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In-vitro Antagonistic Potential of Different Fungi against *Fusarium oxysporum* f. sp. *Capsici*

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Abstract

The current research was conducted in Lab. to assess an antagonistic effect of various fungi against *Fusarium oxysporum* f. sp. *capsici*. In the present research, each treatment (*Trichoderma viride*, *T. harzianum* and *T. Koningii*) with three concentrations viz. $1\times10^{5,}$ $1\times10^{6,}$ 1×10^{7} spores/ml and three replications were assessed through well method in Phytobacteriology lab, Department of Plant Pathology, University of Agriculture, Faisalabad. Spore concentration was adjusted with the help of haemocytometer and data was recorded after 5 days of inoculation. *T. viride*, *T. harzianum* and *T. Koningii* expressed 1.14, 1.31 and 1.57 cm colony growth in 9cm petri plate respectively. *T. viride* exhibited maximum inhibition of *Fusarium oxysporum* f. sp. *capsici* after comparison of mean colony growth.

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The interaction between Treatment and Concentration (T×C) showed that 1×10^5 conidia/ml expressed 1.44, 1.63, 1.88 and 6.14 cm colony growth of *Fusarium oxysporum* f. sp. *capsici* in petri plat through poisoned food technique by *T. viride, T. harzianum, T. Koningii* and control as compared to 1×10^6 conidia/ml showed 1.11 to 1.60 cm and 1×10^7 conidia/ml of *T. Viride* exhibited minimum colony growth (0.881) as compared to *T. harzianum* (0.96) and *T. Koningii* (1.22) cm respectively. Similarly, the interaction between Treatment and Days (T×D) expressed maximum (1.70, 1.96, 2.20 and 6.47) cm colony growth by fungus after tenth day as compared to (1.13, 1.33, 1.56 and 6.27) cm after seventh day and *T. Koningii* (0.94), *T. harzianum* (0.63) and *T. Viride* (0.60) cm after fourth day respectively while interaction between T×C×D expressed that 1×10^7 conidia/ml of *T. viride* (0.36, 0.8, 1.46), *T. harzianum* (0.4, 1.0, 1.6) and *T. koningii* (0.6, 1.26, 1.8) cm exhibited minimum colony growth after day four, seventh and tenth as compared to 1×10^6 conidia/ml (0.56, 0.60, 0.86, 5.70, 1.16, 1.40, 1.60, 6.27, 1.60, 2.00, 2.33 and 6.47) cm and 1×10^5 conidia/ml showed maximum colony growth (0.86, 1.03, 1.36, 5.70, 1.43, 1.60, 1.80, 6.26, 2.03, 2.26, 2.46 and 6.47) cm at fourth, seventh and tenth days respectively. It was concluded that *T. viride* inhibited the colony growth of *Fusarium oxysporum* f. sp. *capsici* most effectively at concentration 1×10^7 conidia/ml.

Keywords: *In-vitro*; antagonistic fungi; poison food technique; *Fusarium oxysporum* f. sp. *Capsici*.

1. Introduction

Chilli pepper (*Capsicum annuum* L.) is an imperative vegetable crop of family solanaceae which is extensively cultivated in Pakistan and approximately grown in every country of the world [1]. It has high medicinal value as it contains many phytochemicals with different antioxidant properties. It also possess carotenoids, phenolic contents, β carotenoids, β cryptoxanthin and zeaxanthin [2]. This vegetable crop is grown on 20% of the total area under cultivation in Pakistan but Sindh and Punjab provinces are mainly focused whereas NWFP and Balochistan occupies only small area for chilli pepper [3]. It exhibited sensitivity to cool and wet abruptly changing weather whereas Pakistan has diverse climatic conditions so it is cultivated at various ecological zones of Pakistan [4]. In the world, it is cultivated on an area of 57.44 thousand hectares with an annual optimum yield of 186.5 thousand tones whereas in Pakistan, its total area under cultivation is 9.18 thousand hectares with an average production of 11.5 thousand tones [5].

It is attacked by several bacterial, viral, fungal diseases and their virulent pathogens in the absence of resistant varieties/advanced lines but Fusarium wilt of chilli pepper caused by *Fusarium oxysporum* (Schlect.) Emend. Synd. and Hans. f. sp. *capsici* Riv. is the most devastating soil born disease in chilli growing areas of the world [6]. The attack of pathogen and spontaneous spread of this disease depends upon favourable climatic conditions and severity of fresh inoculum quantity of *Fusarium oxysporum* f. sp. *capsici* respectively [7]. The severity and intensity of losses caused by this pathogen is increasing in the regions of its cultivation due to widely adopted improper, mishandling and unguided management approaches [8]. Losses of 45-79% occur owing to this disease in some parts of its cultivation in Pakistan [8]. In the world, sever losses of $65300 million revenue with 82 percent disease incidence and 100kg bags/ha of yield losses occurs in case of epidemic form [9] whereas in vegetables 21.9 percent losses followed by16.6 percent with reduction in production of 115.5 to 90.5 thousand tons during 1999-2005 have observed in hot chilli pepper [10].

The characteristic symptoms of Fusarium wilt of chilli pepper is the appearance of yellow leaves, stunting, decaying, browning, sunken appearance with discoloration and girdling of canker at the base. Numerous management strategies are available to minimize the disease incidence but some of those approaches are impracticable owing to heavy cost as well as severe director or indirect implication on human health. Therefore, it dire need to provide such approaches which are ecofriendly as well as easily accessible to farmers [11].

The application of antagonistic organisms is a type of mutualism, to suppress the pathogen population. Various species of *Trichoderma* are used as bio-control agents to manage the soil born diseases. These species suppress the pathogen population either through directly parasitizing on them or competing for nutrients which are essential for their growth [12]. Use of antagonistic species is environmentally oriented, ecologically and economically sound method as depicted in benefit/cost ratio and expresses less environmental haphazard and toxicity as compared to other conventional disease controlling methods. Hence, owing to rapidly increasing environmental degradation by fungicides and other approaches, it is need of this century to exploit their potential against diseases. Therefore, trials on their potential against *Foc* were carried out in the present study to obtain maximum yield of chilli pepper by reducing the losses caused by this pathogen.

2. Materials and methods

The antagonistic effect of fungal species for inhibiting the growth of *Fusarium oxysporum* f. sp. *capsici* (*Foc*) was evaluated by using Well method [12]. For this purpose 6 mm (0.6cm) dia. plugs of *Foc* and antagonist fungal species (*Trichoderma harzianum, T. Viride, T. Koningii*) were taken with the help of sterilized cork borer. These plugs were placed at the opposite sides of the Petri plates of 9cm diameter having potato dextrose agar medium. After inoculation plates were incubated at 25°C for five days. Petri plates with only *Foc* were served as control.

Each treatment was evaluated at three concentrations ($1\times 10^{5,}$ $1\times 10^{6,}$ 1×10^{7}) with three replications and a control. Spore concentration was adjusted with the help of haemocytometer. Mycelial growth of *Foc* in terms of colony diameter was assessed after 5 days of inoculation. T_1 = *T. viride*, T_2 = *T. harzianum*, T_3 = *T. Koningii* and T_4 = Control.

Antifungal potential of *T. viride* (1×10^5, 1×10^6 and 1×10^7 conidia/ml of water) were evaluated at three concentrations under greenhouse conditions through seed treatment. Seeds of susceptible variety Maxi were surface sterilized with 1% solution of sodium hypochlorite (NaClO) for 3 minutes and rinsed with sterile distilled water. Then seeds were sown in a plug trays (plug size 3.4 × 3.4 × 5 cm, 64 plugs) containing sterilized soil. Trays were kept on glasshouse bench.

After 21 days, plugs containing chilli seedlings (three true leaves) were transplanted into (17x13) size pots containing sterile filed soil infested with *Foc* at 1×10^6spores/ml of H_2O [13]. *T. viride* @ 10 ml conidial suspension/pot was applied through soil drenching while control plants were treated with sterilized water. Experiment was conducted with three replication of each treatment with a control under Complete Randomized Design (CRD). Data regarding disease was recorded with seven days interval. T_1 = *T. viridae* (1×10^5, 1×10^6 and

1×10^7 conidia/ml H_2O), T_2 = Control.

2.1. Data analysis

All the statistical tests were performed using SAS/STAT statistical software (SAS Institute, 1990). Means were separated by using Fisher's protected least significant difference (LSD) procedure by taking P = 0.05% probability level [14]. Analysis of variance (ANOVA), interaction of different treatments and their combinations were developed by using SAS/STAT software package.

3. Results

ANOVA indicated that all the treatments (T), concentrations (C), days (D) and their interactions (T×C), (T×D), (C×D) and (T×C×D) expressed significant results (Table 1). *T. viride* exhibited minimum colony growth (1.14) as compared to *T. harzianum* (1.31), *T. Koningii* (1.57) cm respectively (Table 2).

The interaction between T×C expressed that at 1×10^7 conidia/ml of *T. Viride* exhibited minimum colony growth (0.881) as compared to *T. harzianum* (0.96), *T. Koningii* (1.22) cm while at 1×10^6 conidia/ml showed 1.11 to 1.60 cm and at 1×10^5 conidia/ml expressed 1.44, 1.63, 1.88 and 6.14 cm colony growth of fungus respectively (Table 3).

The interaction between T×D exhibited that *T. Viride* (0.60), *T. harzianum* (0.63), *T. Koningii* (0.94) and control (5.70) cm expressed minimum colony growth at fourth day as compared to day seventh (1.13, 1.33, 1.56 and 6.27) cm and tenth day (1.70, 1.96, 2.20 and 6.47) cm respectively (Table 4) while interaction between T×C×D expressed that 1×10^5 conidia/ml of all treatments exhibited maximum colony growth (0.86, 1.03, 1.36, 5.70, 1.43, 1.60, 1.80, 6.26, 2.03, 2.26, 2.46 and 6.47) cm at fourth, seventh and tenth day as compared to 1×10^6 conidia/ml (0.56, 0.60, 0.86, 5.70, 1.16, 1.40, 1.60, 6.27, 1.60, 2.00, 2.33 and 6.47) cm and 1×10^7 conidia/ml (0.36 to 5.70, 0.80 to 6.27 and 1.46 to 6.47) cm respectively (Fig. 1).

Table 1: ANOVA for *in-vitro* evaluation of antagonistic organisms against *Fusarium oxysporum* f. sp. *apsici*

SOV	DF	SS	MS	F	P
Treatments (T)	3	469.969	156.656	29608	0.000*
Concentrations (C)	2	4.064	2.032	384	0.000*
Days (D)	2	22.276	11.138	2105.06	0.000*
T × C	6	1.422	0.237	44.78	0.000*
T× D	6	0.989	0.165	31.17	0.000*
C × D	4	0.065	0.016	3.06	0.000*
T × C× D	12	0.177	0.015	2.78	0.000*
Error	70	0.370	0.005		
Total	107	500.241			

* = Significant at $P < 0.05$

Table 2: *In- vitro* evaluation of antagonistic organisms against colony growth of *Fusarium oxysporum* f. sp. *capsici*

Sr #	**Treatments**	**Colony growth (cm)**
T_1	*T. Viride*	1.14d
T_2	*T. harzianum*	1.31c
T_3	*T. Koningii*	1.57b
T_4	Control	6.14a
	LSD	**0.0395**

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$)

Table 3: Impact of antagonistic organisms and their concentrations on colony growth of *Fusarium oxysporum* f. sp. *capsici*

Treatments	**Colony growth (cm)**		
	Concentrations (conidia/ml)		
	1×10^5	**1×10^6**	**1×10^7**
T. Viride	1.44d	1.11g	0.88 l
T. harzianum	1.63c	1.33e	0.96h
T. Koningii	1.88b	1.60c	1.22f
Control	6.14a	6.14a	6.14a
LSD	**0.0684**		

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

Table 4: Impact of various antagonistic organisms and days on colony growth of *Fusarium oxysporum* f. sp. *capsici*

Treatments	**Colony growth (cm)**		
	Fourth day	**Seventh day**	**Tenth day**
T. Viride	0.60k	1.13 l	1.70f
T. harzianum	0.63k	1.33h	1.96e
T. Koningii	0.94j	1.56g	2.20d
Control	5.70c	6.27b	6.47a
LSD	**0.0684**		

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

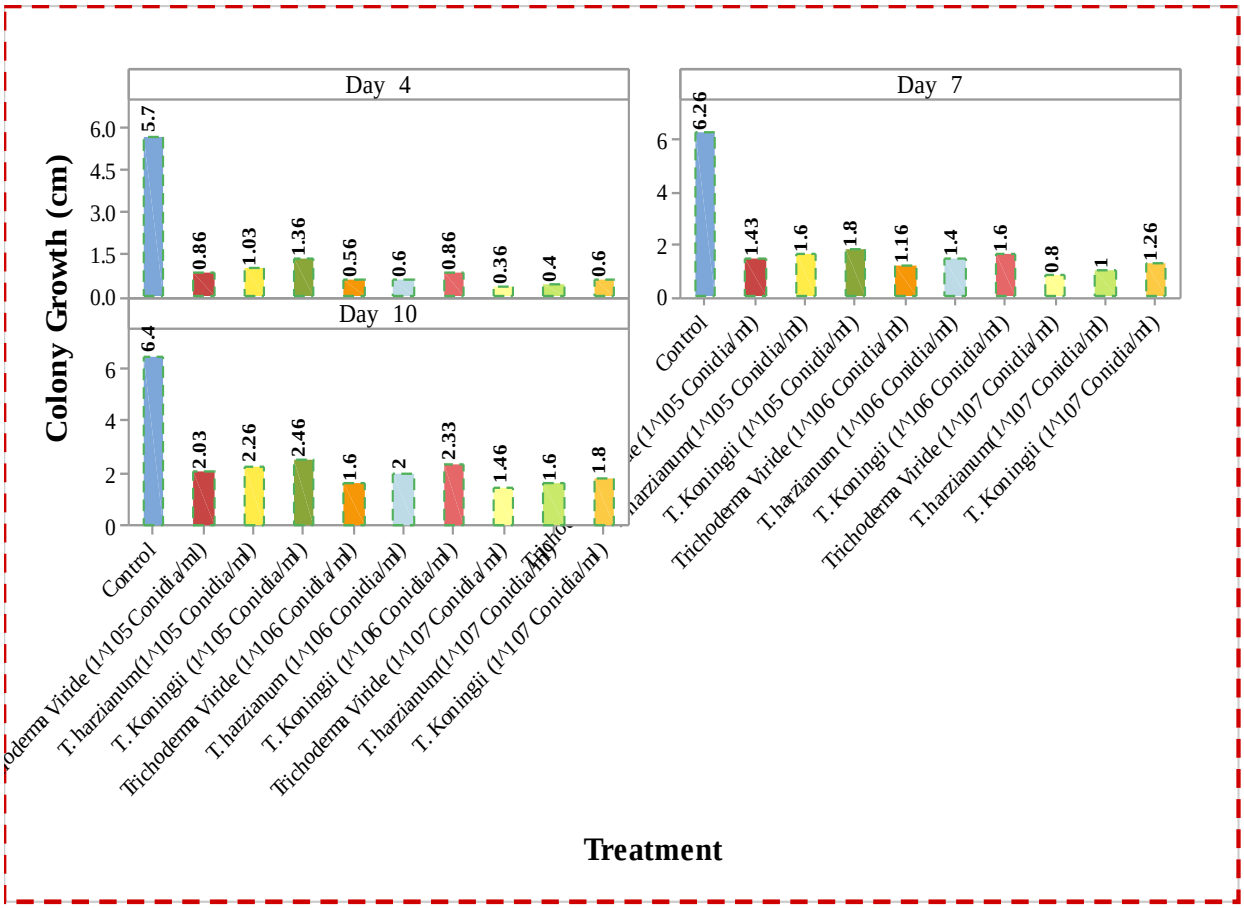

Figure 1: Impact of interaction b/w treatments, concentrations and days on colony growth of *Fusarium oxysporum* f. sp. *capsici* under lab conditions

4. Discussion

Fusarium wilt is the prominent and dominant ailment of chilli pepper caused by *Fusarium oxysporum* f. sp. *capsici* which has attained the economic importance due to heavy losses caused by this pathogen [15]. Due to ubiquitous nature of this pathogen, the disease has caused ephiphytotics in several chilli growing regions of Pakistan and consequently diminishing 45-60 percent yield losses under conducive soil and environmental conditions [16]. Similarly, the losses of $65300.00 million have been reported in the world owing to this disease whereas in Pakistan 21.9 percent on vegetables followed by 16.6 percent on chilli pepper have been observed [17].

Presently much emphasis has given to antagonistic organisms in order to reduce fungicide use for management of this disease and to avoid environmental pollution, cost of management and occurrence of lethal, aggressive and virulent strains of *Foc* [18]. Several biocontrol agents have been found to contain antifungal potential against Fusarium wilt [19]. These antagonistic organisms adopted numerous types of mechanisms such as antibiosis, parasitism/predation, induced resistance, competition for nutrients and lytic enzymes to inhibit the growth of *Foc* [20, 21].

In the current studies, *T. viride* expressed significant results as compared to *T. koningii* by reducing the colony growth of *Foc*. Reference [22] used *T. Viride*, *T. harzianum*, *T. pseudokoningii*, *T. aureoviride* and *T. Koningii* in the lab. against *Foc* and observed 62% reduction in colony growth by *T. viride* similarly [22] another found 50 percent inhibition in radial colony growth by using different strains of *T. harzianum*. Numerous other species of Trichoderma expressed pronounced results when inoculated in small seedlings of chilli pepper against Fusarium wilt [22].

Similar results were also observed by [23]. They observed that *T. viride* and *T. harzianum* are the most significant antagonistic organisms [24] due to secretion of extracellular lytic enzymes and other compounds like harzianien and viridian which enhanced their antagonistic activity against Fusarium wilt of chilli pepper [25]. It has been also visualized that *Trichoderma* spp. hinder pathogenic invasion through release of organic metabolite such as chitinase, pachybasin and volatile inhibitory compounds i.e. acetaldehyde [26]. These findings are also in line with [27] as well as [28] who observed that inhibition of pathogen severity can be minimized undoubtedly due to oozing of extracellular cell degrading enzyme like cellulase, glucanase, chitinase B-1, 3 and lectin which are helpful for mychparasitism to colonize their host. Similarly pathogen inhibition by antagonists may also be due to production of secondary metabolites such as gliotoxin, viridin and glioviridin. It has been also observed that *Trichoderma* spp. excrete unsaturated monobasic acids (Dermadine), extracellular enzymes (chitinase, cellulase) and polypeptides (Alamethicine, Suzukacillin) that reduces soil born plant pathogen population [29]. Similar observations have been described by [30, 31, 32] they proposed numerous possible ways to describe the phenomenon comprising the control of minor pathogens, production of vitamins, production of plant harmones, alteration of non-utilizable materials into such form that can easily utilize by plants and enhance uptake and translocation of minerals, maximize the efficiency of nutrient uptake as well as solubilizing numerous insoluble nutrient elements such as rock phosphate [33].

5. Future perspectives

There is further need to investigate biochemical and physicochemical process as well as formulations of specific antifungal compounds (enzymes and metabolites) produced by *Trichoderma* species which may provide comprehensive mechanism of antagonism and myco-parasitism against soil borne pathogens.

6. Conclusions

Application of antagonistic organisms is an economical approach for the management of Fusarium wilt of chilli pepper cause by *Fusarium oxysporum* f. sp. *capsici*. This approach is ecofriendly and cause less direct or indirect health implications so can be used for suppressing the incidence of disease.

References

[1]. Bajwa, R., Mukhtar, I., & Anjum, T., 2004, “In-vitro biological control of Fusarium solani cause of wilt in Dalbergia sissoo Roxb.” Mycopathology 2(1): 11-14.

[2]. Hernandez D. H., Sonia G. S. A., & Goni I., 2010. “Bioactive compounds of four hot pepper varieties

(Capsicum annuumL.), antioxidant capacity, and intestinal bioaccessibility." Journal of Agriculture Food Chemistry, 58(6): 3399–3406.

[3]. Saleem A., Hamid K., Tariq A. H., & Jamil F.F., 2000. "Chemical control of root and collar rot of chillies." Pakistan Journal of Phytopathology, 12(1): 1-5.

[4]. Hanson L. E., & Howell C. R., 2004. "Elicitors of plant defense responses from biological control strains of Trichoderma virens." Journal of Phytopathology, 94: 171–176.

[5]. Sahi I. Y., & Khalid A. N., 2007. "In-vito biological control of Fusarium oxysporum causing wilt in Capsicum annuum." Mycopathology, 5(2): 85-88.

[6]. Jagtap P. P., Shingane U. S., & Kulkarni K. P., 2012. "Economics of chilli production in India" African Journal of Basic and Applied Sciences, 4(5): 161-164.

[7]. Chohan, S., Perveen, R., Mehmood, M. A., Naz, S., & Akram, N., 2015. "Morpho-physiological studies, management and screening of tomato germplasm against Alternaria solani, the causal agent of tomato early blight." International Journal of Agriculture and Biology, 17: 111-118.

[8]. Amekonnen A, Awoubit D, Aalemu L, Btariku H, 2015. "Assessment of wilt intensity and identification of causal fungal and bacterial pathogens on hot pepper (Capsicum annuum L.) in Bako Tibbe and Nonno districts of west Shewa zone, Ethiopia." International Journal of Phytopathology., XXX.

[9]. Matthew A., Lawal A., Chindo P., & Olalekan B., 2006. "Outbreak of basal stem rot and wilt disease of pepper in northern Nigeria." Journal of Plant Protection Research, 46(1): 7-14.

[10]. Hussain F., & Abid M., 2011, "Pests and diseases of chilli crop in Pakistan" A review. International Journal of Biology and Biotechnology, 8(2): 325-332.

[11]. Bahar, M., Shahab, H., & Nikoo, K., 2012, "Screening of resistance genes to Fusarium root rot and Fusarium wilt diseases in tomato (Lycopersicon esculentum) cultivars using RAPD and CAPs markers." European Journal of Experimental Biology, 2 (4): 931-939.

[12]. Irfan Y. S., & Khalid A. N., 2007. "In-vito biological control of Fusarium oxysporum causing wilt in Capsicum annuum" Mycopathology, 5(2): 85-88.

[13]. Parsa, S., S. T. Sahi, A. Jabbar, A. Rehman, K. Riaz and A. Hannan. 2013. Chemical and biological management of Fusarium oxysporum f. sp melongenae. Pak. J. Phytopath. 25(2): 155-159.

[14]. Steel R. G. D., Torrie J. H., & Dickey D. A., 1997, "Principles and procedures of statistics." A Biometrical Approach. 3rd Ed. McGraw Hill Pub. Co., New York.

[15]. Benitez, T., Rincon, A. M., Limon, M. C., & Codon, A. C., 2004, "Biocontrol mechanisms of Trichoderma strains." International Journal of Microbiology, 7: 249-260.

[16]. Najar A.G., Ganie S. A. & Lone A. H., 2016. "An eco-friendly approach for the management of Fusarial wilt [Fusarium pallidoroseum (Cooke) Sacc.] of chilli." International Journal of Modern Biology & Mediterranean, 7(1): 12-18.

[17]. Bashir M. R., Atiq M., Sahi S. T. and Sagheer M. 2016. "Resistance status of chilli germplasm against Fusarium wilt." Transylvanian Review. 24(6): 636-642.

[18]. Sultana J. N., Pervez Z., Rahman H., & Islam M. S., 2012. "In-vitro evaluation of different strains of Trichoderma harzianum and Chaetomium globosum as biological control agents seedling mortality of chilli." Bangladesh Research Publication Journal, 6(3): 305-310.

[19]. Heydari A., & Pessarakli M., 2010, "A review on biological control of fungal plant pathogens using microbial antagonists." Pakistan Journal of Biological Sciences, 10: 273-290.

[20]. Suprapta D. N., 2012. "Potential of microbial antagonists as biocontrol agents against plant fungal pathogens." J. ISSAAS. 18(2): 1-8.

[21]. Segarra G., Aviles M., Casanova E., Borrero C., & Trillas I., 2013, "Effectiveness of biological control of Phytophthora capsici in pepper by Trichoderma asperellum strain T34." Phytopathological Mediterranian, 52(1): 77−83.

[22]. Diaz J., Silvar C., Varela M. M., & Merino F., 2005. "Fusarium confers protection against several mycelial pathogens of pepper plants." Canadian Journal of Plant Pathology, 54: 773-780.

[23]. Elad Y., Chet I., & Katan J., 1982. "Degradation of plant pathogenic fungi by Trichoderma harzianum." Canadian Journal of Microbiology, 28: 719-725.

[24]. Hajieghrari B., Giglou M. T., Mohammadi M. R., & Davari M., 2008. "Biological potential of some Iranian Trichoderma isolates in the control of soil borne plant pathogenic fungi." African Journal of Biotechnology, 7(8): 967-972.

[25]. Ozbay N., & Newman E. S., 2004, "Effect of T. harzianum strains to colonize tomato roots and improve transplant growth." Pakistan Journal of Biological Sciences, 7: 253-257.

[26]. Bunker R. N. & Kusum M. 2001. "Integration of biocontrol agents and fungicides for suppression of dry root rot of Capsicum frutescens." Journal of Mycology and Plant Pathology, 31: 330-334.

[27]. Muhammad S. and Amusa N. A. 2003. "In-vitro inhibition of growth of some seedling blight inducing pathogens by compost-inhabiting microbes." African Journal of Biotechnology, 2(6): 161-164.

[28]. Shabir R. and Rubina L. 2010. "Biological control of damping-off disease of cabbage caused by Rhizoctonia solani Kuchn." Applied Biological Research, 12: 38-41.

[29]. Champawat R. S. and Sharma R. S. 2003. "Integrated management of nursery disease in brinjal, chilli, cabbage and onion." Journal of Mycology and Plant Pathology, 33(2): 290-291.

[30]. Marnoranjitham S. K., Prakasam V. and Rajappan K. 2001. "Biological control of damping-off disease using talc based formulations of antagonists." Annual Plant Protection Sciences, 8(2): 159-162.

[31]. Srivastava V. K. 2004. 'Trichoderma spp- a boon for better crop health." Pestology. XXVIII(8): 40-45.

[32]. Srideepthi R. & Krishna M. S. R, 2015. "Antimycotic effect of Trichoderma species on Fusarium oxysporum f.sp. capsici inciting vascular wilt in Chilli." In: New Horizons in Biotechnology. (Eds. Viswanath B and Indravathi G) Paramount Publishing House, India, pp. 029-031.

[33]. Aswini A., Sharmila T., Raaga K., Sri Deepthi R. and Krishna M. S. R. 2016. "In-vitro antifungal activity of Trichoderma strains on pathogenic fungi inciting hot pepper (Capsicum annuum L.)." Journal of Chemical & Pharmaceutical Research, 8(4): 425-430.