



## Management of Fusarium wilt of chilli caused by *Fusarium oxysporum* f. sp. *capsici* through nutritional amendments under greenhouse conditions

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### Abstract

Fusarium wilt of chilli is the most important disease in chilli growing areas. This disease has reduced \$65300.00 million revenue with 48 percent disease incidence. Thus, the current research was conducted for its management through non-conventional approaches under greenhouse conditions. Different concentrations of liquid fertilizers namely Nutritop and Compound containing micro and macro nutrients viz. Zn, Br, Fe and NPK alone as well as in combination were used. Most susceptible variety of chilli namely Maxi were sown in plastic tray. Fifteen days old seedlings were transplanted in plastic pots (17×13cm) containing formalin (1:320) sterilized soil. The interaction of treatments with concentrations and days were recorded after seven days. Means of all treatments expressed that Nutritop (Zn, Br and Fe) exhibited maximum disease incidence (36.96) followed by Compound (NPK) 30.31 and Compound + Nutritop (27.66) as compared to control (50.99) percent. The concentration 5000ml/ha of Compound + Nutritop exhibited minimum disease incidence of 22.77% as compared to other concentrations viz. 3000 and 4000ml/ha respectively. Likewise, interaction between treatments and days expressed minimum disease incidence of 31.47 percent by Compound + Nutritop after seventh day as compared to fourteenth (27.53) and twenty one days (23.97) percent respectively. It was concluded that concentration 5000ml/ha of compound + Nutritop minimizes the disease incidence with the passage of time which is an apt management for farmers to overcome the losses cause by Fusarium wilt of chillies.

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## Introduction

Chilli pepper (*Capsicum annum* L.) is an important vegetable and cash crop of Pakistan (Shakouri *et al.*, 2014). It contains proteins, vitamins (A and C), minerals i.e. calcium, phosphorous and irons (Chowdhury *et al.*, 2015). Total area under its production is 9.18 thousand hectares with annual production of 11.50 thousand tons (Sahi and Khalid, 2007). Fusarium wilt of chilli pepper caused by *Fusarium oxysporum* f. sp. *capsici* is one of the most nefarious disease in chilli growing areas of Pakistan (Bashir *et al.*, 2016).

The most visualized characteristic symptoms of Fusarium wilt is slight vein clearing on your leaves, epinasty of older leaves, stunted growth, yellowing adventitious roots bunch formation, wilting of leaves and stem, defoliation, marginal necrosis, browning of vascular tissues, blockage of vascular system and finally death of complete plant (Sahi and Khalid, 2007).

Nutrients are much essential for plant health. Type of disease as well as amount and form of elements are crucial components on the disease progression because minerals are the vital part of plant nutrition. Deficiency of nutrients causes various illnesses in the plants either through creating problems in metabolism or disturbing physiology of the plants by favoring plant pathogens (Asma *et al.*, 2009). Foliar application of nutrients reduces the disease incidence/disease severity by inducing systemic acquired resistance, reducing amino acid and starch formation, inhibiting the sporulation, spore germination and reducing the sensitivity of plants to fusaric acid by creating physical barrier (Hilal *et al.*, 2001; Ma and Yamaji, 2006). The excessive application of these nutrients increases the level of sugar and carbohydrates which makes the plants susceptible for the attack of pathogen (Saikia *et al.*, 2009). The upsurge of nutrients enhances or diminishes pH, NH<sub>3</sub> level in the soil. Their presence spontaneously causes serious impact on microconidia, macroconidia and chlamydospores density which consequently hinder the germination of Fusarium wilt pathogen (Chellemi and Lazarovits, 2002).

The foremost advantage of fertilizers is the short interval during which they supply nutrients to soil microbe and played crucial role for the decomposition process of soil organic matter and ultimately maintained the soil health though enormous availability of nutrients. These nutrients after translocation instigate the resistance mechanism of plants against virulent races of pathogen which resultantly inhibit the disease intensity (Scheuerell, 2004). The balanced amount of nutrients is much pivotal for plant health to compete the different challenges. It is dire need to find the most compatible combination of nutrients for strengthen the plants and to save the environment from hazardous impact of soil, water and air pollution. Therefore, in the current research various nutrients were evaluated to find out their most suitable combination and application dozes for the management of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *capsici* under greenhouse conditions.

## Materials and methods

### *Isolation, purification and identification of Fusarium oxysporum f. sp. capsici*

Plants showing characteristic symptoms of chilli wilt were collected with fibrous tertiary roots on the basis of visual observations and brought in the laboratory for isolation of *Foc*. Infected roots were washed thoroughly and cut into small pieces, and surface sterilized with 1% HgCl<sub>2</sub>. The roots were dried on sterilized filter paper and placed on watch glass. At least two pieces of infected roots were placed in petri plat containing Potato Dextrose Agar (PDA) medium. The plates were incubated at ± 25°C for 48-72 hours for fungal growth (Sarwar *et al.*, 2005). Then colonies of *Foc* were purified. Identification of *Foc* was done under stereomicroscope through morphological characteristics such as mycelium with white and purple colour and microconidia on short conidiophore (Soesanto *et al.*, 2011).

### *Growing and transplanting of nursery*

Seeds of susceptible chilli pepper variety namely Maxi was obtained from National Agricultural Research Counsel (NARC). The seeds were sown in plastic tray and placed on bench under greenhouse controlled conditions.

After germination, fifteen days old seedlings were transferred to plastic pots (17×13cm) containing formalin sterilized soil (1:320) and arranged them on greenhouse wooden table under completely randomized design (CRD) with three replications.

#### *Inoculation of young seedlings with Fusarium oxysporum f. sp. capsici active culture*

After fifteen days of transplantation, seedlings were inoculated @ 40ml/pot with fungal spore suspension ( $1 \times 10^6$  spores/ml of H<sub>2</sub>O) near the root zone by making 3-4 holes at the depth of 2cm.

After inoculation these holes were covered with soil to minimize evaporation losses of spore suspension.

#### *Nutrients application and Data recording*

Different concentrations of nutrients mixtures i.e. Compound (NPK) and Nutritop (Zn, Br and Fe) in alone and in combination were applied through soil drenching (Ashfaq, 2007). Data regarding disease incidence was recorded with one week interval by using Monaim and Ismail's (2010) scale as given (Table 1).

**Table 1.** Disease rating scale for *Fusarium* wilt of chilli pepper.

Disease rating	Description	Response	Symbol
0	0	Immune	I
1	1-20 %	Resistant	R
2	21-40 %	Moderately resistant	MR
3	41-50 %	Moderately susceptible	MS
4	51-70 %	Susceptible	S
5	71-100 %	Highly Susceptible	HS

(Monaim and Ismail, 2010).

Disease incidence was calculated by using following formula.

$$\text{Disease Incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100$$

#### *Statistical analysis*

Experiment was conducted under completely randomized design (CRD). Statistical analysis was performed using Minitab statistical software version 2015. Fisher's least significant difference (LSD) test was used to separate means of data regarding disease incidence (Steel *et al*, 1997).

**Table 2.** Impact of nutrient mixtures on the development of *Fusarium* wilt of chilli pepper under greenhouse conditions.

Sr.	Treatments	Disease Incidence (%)
T <sub>1</sub>	Compound	30.31c
T <sub>2</sub>	Nutritop	36.96b
T <sub>3</sub>	Compound + Nutritop	27.66d
T <sub>4</sub>	Control	50.99a
	LSD	0.2351

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

#### **Results**

Compound along with Nutritop exhibited minimum disease incidence (27.66) as compared to Compound (30.31), Nutritop (36.96) and control respectively (Table 2).

The interaction of treatments and concentrations expressed 24.37 percent disease incidence was expressed by Compound @ 6000 ml/ha as compared to 5000 (31.53 %) and 4000 ml/ha (35.03%) while 1000,

1200 and 1400 ml/ha concentrations of Nutritop exhibited 40.40, 37.40 and 33.08 % disease incidence respectively. Minimum disease incidence (31.33, 28.8 and 22.77) % was observed when (Compound + Nutritop) was applied @ 3000, 4000 and 5000 ml/ha respectively (Table 3). Interaction between treatments and days expressed 33.73, 30.47 and 26.73 percent disease incidence by applying Compound (NPK) whereas Nutritop exhibited 40.07, 37.47 and 33.34 % while (Compound + Nutritop) showed 31.47, 27.57 and 23.97 percent disease incidence as

compared to control after seventh, fourteenth and twenty first days (Table 4). Similarly, interaction between treatments, days and concentrations expressed that 38.40, 34.50, 28.30 % after seven days, 35.40, 31.60, 24.40 % after fourteen and 31.30, 28.50 and 20.40 % disease incidence after twenty one day @ 4000, 5000 and 6000 ml/ha while Nutritop expressed 44.30, 40.30, 35.60% @ 1000 ml/ha, 40.50, 37.60, 34.30 @ 1200 ml/ha and 36.40, 34.30 and 29.33 % disease incidence after seventh, fourteenth and twenty one day respectively.

**Table 3.** Impact of interaction between treatments and concentrations (T×C) on the development of Fusarium wilt of chilli pepper.

Treatments	Disease incidence (%)		
	Concentrations		
	I	II	III
Compound	35.03e	31.53g	24.37i
Nutritop	40.40c	37.40d	33.08f
Compound + Nutritop	31.33g	28.87h	22.77j
Control	34.73e	69.87a	48.37b
LSD	0.4072		

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ( $P \leq 0.05$ ). Compound @ (4000, 5000 and 6000ml/ha), Nutritop @ (1000, 1200 and 1400ml ha) and Compound + Nutritop @ (3000, 4000 and 5000ml/ha).

Minimum disease incidence was observed when Compound + Nutritop applied @ 3000, 4000 and 5000 ml/ha after seven days (34.40, 32.60 and 27.40), fourteen days (31.30, 28.70 and 22.60) and twenty first day (28.30, 25.30 and 18.30) % respectively as compared to other treatments (Fig. 1).

## Discussion

### *Disruption in plant physiological functions and utilization of nutrients*

The attack of pathogen demolishes the physiological functions of plants such as uptake of essential nutrient, assimilation, translocation from root system to shoot system and their utilization (Stewart *et al.*, 2005; Khan *et al.*, 2012). These nutrients maintain plant health through activation of metabolism as well as by regulating various cellular functions that empower the plant to tolerate the attack of plant pathogens (Saikia *et al.*, 2009).

Adequate availability of macro (NPK) and micro nutrients (Zn, B and Fe) minimize the incidence of plant diseases (Mahmood and Bashir, 2011) because most of them are catalytically active co-factors in enzymes whereas some nutrients structurally stabilize the protein molecule (Allabi, 2006; Suharja and Sutarno, 2009). Pathogen attack reduces nutrient availability and deteriorates defense system, growth and development of plant (Sanjeev and Eswaran, 2008). In present studies, mixture of NPK (Compound) and Zn, B and Fe (Nutritop) alone and in combination (Compound + Nutritop) were evaluated against *Foc*. Compound alone and in combination with Nutritop expressed significant results against *Fusarium* wilt of chilli pepper.

*Implication of macro nutrient's in plant health*

Nitrogen is the integral part of proteins, amides, amino acids, nucleotides, nucleic acids, coenzymes, chlorophyll, auxin and cytosine (Guertal, 2000). Plants absorb this nitrogen from soil in the form of nitrate ( $\text{NO}_3^-$ ) and ammonium ion ( $\text{NH}_4^+$ ).

*Foc* creates disturbance in uptake of these ions and consequently hinder plant growth (Chellemi and Lazarovits, 2002). Phosphorus is a crucial component of ATP (Adenosine triphosphate), nucleoprotein, RNA, DNA, phosphoprotein and cell membrane (Suntoro, 2002).

**Table 4.** Impact of interaction between treatments and days (T×D) on the development of Fusarium wilt of chilli pepper under greenhouse conditions.

Treatments	Disease incidence (%)		
	Days		
	D <sub>7</sub>	D <sub>14</sub>	D <sub>21</sub>
Compound	33.73 <sup>f</sup>	30.47 <sup>h</sup>	26.73 <sup>j</sup>
Nutritop	40.07 <sup>d</sup>	37.47 <sup>e</sup>	33.34 <sup>f</sup>
Compound + Nutritop	31.47 <sup>g</sup>	27.53 <sup>i</sup>	23.97 <sup>k</sup>
Control	47.78 <sup>c</sup>	51.68 <sup>b</sup>	53.51 <sup>a</sup>
LSD	0.4072		

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

*Impact of micro nutrients to strengthen plants against pathogen attack*

Zinc is an important element that plays crucial role in the synthesis of protein and starch. Its low concentration inhibits amino acid production and reduces the sugar level in plant tissues. It involves in the cell membrane protection against oxidative damage by detoxifying the superoxide radicals (Cakmak, 2000). Free radicals cause destruction of cell membrane and leakage of low molecular weight compounds which provide food for pathogen (Mengel and Kirkby, 2001). The adequate application of Zinc in the soil reduces the infection of *Fusarium* spp. (Grewal *et al.*, 1996). Boron is an essential micronutrient for healthy and vigorous plant. Its deficiency in plants is the major problem in the world (Brown *et al.*, 2002). It plays an important role in diminishing disease severity by enhancing stability and rigidity of cell wall (Dordas and Brown, 2005). Iron (Fe) promotes the synthesis of antimycosis. It activates the enzymes that are involved in the defense system of host plant. Similarly, the synthesis of siderophores by soil microorganism can lower the level of Fe which inhibits the chlamydo spores germination of *Fusarium oxysporum* f. sp. *capsici* (Mann *et al.*, 2004).

**Conclusion**

Liquid fertilizers alone and in combination *viz.* Compound (NPK) + Nutritop (Zn, Br and Fe) @ (3000, 4000 and 5000ml/ha) against Fusarium wilt of chilli pepper caused by *Fusarium oxysporum* f. sp. *capsici* expressed statistically significant results which can be used in future nutritional management program if all other agronomic practices are maintained adequately.

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