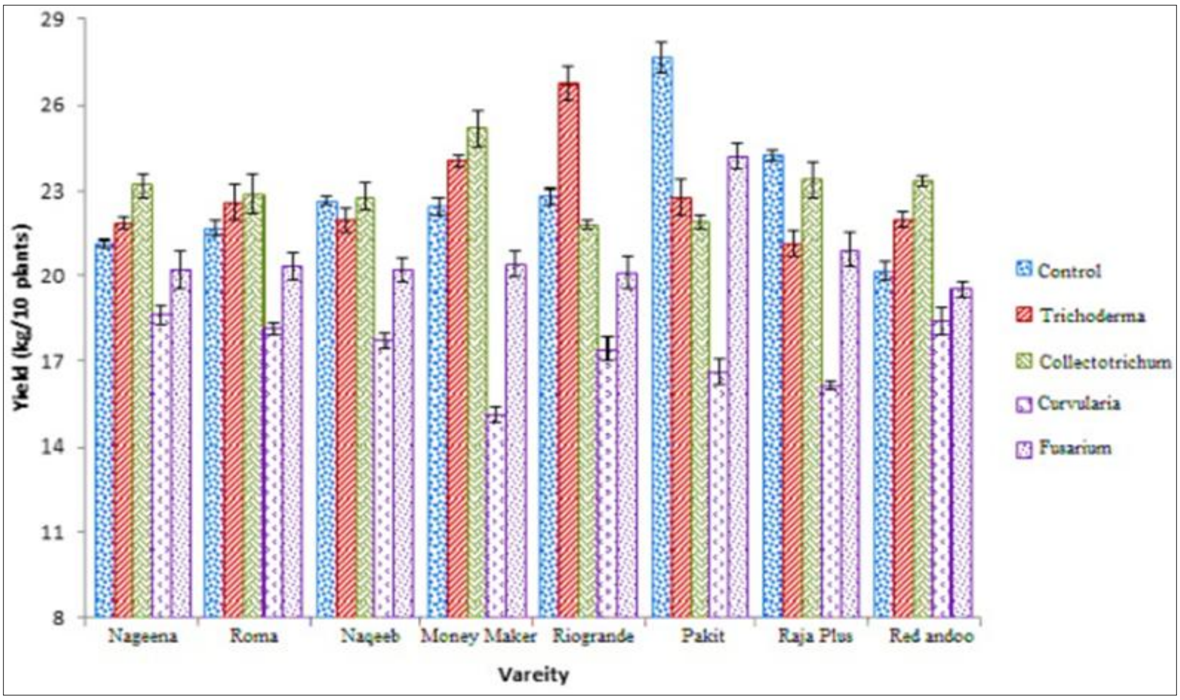

Fig 3: Yield (kg/10 plants) under heat stress

3.4 Shoot length at different time intervals under heat stress

Analysis of variance table for shoot length depicted that all treatments were highly significant in enhancing shoot length of tomato varieties at different time intervals (Fig. 4). Shoot length was less in all the varieties after day 1 which significantly increased after day 2 and day 3. In variety Nageena, shoot length after day 1 was 12.31 which increased to 17.21 and 20.41 respectively after day 2 and day 3. Similarly in variety Roma, shoot length after day 1 was 12.61 and after day 2 and 3 was recorded 17.41 and 20.51 respectively. Variety Naqeeb showed 13.11 shoot length after day 1 and after day 2 and 3 it increased to 17.51 and 20.91 respectively. After 3 days, Money maker showed shoot length 13.31, 17.81 and 21.11 respectively while Riogrande showed shoot length as 13.81, 18.21 and 21.51 respectively. Maximum shoot length 14.74, 19.81 and 24.21 after 3 days was observed in variety Pakit among all the varieties.

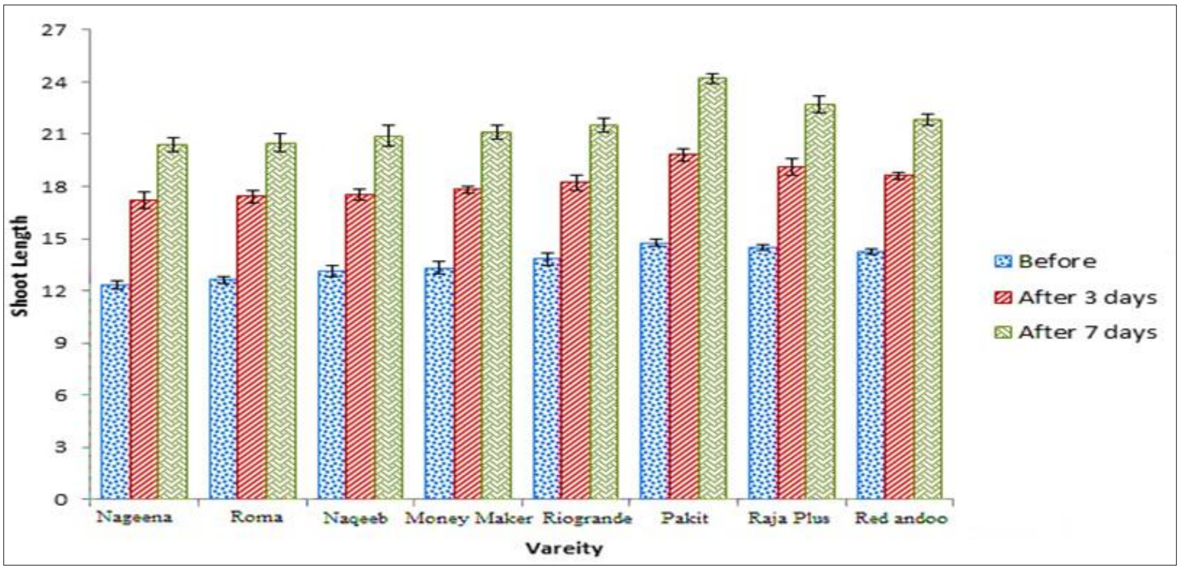

Fig 4: Shoot length under heat stress

3.5 Root length at different time intervals under heat stress

According to ANOVA all treatments were highly significant. Root length was less in all the varieties after one day which was significantly increased after two and three days (Fig. 5). In Nageena, root length after one day was 8.55 which increased to 20.48 and 22.14 respectively after two and three days. Similarly in Roma, root length after one day was 8.35 and after two and three days was recorded 20.18 and 21.74 respectively. Naqeeb showed 9.25 root length after one day and then increased after two and three days as 21.08 and 22.34 respectively. After three days, Money maker showed root length of 9.65, 21.58 and 22.74 respectively while Riogrande showed root length of 9.85, 22.08 and 23.44 respectively. Maximum root length 12.25, 23.58 and 25.14 after three days was observed in Pakit among all the varieties.

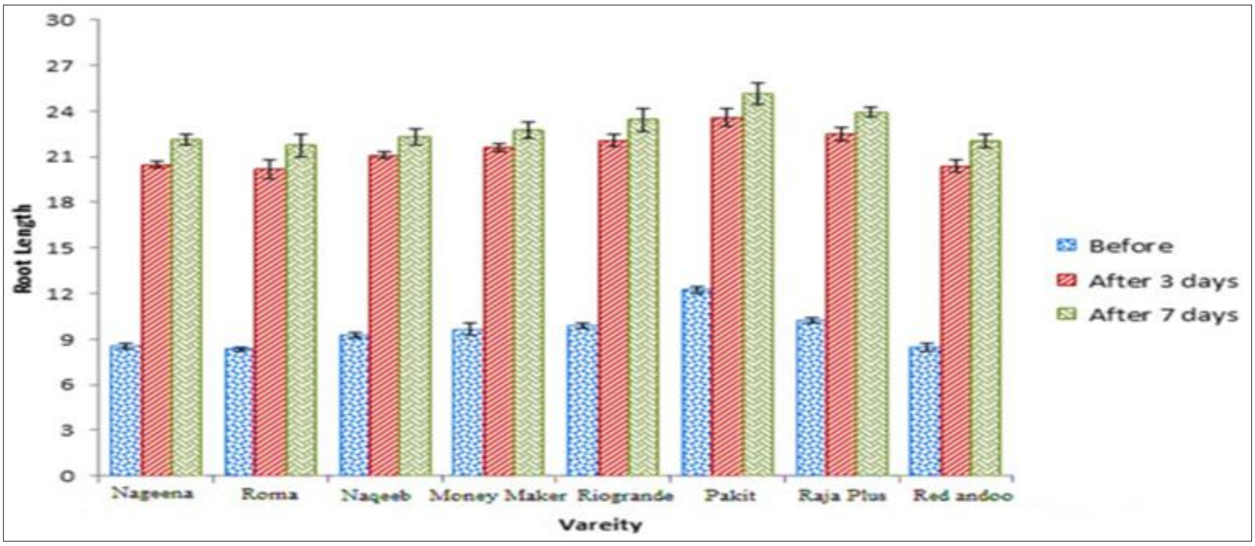

Fig 5: Root length under heat stress

3.5 Shoot weight at different time intervals under heat stress

ANOVA table showed that all treatments were highly significant in enhancing shoot weight of tomato varieties at different time intervals (Fig. 6). Shoot weight was less in all the varieties after one day which significantly increased after second and third day. In Nageena, shoot weight after one day was 7.10 which increased to 11.70 and 16.30 respectively after second and third day. Similarly in Roma, shoot Weight after one day was 7.40 and after second and third day was recorded 11.90 and 16.40 respectively. Naqeeb showed 7.90 shoot weight after one day and after second and third day it increased to 12.00 and 16.80 respectively. After three days, Money maker showed shoot weight of 8.10, 12.30 and 17.00 respectively while Riogrande showed shoot weight of 8.60, 12.70 and 17.40 respectively. Maximum shoot weight 9.53, 14.30 and 20.10 after three days was observed in Pakit among all the varieties.

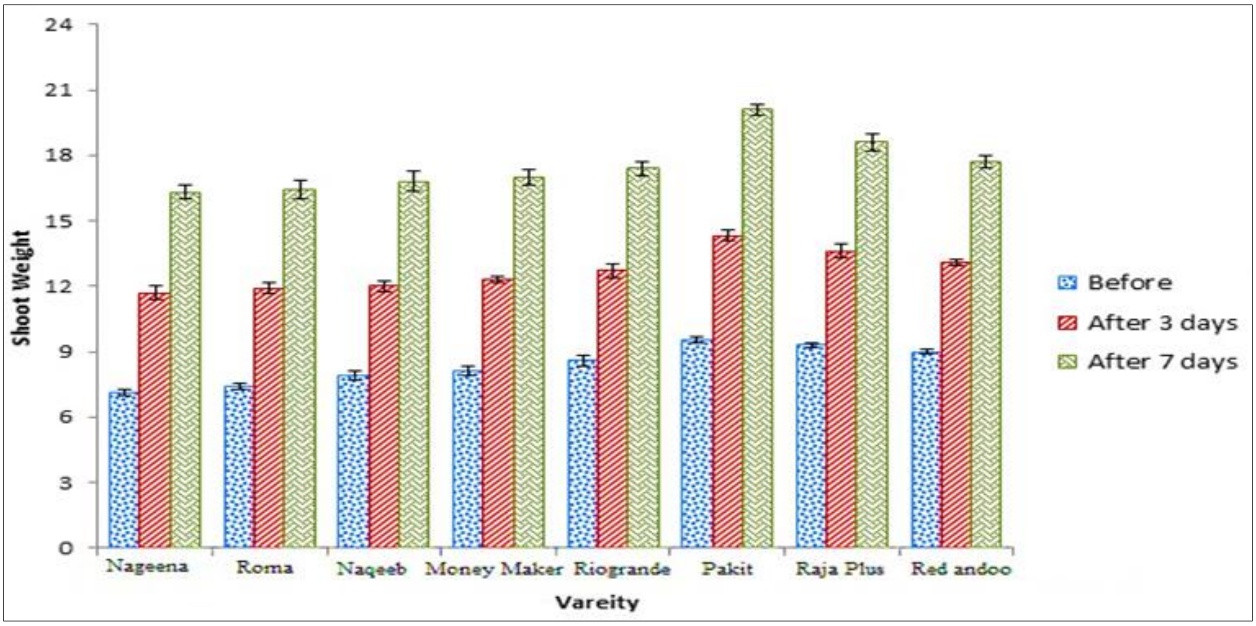

Fig 6: Shoot weight under heat stress

3.6 Root weight at different time intervals under heat stress

ANOVA table for root weight represented that all treatments were highly significant in enhancing root weight of tomato varieties at different time intervals (Fig. 7). Root weight was less in all the varieties after one day which was significantly increased after second and third day. In Nageena, root weight after one day was 12.31 which increased to 17.21 and 20.41 respectively after second and third day. Similarly root weight after one day was 12.61 and then second and third day was recorded 17.41 and 20.51 respectively in Roma. Naqeeb showed 13.11g root weight after one day and increased to 17.51 and 20.91 respectively after second and third day.

After three days interval, Money maker showed root weight of 13.31, 17.81 and 21.11 respectively while Riogrande showed root weight of 13.81, 18.21 and 21.51 respectively. Maximum root weight 14.74, 19.81 and 24.21 after one, two and three days was observed in Pakit among all the varieties.

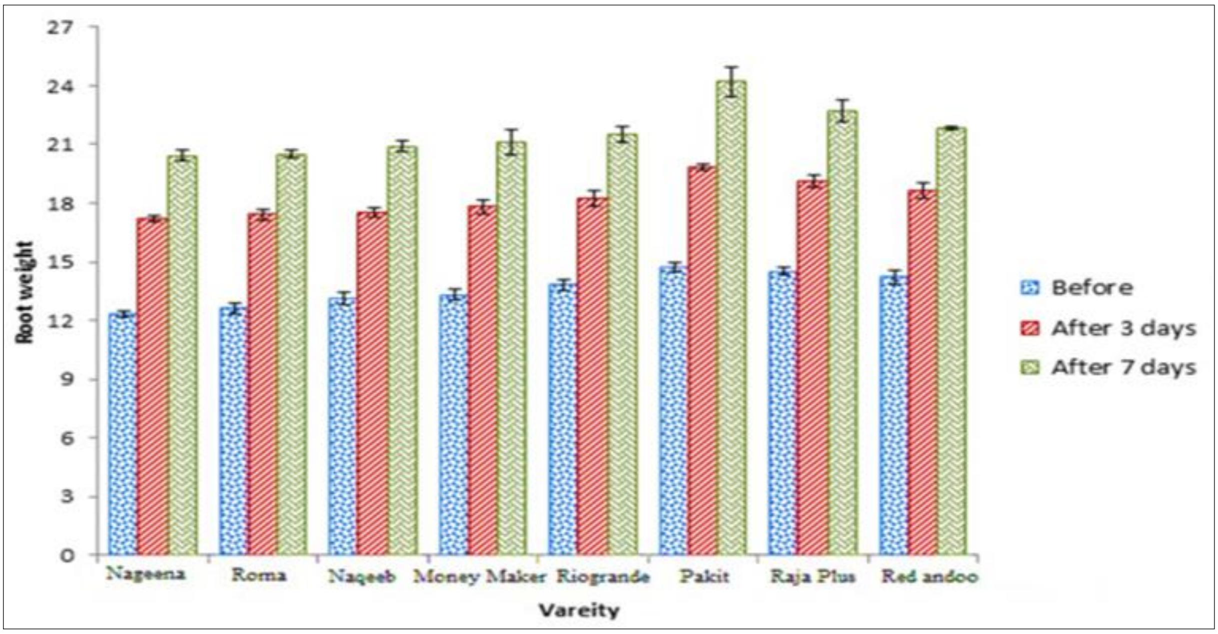

Fig 7: Root weight under heat stress

3.7 Fresh shoot weight under drought stress:

ANOVA table for fresh shoot weight depicted that all treatments were highly significant in enhancing fresh shoot weight of tomato varieties and also differs significantly among themselves. Curvularia gave maximum fresh shoot weight in Nageena, Naqeeb, Money maker, Riogrande, Raja plus and Red andoo with mean values of 43.20, 40.40, 41.10, 45.10, 44.30 and 43.60 respectively. However in variety Roma, colletotrichum gave better results (42.00) followed by curvularia (40.40) and trichoderma (40.20). In case of Pakit, maximum fresh shoot weight (43.30) was observed in plots treated with trichoderma followed by colletotrichum (41.00) and curvularia (39.67). Graph also depicted that untreated check gave better results than fungus fusarium in all varieties except for variety Red andoo which showed negative interaction of fusarium with all varieties except for Red andoo.

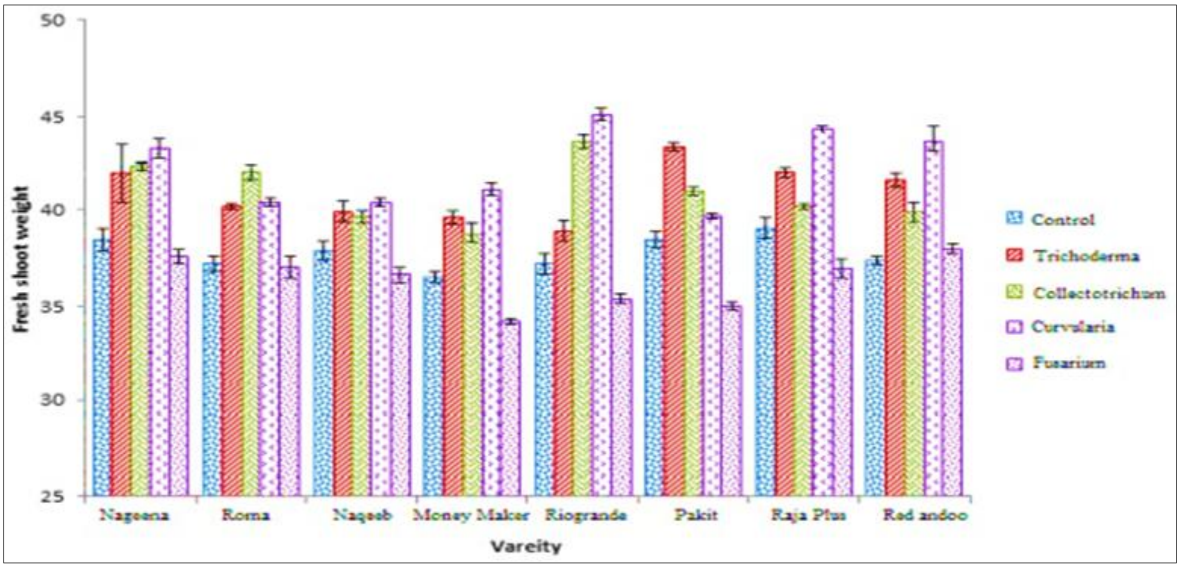

Fig 8: Fresh shoot weight under drought stress

3.8 Fresh root weight under drought stress:

ANOVA table for fresh root weight depicted that all treatments were highly significant in enhancing fresh root weight of tomato varieties and also differs significantly (Fig. 9).Nageena, Money maker, Pakit and Raja plus show maximum fresh root weight treated with trichoderma with mean values of 11.00, 11.70, 13.30 and 12.60 respectively followed by colletotrichum and curvularia. In case of Roma, the maximum results 10.50 were observed by curvularia followed by colletorichum (10.00) and trichoderma (9.60). Colletotrichum gave maximum results than other treatments in case of Naqeeb, Riogrande and Red andoo with mean values of 11.90, 13.70 and 12.90 respectively.

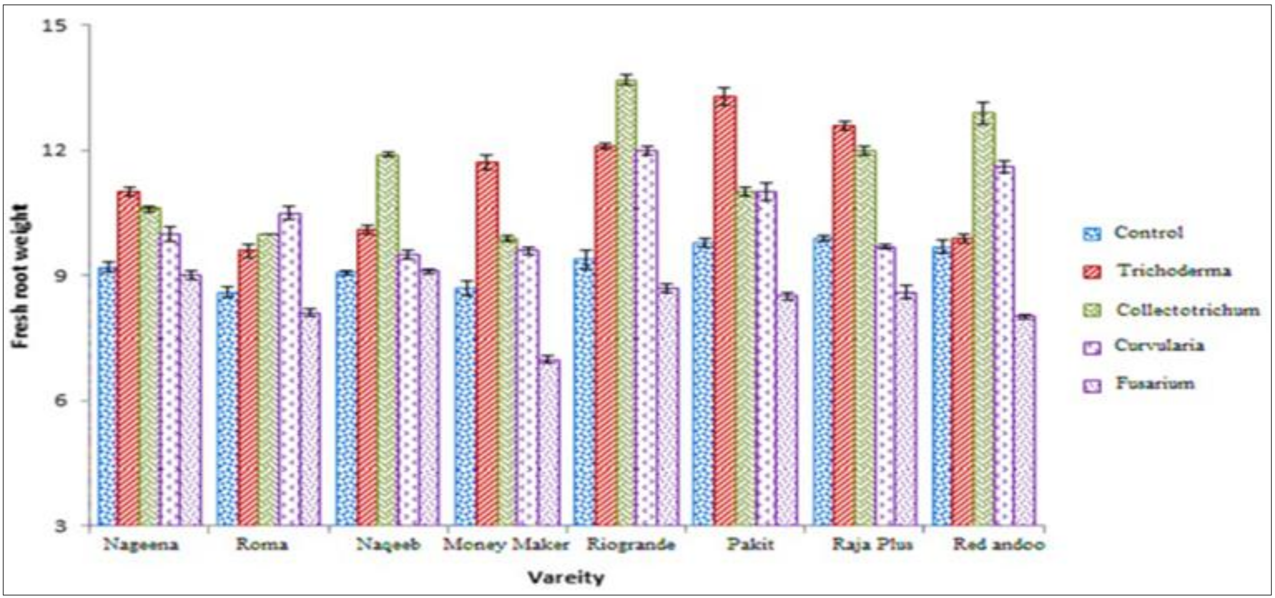

Fig 9: Fresh root weight under stress

3.9 Yield (kg/10 plants) under drought stress

ANOVA table for yield (kg/10 plants) depicted that all treatments were highly significant in enhancing yield of tomato varieties and also differs significantly (Fig. 10). In case of Nageena, Money maker and Red andoo, maximum yield (kg/10 plants) was obtained in plots treated with curvularia with mean values of 20.30, 22.30 and 20.43 respectively. Trichoderma gave better yield in case of Naqeeb (20.50), Pakit (25.50) and Raja plus (22.10). In case of Roma and Riogrande, the maximum yield was observed by colletotrichum with mean values of 2.30 and 24.50 respectively. Overall minimum yield was observed in plots treated with fusarium which was even less than untreated check. This confirmed the negative interaction of fusarium with all the tested varieties.

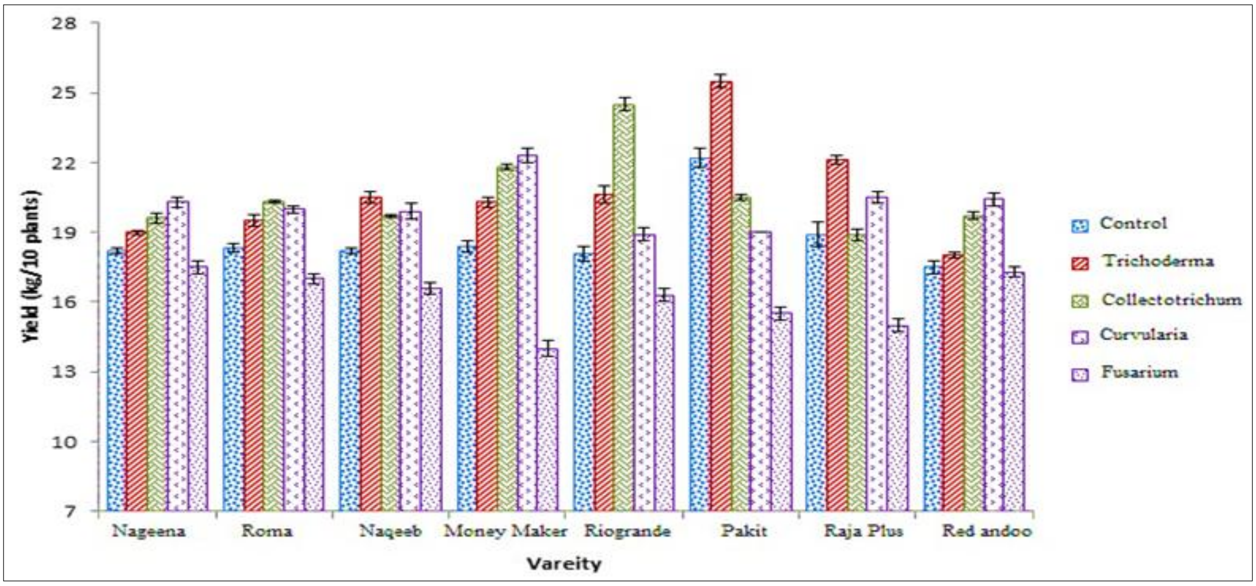

Fig 10: Yield (kg/10 plants) under drought stress

3.10 Shoot length after different day intervals under drought stress:

ANOVA table for shoot length at different time intervals depicted that all treatments were highly significant in enhancing shoot length of tomato varieties at different time intervals (Fig. 11, 12, 13). Graph depicted that all fungus increased shoot length significantly than the control treatment and also differs significantly among them. After one day curvularia and trichoderma showed satisfactory results than the other treatments while after second day, similar results were obtained. A significant enhancement had been observed in shoot length after second day as compared to first day. Similar trend had also been observed after third day. These results confirmed the colonization of endophytic fungus in the treated plants.

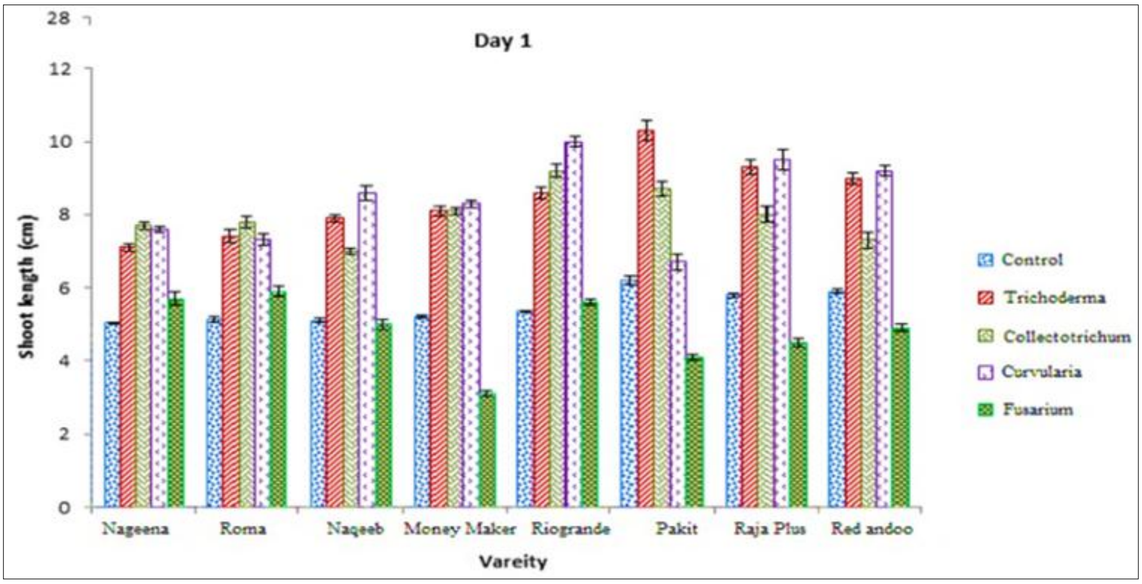

Fig 11: Shoot length after day 1 under drought stress

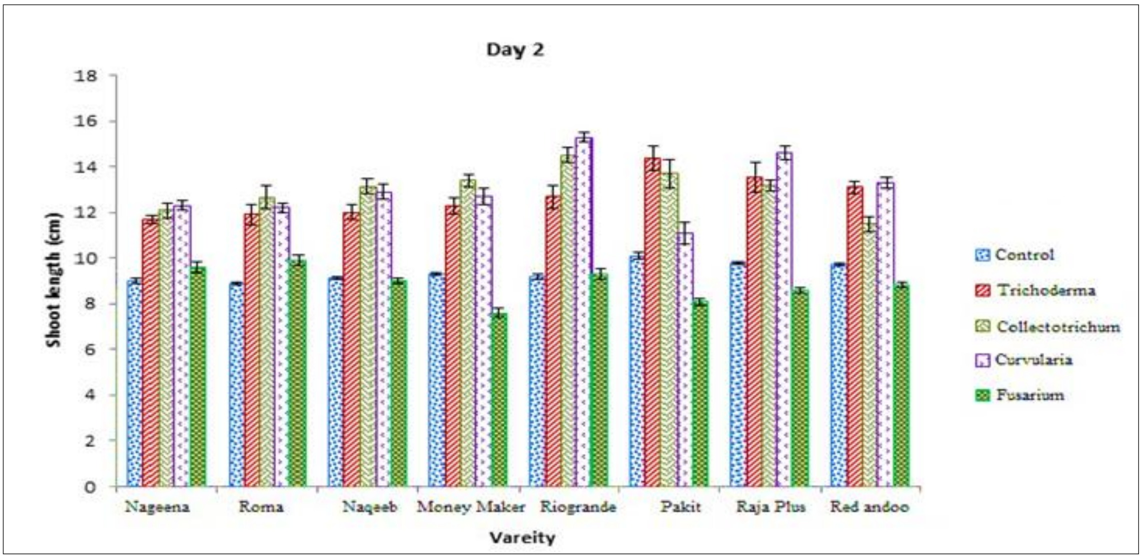

Fig 12: Shoot length after day 2 under drought stress

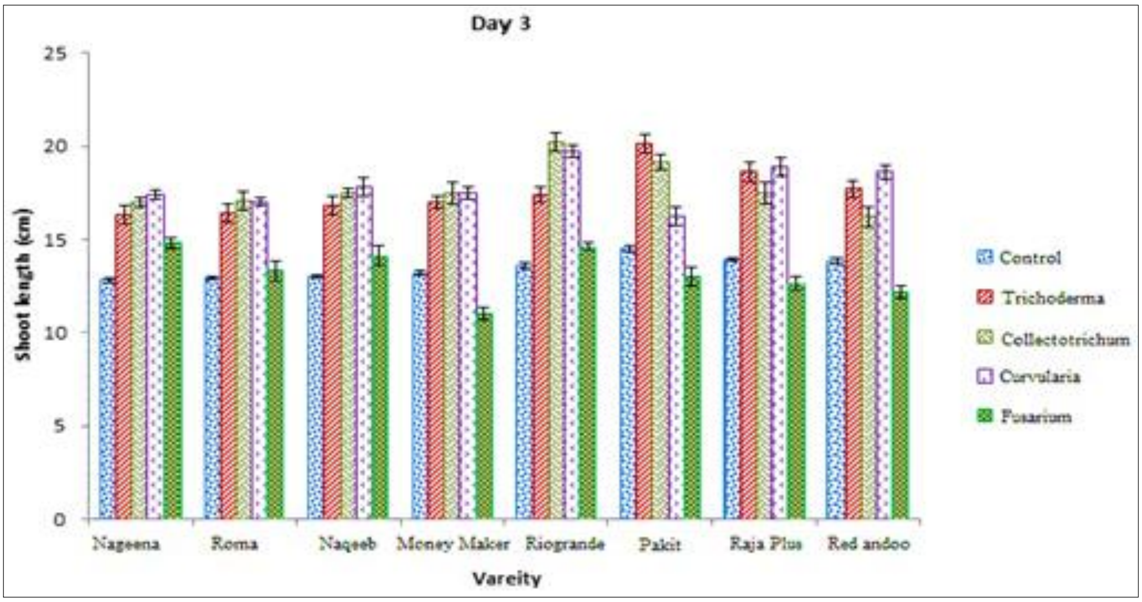

Fig 13: Shoot length after day 3 under drought stress

3.11 Root length after different day intervals under drought stress

Significant differences were observed in the root length of all tomato varieties at different time intervals when treated with endophytic fungi as compared to the untreated check (Fig. 14, 15, 16). Analysis of variance table for root length at different time intervals depicted that all treatments were highly significant in enhancing root length of tomato varieties at different time intervals. Graph depicted that all fungal species increased root length significantly than the control treatment and also differs significantly among all. After one day curvularia gave satisfactory results than the other treatments in case of Nageena, Roma, Naqeeb and Red andoo. In Money maker and Riogrande, colletotrichum showed maximum enhancement in root length after one day while in Pakit, trichoderma showed better results than other fungi. After second day, trichoderma, colletotrichum and curvularia showed significant enhancement in root length in all varieties and almost statistically at par with each other. Fusarium also gave better results than untreated check in all 8 varieties. Similar trend has been observed in all varieties for enhanced root length after third day. All fungi showed prominent enhancement in root length as compared to second day. These results confirmed the colonization of all endophytic fungi in all sown varieties and also showed that fungi played its role even after long time of their application.

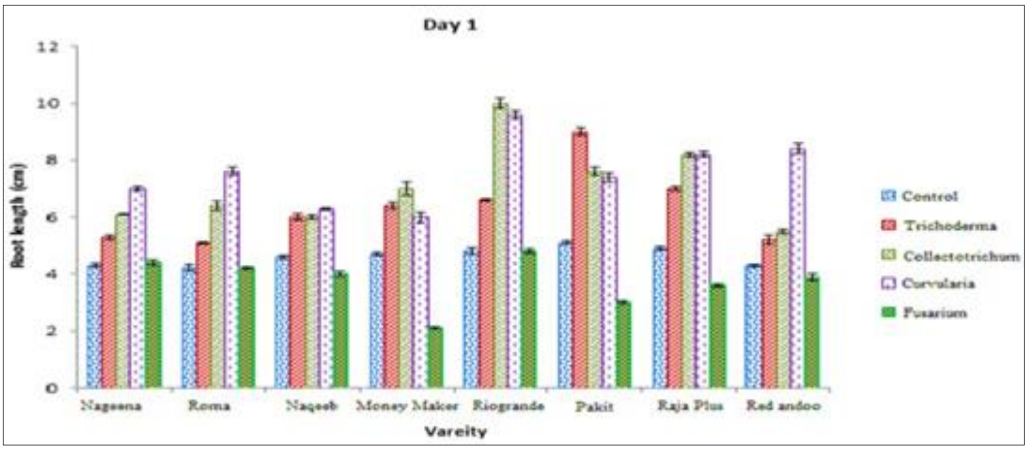

Fig 14: Root length after day 1 under drought stress

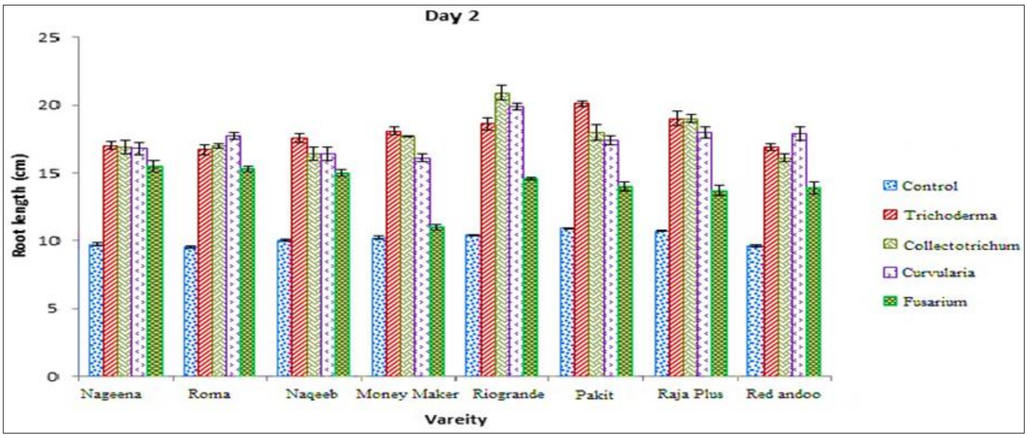

Fig 15: Root length after second day under drought stress

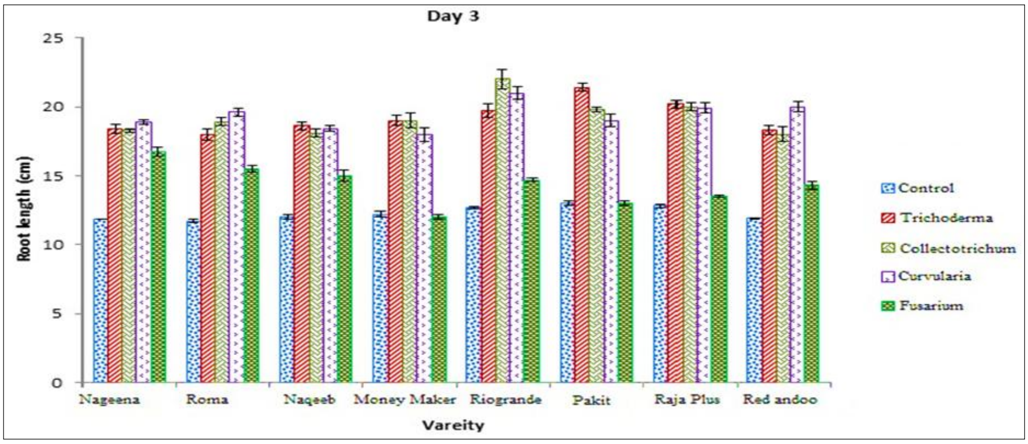

Fig 16: Root length after third day under drought stress

4. Discussion

The present research was undertaken to study the influence of certain endophytic fungi on growth parameters of tomato crop under drought and heat stress conditions. The endophytic fungi have great potential to enhance plant growth under abiotic stress conditions. Ernst *et al*., (2003) [12]; Macia'-Vicente *et al*., (2008b) [18], confirming the present findings in which endophytic fungi increased plant root and shoot biomass as well as enhanced yield of tomato crop. The role of endophytic fungi in conferring tolerance to a wide range of abiotic and biotic stresses such as disease, drought, desiccation, heat and salinity had also been extensively studied by (Danielsen and Jensen, 1999 [11]; Redman *et al*., 2001, 2002 [27, 28]; Narisawa*et al*., 2002; Campanile *et al*., 2007 [9]; Márquez*et al*., 2007).

The result of the experiment showed that endophytic fungi had a positive influence on the growth of roots of treated crop and enhanced root length and weight significantly than the untreated check during water deficit conditions. These results were in harmony with the finding of Malinowski and Belesky (2000) [19]. who studied that endophytic fungus *N. coenophialum*enhance the root growth and development of host plant that assist plants to attain better soil moisture, resulting in drought tolerance or quicker recovery from water stress. Present results were also supported by Barrow (2003) [5]. who found that root inhabiting septateendophytic fungus aid water and other nutrient transport under prolonged water deficit conditions.

Data regarding shoot biomass also suggested that endophytic fungi had a positive impact on shoot growth of treated tomato varieties. These results had been confirmed by the previous published data of (Ernst *et al*., 2003 [12]; Mucciarelli*et al*., 2003) who studied that Class 2 endophytes not only increased the plant root and shoot biomass but also required for normal plant growth and development under abiotic stress conditions. Furthermore (Tudzynski and Sharon, 2002) [33]. confirmed that increased host root and shoot biomass was attained due to presence of certain plant hormones which were synthesized in the host plant by the inhabitation of endophytic fungi. (Redman *et al*., 2001) [27]. further proved the results and stated that *Colletotrichum magna* and *C. protuberate* 4666D confer significant tolerance to wheat, tomato and watermelon plants against water deficit conditions.

Results also depicted increased plant yield with the application of endophytic fungi in drought stress conditions. These reslts were supported by (Waller *et al*., 2005; Achatz*et al*., 2010) who found interactions of endophytes with the plants beneficial for the plant which lead to increased plant biomass leading to higher yield. In addition to this (Tsimilli-Michael and Strasser, 2013) [32]. documented the role of *P. indica*in diminishing the deleterious effect on photosynthetic activity of the host plants during stress conditions.

Similar kind of increased growth had also been observed in results with the colonization of endophytes in the treated plants under heat stress conditions. Data regarding root, shoot growth and yield also gave better results than control treatment. Results were supported by (Pennisi, 2003) [24]. who found that grass seedlings which were inoculated with the foliage and root colonisingendophyte were able to endure constant soil high temperature of 50 °C for 3 days while the untreated plants were chlorotic and shrivelled. Fuhteremore when these inoculated and non-inoculated plants were exposed to intermittent soil temperatures of 65 °C for 10 days, inoculated seedlings survived whereas all non-inoculated seedlings died. Further inoculation studies confirmed that the same fungus conferred enhanced drought and heat tolerance on various horticultural and agricultural crop plants. Redman *et al*. (2002) [28]. advocated that the fungal melanin have a key role in heat dissipation or may also form complexes with oxygen radicals during abiotic stress conditions.

Results also showed the negative interaction of certain fungi on various varieties. In these cases besides the application of endophytic fungi untreated check gave better results that the plots treated with endophytic fungi. A significant reduction has been observed in root and shoots biomass and also yield of crop. Results were in harmony with the findings of (Arnold and Engelbrecht, 2007) [2]. who demonstrated that some plants lose water more quickly under severe drought when natural assemblages of Class 3 endophytes are present thus hampered plant growth.

Hence it was concluded that microorganisms assist agricultural plants to confer tolerance to abiotic stresses. The dynamic and complex relations between plant roots and microorganisms under abiotic stress conditions affect not only the plant growth but also alter the chemical, physical and structural properties of soil. The probability of alleviation of abiotic stresses in plants opens a new chapter in the application of microorganisms in agriculture. Some microbial species and strains could play an important role for understanding plant tolerance to stress, adaptation to stress, and mechanisms that develop in plants under stress conditions. Selection of microorganisms from stressed ecosystems may contribute to the concept of biotechnology application in agriculture.

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