AGRICULTURAL AND BIOLOGICAL SCIENCES

ISSN (Print): 24490954
ISSN (Online): 26364972

IN VITRO RESPONSE AND MORPHOLOGICAL VARIABILITY OF FOUR EXOTIC TOMATO VARIETIES (*Solanum lycopersicum L.*) TO TOXIN FILTRATES OF TOMATO WILT FUNGI

***Okogbaa, J.I., Terna, T.P., and Ogah, J.**

Department of Botany, Federal University Lafia. P.M.B 146 Lafia, Nasarawa State, Nigeria.

Correspondence Email: jamesokogbaa@yahoo.com.

Received 11/04/2017 Accepted: 12/10/2017 Published: December 2017

ABSTRACT

A study was carried out to evaluate morphological variability and response of four exotic tomato varieties, namely; Copernic f1, Lindo f1, Cobra f1 and Kiara f1 to fungal wilt in Lafia, Nasarawa State, Nigeria. Healthy leaves collected from 8 weeks old tomato seedlings were inoculated with 21-day old culture filtrates of *Colletotrichum gloeosporiodes, Pythium* spp. and *Fusarium oxysporum*, and observed for 3 days for the development of leaf necrosis and yellowing. All four varieties were susceptible to disease initiation by crude toxin filtrates of the wilt fungi. Lindo f1 was the least susceptible with 33.33% leaf necrosis and 29.17% leaf yellowing. Leaves of Cobra f1 were the most susceptible to necrotic symptoms (72.92%), while the highest incidence of leaf yellowing (79.17%) was observed in filtrate-inoculated leaves of the Kiara f1 tomato variety. Differences in mean disease incidence among the evaluated tomato varieties were significant ($P \leq 0.05$). *Fusarium oxysporum* was the least pathogenic, inducing 30.00% leaf necrosis and 36.25% leaf yellowing. *Pythium* spp. induced the highest incidence of leaf necrosis and leaf yellowing (56.00% respectively). Kiara f1 had the highest plant height (34.40cm), leaf area (40.70cm2) and stem girth (6.20mm). Copernic f1 had the highest number of leaves (91), number of fruits (4.40) and least plant height (23.3cm). Lindof1 had the least leaf area (20.00cm2) and stem girth (4.40mm), while Kiara f1 had the least number of fruits (0.40). Differences in morphological variations were significant ($P \leq 0.05$) except in plant height. Lindo f1 was the most disease resistant of the tomato varieties and could be selected for enhanced yield and productivity.

Keywords: *Exotic tomato varieties, Morphological variations, Wilt fungi, Toxin filtrates, Disease incidence, Disease resistance*

INTRODUCTION

Tomato (*Solanum lycopersicum L.*) belongs to the family solanaceae and is one of the most remunerable and widely grown vegetables in the world (Cox, 2000). Tomatoes have, undoubtedly assumed the status of a food with functional properties, considering the epidemiological evidence of reducing the risk of certain types of cancer (Golia, 2008). Consumption might be beneficial for reducing cardio vascular risk associated with type 2 diabetes (Shidfar *et al.*, 2011). It contains many nutrients, anti-oxidants and secondary metabolites such as vitamin C and E, B-Carotene, flavoniodes, organic acids, phenolics, and chlorophyll II which are important for human health (Giovianelli and Paradisco, 2002; Radwan and Salama, 2006; Demirbas, 2010).

With a total cultivated area of one million hectares and annual yield of about 2.5 million tons, Nigeria is ranked the second largest producer of tomato in Africa and 13th in the world (Adegbola *et al.,* 2012). In spite of this huge annual production capacity, Nigeria currently ranks as the largest importer of tomato paste from China and Italy, largely because of frequent crop losses to disease and other production challenges in the tomato value chain.

Several studies have been carried out by a number of researchers on the pathogenicity of various fungal pathogens on local and improved varieties of tomato in the North-Central Region of Nigeria (Bem, 2009; Terna *et al.,* 2015), but none has focused on the role of fungal toxins in the development of disease symptoms. The purpose of this work is to determine the susceptibility of four exotic tomato varieties to disease initiation by secondary metabolites of selected wilt fungi. The findings of this study shall serve as veritable means of selecting better resistance in tomato varieties against fungal pathogens that cause huge annual yield losses in the study area.

MATERIALS AND METHODS

Certified seeds of four exotic tomato varieties (Copernic f1, Lindof1, Cobra f1 and Kiara f1) were purchased from Da-ALLGREEN SEEDS LIMITED, Kaduna State, Nigeria. The tomato wilt fungi used in the study (*Colletotrichum gloeosporiodes, Pythium* spp *and Fusarium oxysporum*) were obtained from the culture bank of the Plant Pathology Unit of the Department of Botany, Federal University Lafia, Nasarawa State.

Five kilograms each of sandy-loamy soil in metal drum, were sterilized at 180-200^{0}F (82.2-93.3^{0}C) for 45min and dispensed into sterile polyethylene bags and allowed to cool. Sterilized soils were moistened with water and incorporated with healthy tomato seeds at the rate of about 30 seeds per pot, and 10 pots for each variety (4 varieties in total). The potted plants were placed under a shade and watered twice daily (morning and evening) to ensure seedling growth.

Four weeks old seedlings of tomato were transplanted onto fresh sterile potted soils at the rate of 2 seedlings per pot. Transplanting was done in the evening to prevent excessive loss of water and wilting due to the desiccating effects of the heat of the sun.

Measurement of morphological parameters such as number of leaves, stem height, stem girth and leaf area using of the tomato varieties were carried out six (6) weeks after transplanting. Leaf area was determined as reported by Osei-Yeboah *et al.*,(1983) thus:

Leaf Area= L × W × 2.325 (Correction factor).

The method of (Zheng *et al.,* 2010) was used for Fungal cultures and toxin production as follows: Cultures of 3 wilt fungi of tomato (*Colletotrichum gloeosporiodes, Pythium* spp and *Fusarium oxysporum*) were grown on Potato Dextrose Agar (PDA) plates at 25°C for 7 days and then stored at 4°C. Four 6-cm-diameter mycelial plugs were obtained from cultures of test fungi using a sterile cork borer, and transferred into separate conical flasks each containing 200 ml of sterile Potato Sucrose Broth (PSB). Inoculated broths were incubated at 25°C in the laboratory under continuous diffused light for 21 days, and culture filtrates obtained by passing the liquid through four layers of cheesecloth and Whatman no. 1 filter paper. The biological activities of culture filtrates of the wilt fungi were determined by leaf necrosis assay using the methods of Zheng *et al.,* (2009). Petioles of excised tomato leaves of the different varieties were separately wrapped in sterile cotton wool, moistened with 2ml of fungal filtrates in sterile Petri dishes, and observed for the appearance of disease symptoms for a duration of 3 days. Tomato leaf petioles wrapped in sterile cotton wool and moistened in 2ml sterile PSB served as controls.

The treatments were laid out in Completely Randomized Design (CRD) with 3 replicates in a 3x4x4 layout. Data obtained from assessment of disease incidence and morphological variations were subjected to analysis of variance (ANOVA) at 5% level of probability.

RESULTS AND DISCUSSION

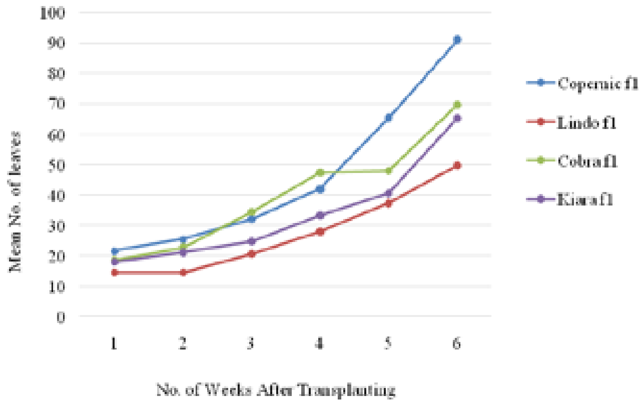

Fig 1: Variations in Mean Number of Leaves among the Evaluated Tomato Varieties

Variations were observed in the mean number of leaves of the four evaluated tomato varieties in the field after transplanting (Fig.1.). Number of leaves increased steadily in all four varieties, from the 1st week and peaked at the 6th week after transplanting. Copernic f1 had the highest mean number of leaves (91), followed by Cobra f1 (69.6), Kiara f1 (65.4) and Lindo f1 (49.6).

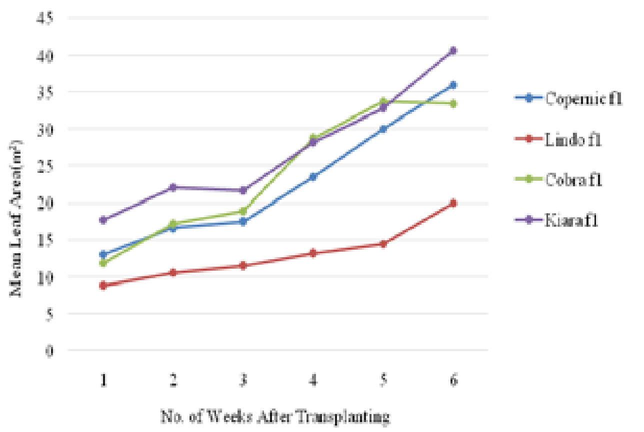

Fig 2: Variations in Mean Leaf Area among the Evaluated Tomato Varieties

Variations were observed in the mean leaf area of the four evaluated tomato varieties (Fig.2.). .Kiara f1 had the highest mean leaf area (40.70m2) followed by Copernic f1 (35.90m^2), Cobra f1 (33.40m^2) and Lindo f1 (20.00m^2).

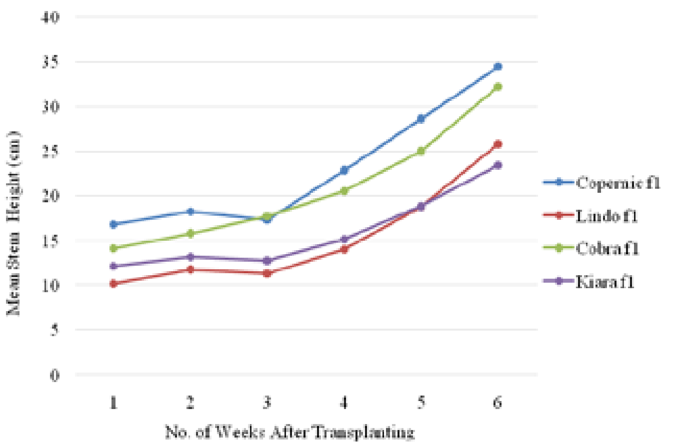

Fig 3: Variations in Mean Stem Height among the Evaluated Tomato Varieties

The stem height increased steadily from the 1st week after transplanting and peaked at the 6th week, in all four tomato varieties (Fig. 3). Copernic f1 had the highest mean stem height of (34.4cm) followed by Cobra f1 (32.2cm), Lindo f1 (25.8cm) and Kiara f1 (23.4cm).

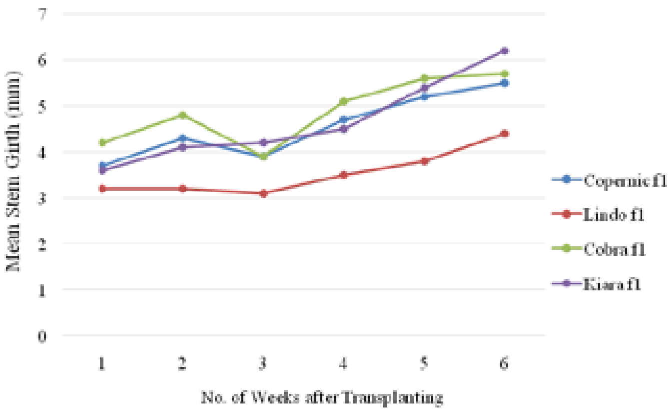

Fig 3: Variations in Mean Stem Height among the Evaluated Tomato Varieties

Variations in the mean stem girth among the four tomato varieties are presented in Figure 3. Kiara f1 had the highest mean stem girth of (6.2mm) followed by Cobra f1 (5.7mm), Copernic f1 (5.5mm) and Lindo f1 (4.4mm). All evaluated tomato varieties showed the highest stem girth at the 6th week after transplanting.

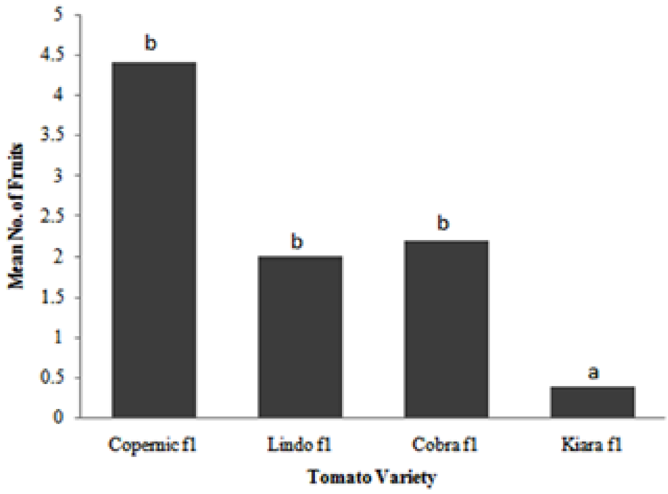

Fig. 4. Variations in Number of Fruits among the Evaluated Tomato Varieties.
Note: Columns with same alphabets are not significantly different ($P \leq 0.05$)

Variations in number of fruits produced by the evaluated tomato varieties are presented in Figure 4 and Plate 4. Copernic f1yielded the highest mean number of fruits (4.40) followed by Cobra f1(2.20), Lindo f1(2.00) and Kiara f1(0.40). Differences in mean number of fruits were significant between Kiara f1 and the other studied varieties ($P \leq 0.05$).

Plate 4. Copernic f1 (A), Lindo f1 (B), Cobra f1 (C) and Kiara f1 (D) Tomato Varieties Showing Variations in Fruit Yield 10 Weeks after Transplanting.

Table 1. Comparative Disease Susceptibility of Tomato Varieties to Inoculated Fungal Pathogens

Disease Incidence (%)		
Variety	**Necrosis**	**Yellowing**
Copernic f1	45.83b	53.33c
Lindo f1	33.33b	29.17b
Cobra f1	72.92c	77.08d
Kiara f1	50.00b	79.17d
Control	0.00a	0.00a

Means followed by same superscripts within same column are significantly different ($P \leq 0.05$).

Varying degrees of leaf necrosis and yellowing were observed invitro on tomato leaves inoculated with toxin filtrates of fungal pathogens (Table 1 and Plates 1-3). Cobra f1 had the highest incidence of leaf necrosis (72.92%) followed by Kiara f1 (50.00%), Copernic f1 (45.83%) and Lindo f1 (33.33%). Differences in mean disease incidences were significantly different($P \leq 0.05$). The highest incidence of leaf yellowing was observed on leaves of Kiara f1 (79.17%), followed by Cobra f1 (77.08%), Copernic f1 (53.33%), and Lindo f1 (29.17%). Differences in incidence of foliar disease symptoms among the evaluated tomato varieties were significant ($P \leq 0.05$).

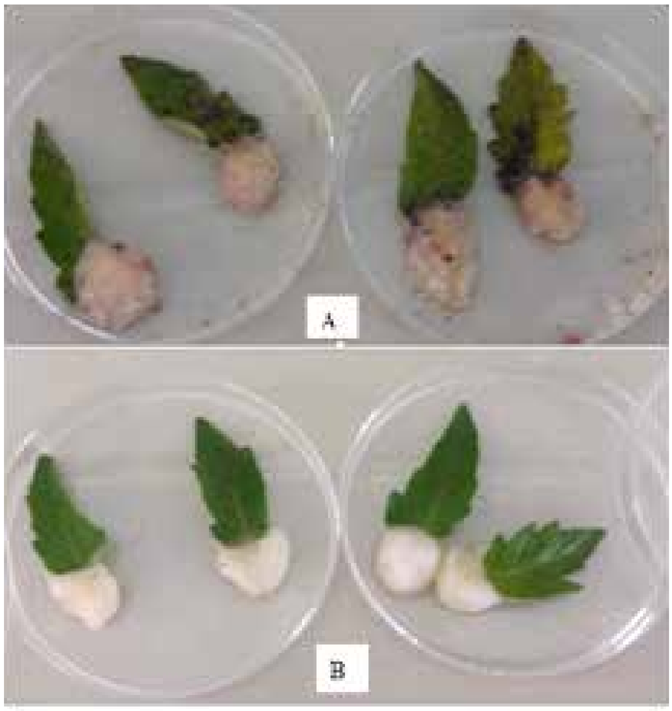

Plate 1: Lindo f1 Leaves Showing Symptoms of Necrosis and Yellowing, 3 Days after Inoculation with Culture Filtrates of Fusarium oxysporum (A); Control Experiment Showing Absence of Disease Symptoms 3 Days after Inoculation with Sterile Potato Sucrose Broth (B).

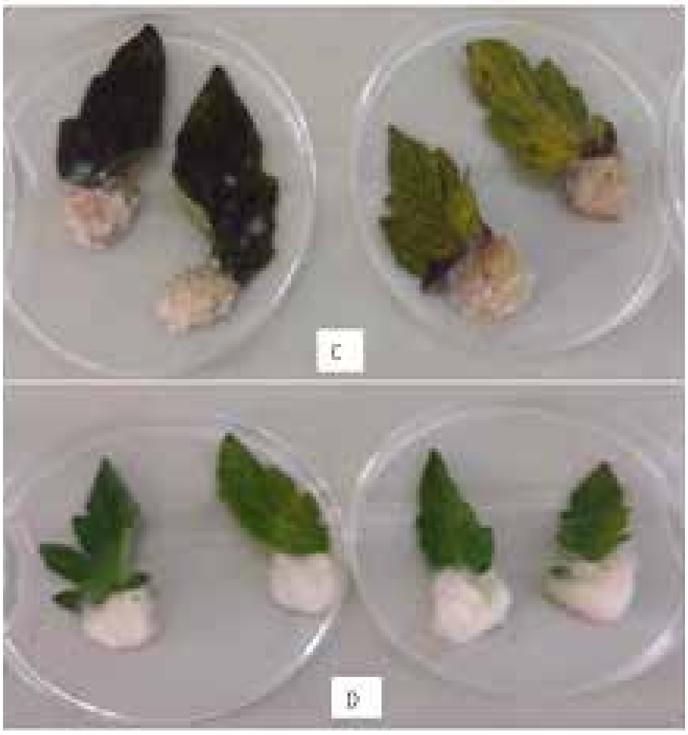

Plate 2: Cobra f1 Leaves Showing Necrotic Lesions and Yellowing 3 Days after Inoculation with Culture Filtrates of Colletotrichum gloeosporiodes(C); Control Experiment Showing Absence of Disease Symptoms 3 Days after Inoculation with Sterile Potato Sucrose Broth (D).

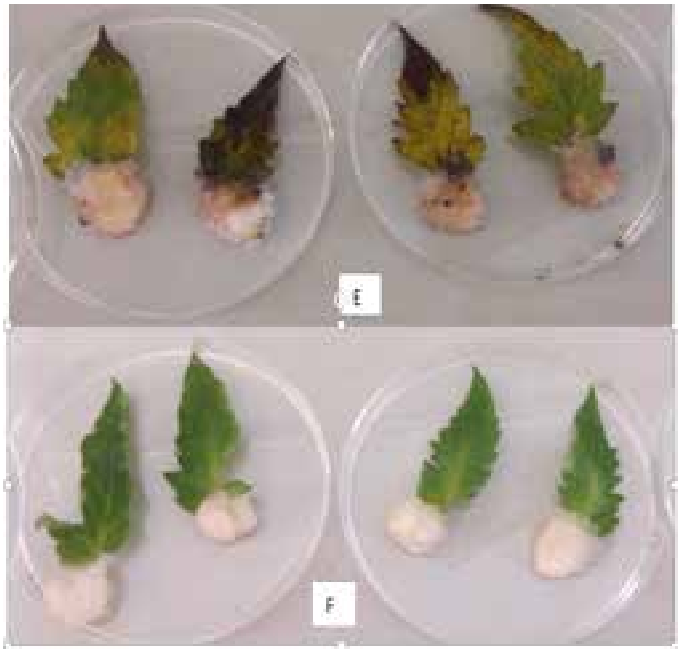

Plate 3: Kiara f1 Leaves Showing Various Degrees of Necrosis and Yellowing 3 Days after Inoculation with Culture Filtrates of Pythium spp (E); Control Experiment Showing Absence of Disease Symptoms 3 Days after Inoculation with Sterile Potato Sucrose Broth (F).

Table 2. Overall Pathogenicity of Fungal Pathogens on Inoculated Tomato Varieties

Disease Incidence (%)		
Pathogen	**Necrosis**	**Yellowing**
Colletotrichum gloeosporiodes	35.00a	53.75b
Pythium spp	56.25b	56.25b
Fusarium oxysporum	30.00a	36.25a

Means followed by same superscript within same column are significantly different (P≤ 0.05).

Results of overall pathogenicity of fungal pathogens on inoculated tomato varieties (Table 2) revealed that *Pythium* spp induced the highest incidence of both leaf necrosis (56.25%) and leaf yellowing (56.25%). Tomato leaves challenged with culture filtrates of *Fusarium oxysporum* showed the least incidence of necrosis (30.00%) and yellowing (36.25%). Differences in mean total diseases induced by culture filtrates of the test fungi were significant (P≤ 0.05).

Significant differences in susceptibility to yellowing and necrosis induced under in vitro conditions by culture filtrates of the test fungi, were observed among the evaluated tomato varieties. Ploetz (2006) also noted similar symptoms on tomato plants infected by fungal pathogens. Variability in disease susceptibility observed in the study could be a result of inherent genetic properties which confer varying degrees of disease tolerance in the studied tomato varieties.

Pythium spp was the most virulent fungal pathogen on the tested tomato varieties. In related studies by Larkin *et al.,* (1993) and Lemanceau et al. (1993) the ability of Pythium spp to induce severe necrosis, leaf yellowing and damping-off of young seedlings was reported. Furthermore, studies on greenhouse crops, hydroponic peppers and lettuces by a number of researchers (Jarvis, 1992; Owen-Going, 2006; Bagnall and Roger, 2007), also emphasized the aggressive nature of the virulence pattern of *Pythium* spp on infected plants. The mechanisms of pathogenicity by *Pythium* spp on infected plants are related to their ability to interfere with water and nutrient supply, resulting to wilting of infected plant tissues and organs.

Significant morphological variations were observed among the four exotic tomato varieties evaluated in the study. Variations were observed in growth and yield parameters such as number of leaves, plant height, leaf area, stem girth/width, and number of fruits. These variations could result from genotypic characteristics that enhance the selective performance of individual tomato varieties under the prevailing growth conditions. In related studies by Shuaib *et al.,* (2000), Fufa *et al.,* (2005), Tasisa *et al.,* (2011), Ullah *et al.,* (2012), and Nwosu *et al.,* (2014), significant differences in morphological variations among different crop plants were also attributed to peculiar variations in their genetic make-up.

CONCLUSION

The evaluated tomato varieties showed significant variations in their pre-and post- infection growth and yield attributes. Lindo f1 was the most disease tolerant of the four tomato varieties and could be selected for enhanced productivity and crop yield.

REFERENCES

Abdussamee, H., Hussain, M., Ali, M., Siddique, M.S., Jatoi, S.U.K., Sahi, S.T., and Hannan, A.(2014).Genetic response of tomato germplasm against early blight and its management through fungicides. *Applied Science Report, 6*: 119-127.

Adegbola, J.A., Awagu, F., Adu, E.A., Anugwom, U.D., Ishola, D.T., and Bodunde, A.A. (2012). Investment Opportunities in Tomato Processing in Kano, Northern Nigeria. Global Adv. Res. *Journal of Agricultural Science, 1(10):* 2882-97.

Akhtar, S., and Harzra, P. (2013). Nature of gene action for fruit characters of tomato (*Solanum lycopersicum*). *African Journal of Biotechnology, 12:* 2869-2875.

Arunakumara, K.T. (2006). Studies on Alternaria solani and growth causing early blight ofto mato. M.Sc. Thesis, University of Agricultural Science, Dharwad.

Calvo, A.M., Wilson, R.A., Bok, J.W., and Keller, N.P. (2002). Relationship between secondary metabolism and fungal metabolism. *Microbial Mol Biol Rev*., 66: 447-459.

Bagnall, R., (2007). Control of Pythium wilt and root rot hydroponically grown lettuce by means of chemical treatment of the nutrient solution. M.Sc. Thesis, University of Pretoria.

Baranska, M., Schultze, W., and Schutze, H. (2006). Determination of Lycopene and Beta-carotene content of Tomato Fruits and related Products. *Anal.Chem., 78*(24): 8456-8461.

Bennett, J.W., and Klich, M. (2003). Mycotoxins. *Clin Microbiol Rev., 16:* 497-516.

Bhowmik, D., Kumar, K.P.S., Paswan, S., Srivastava, S. (2012). Tomato- A Natural Medicine and its Health benefits. *Journal of Pharmacognosy and Phytochemistry, 1(*1): 1-11.

Demirbas, A. (2010). Micronutrient and heavy Metals Contents of Tomatoes. *Food Chem., 118*:504-507.

Etem, O., Ibrahim, I.O., Zeliha, L., Goskel, D., and Memduh, S. (2012). Determination of heavy metal concentration in tomato grown in different Station types. *Romanian Biotechnological Letters,* 17(1): 6962-6971.

Fufa, H., Baenziger, B.S., Beecher, B.S., Graybosch, R.A., Eskridge, K.M., and Nelson, L.A.(2005). Genetic improvement trends in agronomic performances and end use quality characteristics among hard red winter wheat cultivars in Nebraska. *Euphytica, 144*(1-2):187-198.

Fox, E.M., and Howlett, B.J. (2008). Secondary metabolism: regulation and role of fungal biology. *Curr Opin Microbiol., 11*(16): 481-487.

Giovanelli, G., and Paradiso, A. (2002). Stability of dried and Intermediate moisture tomato pulp during storage. *J. Agr. Food Chem., 50:* 7277-7281.

Golia, E.E., Damirkou, A., and Mitsios, I.K. (2008). Influence of some soil parameters on heavy metals accumulation by vegetables grown in agricultural soils of different soil orders. *Bull Environ Contain Toxicol., 81*: 80-84.

Jarvis, W.R. (1992) Managing diseases in greenhouse crops. St. Paul, Minnesota: APS Press.

Keller, N.P., Turner, G., and Bennet, J.W. (2005). Fungal secondary metabolism – from biochemistry to genomics. *Nat Rev Microbiol., 3*: 937-947.

Larkin, R.P., Hopkins, D.L., and Martin, F.N. (1993). Effect of successive watermelon plantings on *Fusarium oxysporum* and other microorganism in soil suppressive and conducive to *Fusarium* wilt of watermelon. *Phytopathology, 83*: 1097-2000.

Lemanceau, P., Bakker, P.A., De, H.M., Kogel, W.J., Alabouvette, C., and Schippers, B. (1993). Antagonistic effect of nonpathogenic Fusarium oxysporum Fo47 and Pseudobactin 358 upon pathogenic *Fusarium oxysporum f.*sp. *dianthi. Applied and Environmental Microbiology,* 59:74-82.

Osei-Yeboah, S., Lindsay, J.I., and Gumb, S.F.A. (1983). Estimating leaf area of cowpea (*Vigna unguiculate (L.)* Walp) from linear measurement of terminal leaflets. *Tropical Agriculture, 60*(2): 149-150.

Owen-Going, T.N. (2006). Quantitative investigations of phenolic compounds associated with root rot of hydroponic pepper, Capsicum aphanidermatum (Edison) Fitzp. Ph.D Thesis, University of Gulph.

Nwosu, D.J., Onakoya, O.A., Okere, A.U., Babatunde, A.O., Popoola, A.F., (2014). Genetic Variability and Correlations in Rainfed Tomato (Solanum spp.) Accessions in Ibadan, Nigeria. *Greener Journal of Agricultural Sciences, 4*(5): 211-219.

Ploetz, R.C. (2006). Fusarium wilt of Banana is caused by several pathogens referred to as *Fusarium oxysporum f.* sp.*cubense*. *Phytopathology, 96*: 653-656.

Radwan, M.A., and Salama, A.K. (2006). Market Basket Survey for some heavy metals in Egyptian fruits and vegetables. *Food and Chemical Toxicology, 44*: 1273-1278.

Renshaw, J.C., Robson, G.D., and Trinci, A.P.J. (2002). Fungal siderophores: structures, functions and applications. *Mycol. Res*., 106: 1123-1142.

Shidfar, S., and Gobari, M. (2011). The Effects of Tomato Consumption on Serum Gilucos, Apolipoprotein B, Apolipoprotein A-1, Homocysteine and Blood Pressure in type 2 Diabetic Patients. *International Journal of Food Sciences and Nutrition, 63(*3): 289-294.

Shuaib, A., Yang, Y., and Li, Q. (2000). Evaluating the efficacy of citicoline in embolic ischemic stroke in rats: neuroprotective effects when used alone or in combination with urokinase Exp. *Neurol, 161:* 733-739.

Tasisa, J., Belew, D., and Bantte, K. (2011) Variability, heritability and genetic advance in tomato (*Lycopersicon esculentum Mill.*) genotypes in West Shoa, Ethiopia. American-*Euroasian J. Agr. & Environ. Sci,, 11*(1): 87-94.

Ullah, M.Z., Hassan, M.J., Chowdhury, A. Z.M.K.A., Saki, A.I., and Rahman, A.H.M.A. (2012). Genetic variability and correlation in exotic cucumber (Cucumis sativus L.) varieties. *Bangladesh Journal of Plant Breeding and Genetics, 25*(1): 17-23.

Yu, J.H., and Keller, N. (2005). Regulation of secondary metabolism in filamentous fungi. *Annu Rev Phytopathol.,43*: 437-458.

Zheng,, L., Lv, R.J., Hsiang, T., and Huang, J.B. (2009). Host range and phytotoxicity of *Stemphylium solani*, causing leaf blight of garlic (*Allium sativum*) in China. *Eur. J. Plant Pathol. 124*: 21-30.

Zheng, L., Lv, R., Huang, J., Jiang, D., and Hsiang, T. (2010). Isolation, purification, and biological activity of a phytotoxin produced by *Stemphylium solani. Plant Dis., 94*: 1231- 1237.