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COMPARATIVE EFFICACY OF DIFFERENT FUNGICIDES AGAINST FUSARIUM WILT OF CHICKPEA (*CICER ARIETINUM* L.)

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Abstract

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (*Foc*) is the most serious and widespread disease of chickpea, causing a 100% loss under favorable conditions. Fourteen fungicides were evaluated against wilt pathogen *In vitro* with five different concentrations ranging from 1-10000 ppm. Among these only Carbendazim and Thiophanate-methyl was found as the most effective at all used concentrations. Other fungicides like Aliette, Nativo, Hombre-excel and Dividend star were found to be moderately effective. Whereas, remaining fungicides were ineffective against the targeted pathogen. Generally, a positive co-relation was observed between increasing concentrations of the tested fungicides and inhibition of *Foc*. Based on *In vitro* results Carbendazim, Thiophanate-methyl, Aliette, Dividend-star, Hombre-excel, Score and Nativo were selected for pot and field experiments. The higher concentrations of the few fungicides completely inhibited the pathogen as well as found to be phytotoxic and suppressed the plant growth while lower concentrations promoted the growth of chickpea plants. On over all bases, the Carbendazim and Thiophanate-methyl, followed by Aliette and Nativo were more effective in reducing the impact of pathogen as well as enhancing the plant growth in greenhouse experiment. Under field conditions, all fungicides except Score remarkably decreased the disease development and subsequently increased the plant growth as well as grain yield as compared to untreated plants.

Key words: *Fusarium oxysporum* f. sp. *ciceris*, Chemical control, Chickpea.

Introduction

Chickpea (*Cicerarietinum* L.) is an important pulse crop of family Leguminaceae. It is used as a big source of protein in the human diet. Kabuli and Desi are the two major groups of chickpea cultivars grown throughout the world. Chickpea was originated from West Asia and now cultivated in 55 countries of the world. Worldwide it is grown on an area of 13.5 million hectares with a production of more than 13 million tons. It is an important crop of Indian sub-continent that usually contributes more than 66% in terms of global production, while Pakistan ranked seventh and producing 2.5% of the world production (Anon., 2013). Per capita consumption of chickpea in Pakistan is very high; therefore, domestic production is not enough to meet local requirements. Moreover, per hectare yield in Pakistan is very low (757 Kg/ha) as compared to high yielding (3333 Kg/ha) country China. Low yield and increasing demand of chickpea has adversely affected the supply situation. As a result, Pakistan becomes net importer of chickpea and imported 279650 tones during 2011 to meet domestic requirements (Anon., 2013).

Annual yield losses in chickpea were estimated to be 4.8 million tones worldwide due to biotic stresses, including infectious plant diseases (Ryan, 1997). Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (Padwick) Matuo & K. Sato, is considered most serious and widespread disease of chickpea throughout the chickpea growing areas of the world (Haware, 1990; Jalali & Chand, 1992; Nene & Reddy, 1987; Trapero-Casas & Jimenez-Diaz, 1985). Under severe conditions, the wilt infection can damage the crop completely and cause 100% yield loss (Navas-Cortés *et al*., 2000; Halila & Strange, 1996). However, yield losses of 10-15% were reported as a regular feature of this destructive disease (Haware, 1990; Campbell & Madden, 1990). In India, annual yield losses due to Fusarium wilt were estimated about 10% (Trapero-Casas & Jiménez-Díaz, 1985) and in Tunisia 40% (Bouslama, 1980). The same disease has reduced the share of chickpea from 50% in 1950s to 10% in 1990s on irrigated lands of Pakistan (Haqqani *et al*., 2000).

The chickpea wilt fungus *Foc* is a vascular pathogen that perpetuates through seed and soil. It can survive in soil, even in the absence of a host for 3-6 years (Ayyub *et al*., 2003; Haware *et al*., 1996). This pathogen can cause infection at all stages of plant growth with more incidences in flowering and poding stage. Relatively high temperature with drought may cause upto 80% plant mortality (Govil & Rana, 1994). As a result of wilt infection, the complete plant or plant parts may die within few weeks of infection. In field conditions, the typical wilting can be appeared within 3-4 weeks after sowing, if the variety is susceptible (Haware, 1990).

Diseases are controlled through different strategies such as use of resistant cultivars, cultural practices, use of chemicals and by bio-control agents. Although each of these methods of disease management practices has their own importance, yet none is completely successful when applied alone for disease control (Chandel & Deepika, 2010). Despite many attempts to control chickpea wilt fungus *Foc*, the problem is still important throughout the world. The chemical control based on the use of fungicides is most effective and reliable method. No economical and eco-friendly control measures are available to combat this devastating threat (Bakhsh *et al*., 2007). New fungicides with novel chemistry are being introduced and evaluated for plant disease control. Their application in the farmer fields can only be recommended against the causal pathogens after a successful laboratory

evaluation. It, therefore, needs a constant watch and effort to evolve new fungicides along with some important non-chemical methods of controlling the diseases (Jamil & Kumar, 2010). The present study is carried out to evaluate different fungicide against chickpea wilt fungus *Foc* and their effect on plant growth and disease development.

Material and Methods

***In vitro* efficacy of fungicides:** Different systemic and contact fungicides were tested by the food poisoning method at 1, 10,100, 1000 and 10000 ppm concentrations (Borum & Sinclair, 1968). The detail of fungicides including their trade name, chemical name, active ingredient, formulation, chemical group and mode of action are given in Table 1. The required concentrations were added in the sterilized medium at the time of pouring. PDA medium without fungicide served as control. After solidifying the medium, one cm disc of pure culture of test fungus was placed in the center of Petri-dishes and incubated at 25°C, for 7 days. There were five replications of each treatment. Radial mycelial growth of the test fungus was recorded in millimeter after each 24 hours till the control plates were full with the mycelial growth of the fungus. The effectiveness of each fungicide against the test pathogen was assessed by determining the percent inhibition of radial growth (PIRG) by using the following formula:

$$PIRG = (R1 - R2)/R1 \times 100$$

where;

R1: Radial growth of test pathogen in control plate.
R2: Radial growth of test pathogen in the treated plate

Effect of fungicides on plant growth and disease development: Seven fungicides *viz*., Carbendazim, Thiophanate-methyl, Aliette, Dividend-star, Hombre-excel, Score and Nativo were selected for *In-vivo* studies. Efficiency in *In vitro* assay against *Foc* was the selection criteria of fungicides.

Greenhouse experiment: A pot experiment conducted to evaluate the effect of selected fungicides on plant growth and disease development of chickpea plants. Three different concentrations (10, 100 and 1000 ppm) of each fungicide were tested. For this purpose seeds of the of the commonly growing chickpea variety 'Rabbat' were surface sterilized with 5% commercial bleach (Sodium hypochlorite) for 1-1.5 minutes. Ten surface sterilized seeds were sown in 20 cm diameter earthen pots containing 2 Kg steam sterilized soil (sandy clay loam). The soil artificially infested with pathogen inoculum at 10^5 conidia/g of soil. Ten seeds were sown in each pot. The seeds were then covered with a thin layer of soil. After 7 days of sowing, the required concentrations of fungicides (10, 100 and 1000 ppm) were drenched into the soil. The experiment arranged as RCBD with three replications. The pots irrigated whenever needed with sterilized water. After 45 days of sowing, the plants were uprooted and data on plant mortality, root infection, plant length (cm) and weight (g) as root and shoot separately were recorded.

Field experiment: The field trial was conducted using chickpea variety 'Rabbat' at the experimental field of Plant Pathology Section, Agriculture Research Institute, Tandojam, during Rabi season 2012-2013. Chickpea seeds were obtained from Pulses Station, Agriculture Research Institute, Tandojam. The experiment was designed as Randomized Complete Block Design (RCBD) with four replications. The plot size was 5×3 m^2; there were 10 rows per plot with row-to-row distance of 30 cm.

Seed treatment: The seeds were treated with selected fungicides (Carbendazim, Thiophanate-methyl, Aliette, Nativo, Dividend-star, Hombre-excel and Score at 2g or 2ml per kg of seed). Untreated seeds served as control. The data on plant mortality, plant growth, disease incidence, root infection and grain yields were recorded. All agronomic practices (fertilization and irrigation) were carried out as per recommendations.

Finally the data was analyzed by using Statistix 8.1 software. The results are subjected to ANOVA followed by Duncan's Multiple Range Test to compare treatments means at P = 0.05 (Gomez & Gomez, 1984).

Table 1. Details of fungicides used in the experiments.

Trade name	Chemical name	Active ingredient	Chemical group
Thiophanate-methyl	Thiophanate-methyl	70% Thiophanate-methyl	Thiophanate-methyl
Carbendazim	Carbendazim	50% Carbendazim	Benzimidazole
Aliette	Fosetyl-aluminium	80% Fosetyl-aluminium	Phosphonate
Dithane M-45	Mancozeb	80% Mancozeb	Dithiocarbamate
Copper oxychloride	Copper oxychloride	50% Copper oxychloride	Copper compound
Curzate	Cymoxanil+Mancozeb	8% Cymoxanil+ 64% Mancozeb	Acetamida-Dithiocarbamate
Nativo	Trifloxystrobin+Tobuconazole	25% Trifloxystrobin + 50% Tobuconazole	Trifloxystrobin-Tobuconazole
Hombre-excel	Hombre-excel	TBZ-Tebuconazole+ IMD-Imdacloprid	Tebuconazole+Imdacloprid
Dividend star	Dividend star	Difenonazole.30g/l+ Ciproconazole 6.25g/l	Triazole
Metalaxyl+Mancozeb	Metalaxyl + Mancozeb	8% Metalaxyl +64% Mancozeb	Phenylamide
Score	Difenoconazole	Difenoconazole 250 Ec	Difenoconazole
Antracol	Propineb	70 % Propineb	Dithiocarbomate
Acrobat-Mz	Dimethomorph +mancozeb	9% Dimethomorph + 60% mancozeb	Dimethomorph -mancozeb
Baytanfoliar	Triadimimenal	23% Triadimimenal	Triadimimenal

Table 2. Effect of various fungicides at different concentrations on inhibition of *F. oxysporum* f. sp. *ciceris.*

Fungicides name	Inhibition % of radial growth of *Foc*				
	1ppm	10ppm	100 ppm	1000ppm	10000ppm
Carbendazim	96.5ab	100 a	100 a	100 a	100 a
Curzate	6.5yz	11.62xy	16wxy	49.25opq	70.25jkl
Thiophanate-methyl	66.87klm	93.25	100 a	100 a	100 a
Aliette	26 uvw	67.5 klm	100 a	100 a	100 a
Nativo	69 jkl	84.37 cdefg	91.62 abcd	100 a	100 a
Hombre-excel	54.62 nop	82.87 defgh	96.62 ab	100 a	100 a
Dividend star	38.37 rst	64.62 lmn	84 cdefg	100 a	100 a
Dithane-M	0 z	0 z	14.85 xy	45.62 pqr	63.37 mn
Metalaxyl + Mancozeb	0 z	0 z	16.5 vwxy	49.48 opq	63.12 lmn
Score	56.5 no	73hijkl	77.87fghij	87.25bcdef	100 a
Antracol	17.5vwx	26.12uv	31tu	57.87mno	80.87efghi
Acrobat	0 z	13.12xy	27.87 u	41.75qrs	75.87ghijk
Baytan foliar	40.87qrst	44.37qr	72.75ijkl	83.25cdefg	100 a
Copper oxychloride	6.75yz	13.87xy	19.62stu	91.5abcde	100 a

*Means followed by different letters are significantly different at P=0.05 according to DMRT

Results

***In vitro* efficacy of fungicides:** Based on *In vitro* efficacy against *Foc*, the tested 14 fungicides divided into three groups. The first group consists of Carbendazim and Thiophanate-methyl, which appeared highly effective even at very low concentrations. In case of Carbendazim, the pathogen failed to grow at all used concentrations, except only 1ppm, where it produced negligible growth. Similarly, Thiophanate-methyl completely suppressed the colony growth of *Foc* at 100-10000 ppm, while it produces 67 and 93% inhibition at 1 and10 ppm, respectively. The 2nd group comprised of moderately effective fungicides including Aliette, Nativo, Hombre-excel and Dividend star which were only effective at their very high concentrations but became gradually ineffective with medium and lower concentrations. Fungicides including Curzate, Metalaxyl + Mancozeb, Score, Antracol, Acrobat, Baytan foliar and Copper oxychloride comprised of the third category, which were either completely or to greater extent ineffective against *Foc*. Among these Baytan foliar and Copper oxychloride completely inhibited the colony growth of *Foc* at only 10000 ppm. The lower doses of the third group of fungicides were appeared completely or partially ineffective to check the colony growth of *Foc* (Table 2).

Greenhouse experiment: Fungicides showing the good inhibition in *In vitro* assay were also evaluated against artificially infested chickpea plants with *Foc*. High doses of Carbendazim (100 and 1000 ppm), Hombre-excel (1000 ppm) and Dividend star (1000 ppm) were highly phytotoxic as no plant growth was observed in them. It was noted that in all treatments the root infection could not occurred or significantly reduced as compared to the control (untreated) plants. Root infection was not recovered in plants treated with 10 ppm of Carbendazim, 100 ppm of Thiophanate-methyl, Aliette, Nativo, Hombre-excel, Dividend star, Score and 1000 ppm of Thiophanate-methyl, Aliette, Nativo and Score. The lower doses, i.e., 10 ppm of Score, Nativo, Aliette, Dividend star, Hombre-excel and Thiophanate-methyl were less effective in controlling the pathogen infection in treated plants, which produced 20-70% root infection (Fig. 1a).

Generally, most of the fungicide treatments appeared highly effective in reducing the plant mortality of chickpea plants as compared to the untreated plants (Fig. 1b). The most effective treatments, which created either no mortality or very less mortality ranging from 0-3.33% includes Carbendazim 10 ppm, Thiophanate-methyl 10-1000 ppm, Aliette 100-1000 ppm, Nativo100-1000 ppm, Hombre-excel 100 ppm, Dividend star 100 ppm and Score 100-1000 ppm. The highest plant mortality was observed in untreated plants (93.33%) followed by plants treated with Score 10 ppm (73.33%), Dividend star 10 ppm (76.66%) and Hombre-excel 10 ppm (60%), Aliette 10 ppm and Nativo 10 ppm appeared as moderately effective as they caused only 43.33% and 50% plant mortality, respectively (Fig. 1b).

The dose that not caused the phytotoxicity, stimulated the plant growth as compared to the control plants. Generally, higher concentrations of fungicides (if not phytotoxic) were more effective in promoting the plant growth as compared to lower and medium concentrations. Highest shoot length was recorded in plants treated with 10 ppm of Carbendazim, 100 and 1000ppm of Thiophanate-methyl, Aliette, Nativo, Score, 100 ppm of Hombre-excel and Dividend star. Shoot length was significantly lower in untreated plants (6.72cm), followed by plant treated with 10 ppm of Aliette (9.76cm), Score (10.71cm) and Thiophanate-methyl (11.3cm) (Fig. 1e). More or less similar trends were also observed in terms of root length. Significantly maximum root length ranging from 18.66-18.47cm was observed in plants treated with 10 ppm of Carbendazim, 100-1000 ppm of Thiophanate-methyl, Aliette, Nativo, Score, 100 ppm of Hombre-excel and Dividend star. The root length was remarkable reduced in untreated plants (7.47cm). All other remaining treatments were moderately effective in increasing the root length of chickpea plants (Fig. 1c).

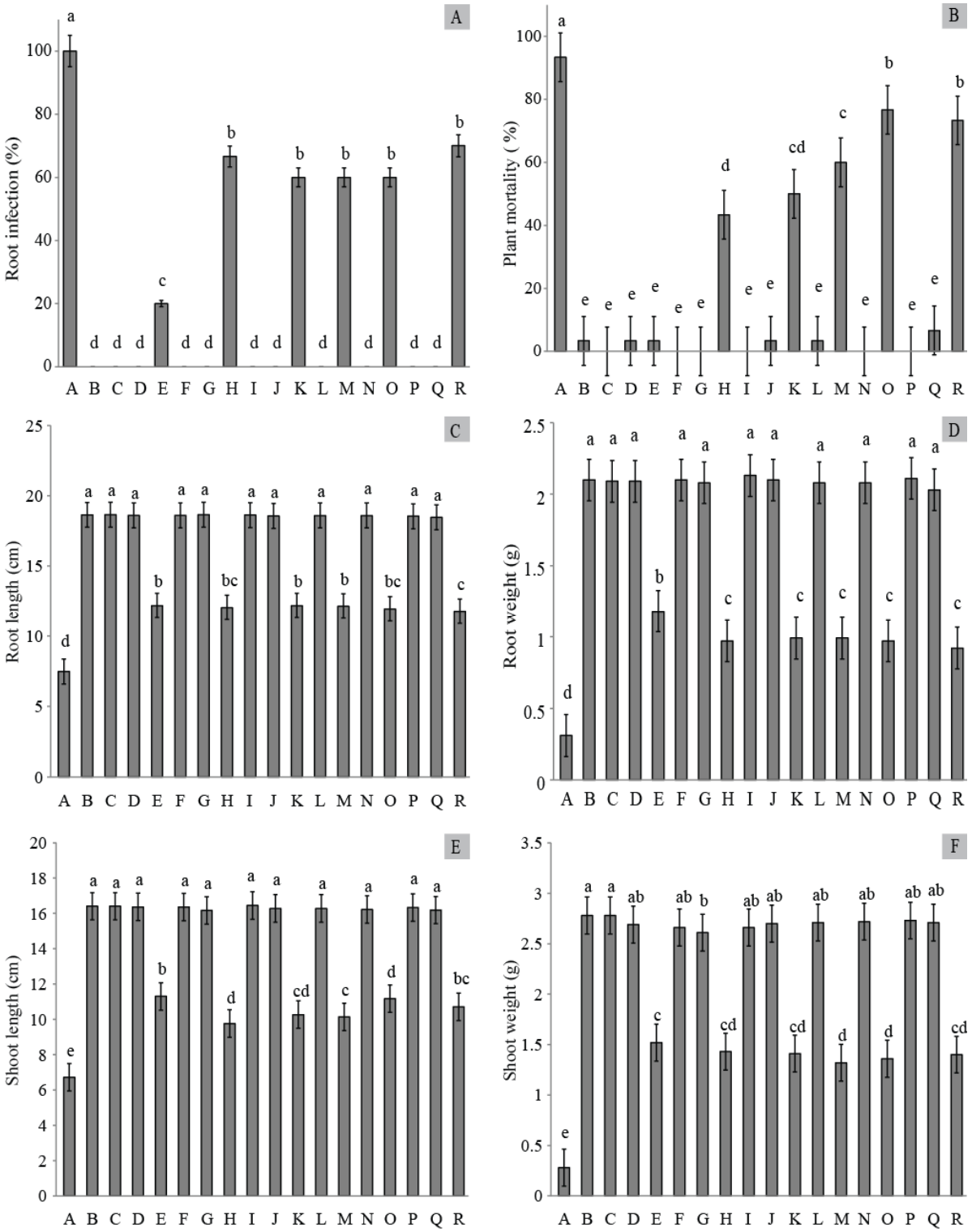

Fig. 1. Effect of different fungicides (applied as drenching) and their different doses on (a) root infection, (b) plant mortality, (c) root length, (d) root weight, (e) shoot length and (f) shoot weight of chickpea plants inoculated with *F. oxysporum* f. sp. *ciceris* in pot experiment. Means followed by different letters in respective bar are significantly different at P=0.05.

where, A: Control, B: Carbendazim 10ppm, C: Thiophanate-methyl 1000ppm, D: Thiophanate-methyl 100ppm, E: Thiophanate-methyl 10ppm, F: Aliette 1000ppm, G: Aliette 100ppm, H: Aliette 10ppm, I: Nativo 1000ppm, J: Nativo 100ppm, K: Nativo 10ppm, L: Hombre-excel 100ppm, M: Hombre-excel 10ppm,N: Divident-star 100ppm, O: Divident-star 10ppm, P: Score 1000ppm, Q: Score 100ppm and R: Score 10ppm. (Initially 3 doses 10, 100 and 1000ppm of each fungicide were applied but in data presentation bars of those doses are excluded in which plants failed to grow because of phytotoxicity caused by higher concentrations).

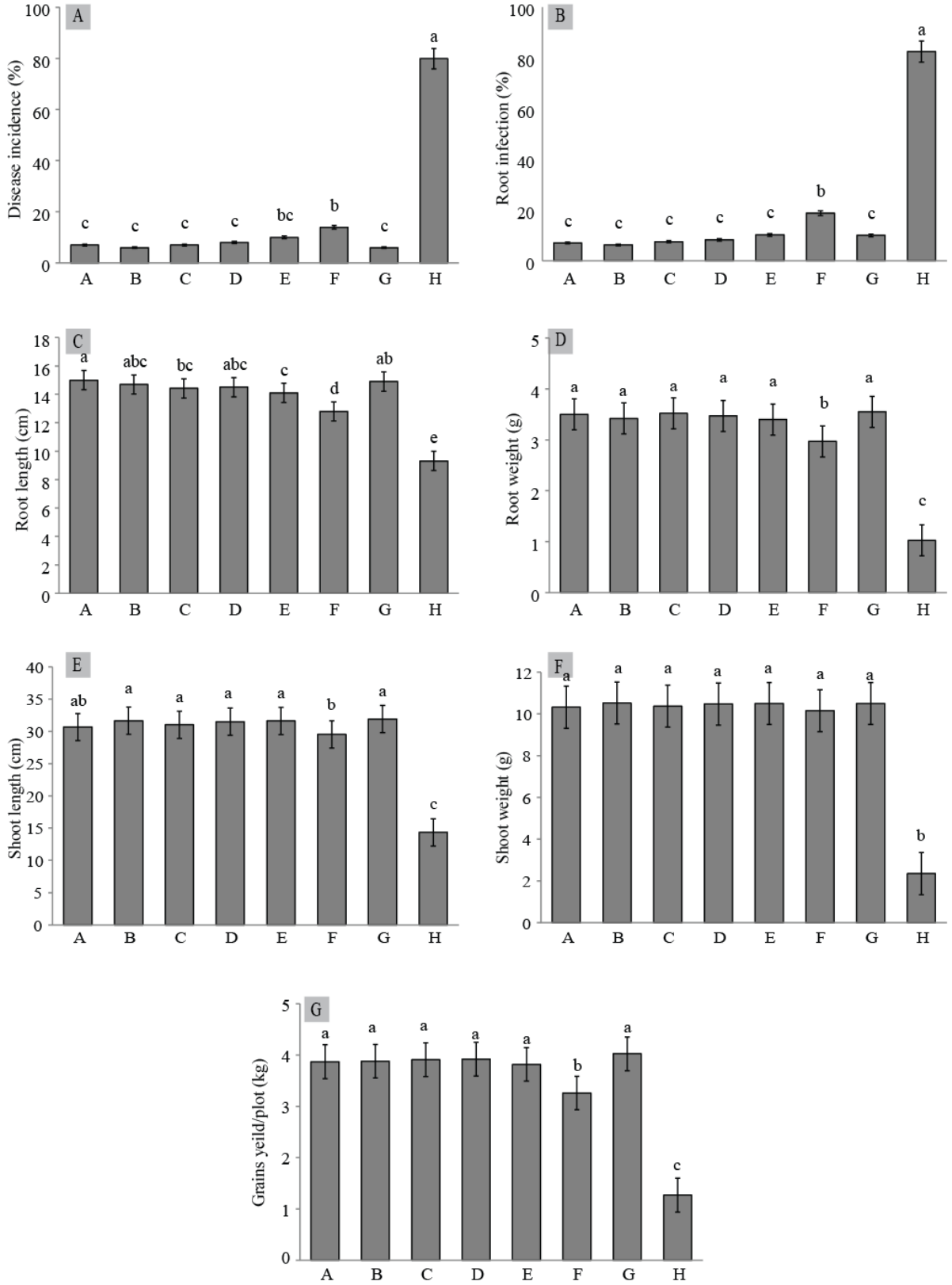

Fig. 2. Effect of different fungicides (applied as seed treatment @ 2g or 2ml/kg of seed.) under field conditions on (a) disease incidence, (b) root infection, (c) root length, (d) root weight, (e) shoot length, (f) shoot weight and (g) grain yield of okra. Means followed by different letters in respective bar are significantly different at P= 0.05.
Where, A: Carbendazim, B: Thiophanate-methyl, C: Aliette, D: Dividend-star, E: Hombre-excel, F: Score, G: Nativo and H: Control.

Consequently, maximum shoot weight was recorded in plants treated with a Carbendazim 10 ppm and Thiophanate-methyl 1000 ppm (2.78g) followed by plants treated with 1000 ppm of Aliette, Nativo, 100 ppm of Score, Thiophanate-methyl, Nativo, Hombre-excel and Score (2.66-2.73g) (Fig. 1f). Among various treatments minimum shoot weight was recorded in plants treated with 10 ppm of Hombre-excel (1.32g), Dividend star (1.36g), followed by Nativo (1.41g), Aliette (1.43g) and Score (1.4g) (Fig. 1f). Consequently, significantly maximum root weight was recorded in plants treated with Carbendazim 10 ppm (2.1g), Thiophanate-methyl 100-1000 ppm (2.0g), Aliette 1000 ppm (2.1g), Aliette 100 ppm (2.08g), Nativo 1000 ppm (2.13g), Nativo 100 ppm (2.1g), Hombre-excel 100 ppm (2.08g), Dividend star (2.08g), Score 1000 ppm (2.11g) and Dividend star 100 ppm (2.03g). The significant reduction in root weight was recorded in control plants (0.31g) followed by plants treated with 10 ppm of Score, Dividend star, Hombre-excel, Nativo and Aliette (0.92-0.97g) (Fig. 1d). Variability in plant weight was found among different fungicides and their doses. No statistically significant difference was observed between shoot weight when treated with 10 ppm of Carbendazim and 100 and 1000 ppm of Thiophanate-methyl, Aliette, Nativo, Score, 100 ppm of Hombre-excel and Dividend star. Lowest shoot weight was recorded in untreated plants. Consequently, significantly maximum root weight was recorded in plants treated with 10 ppm of Carbendazim and 100 and 1000 ppm of Thiophanate-methyl, Nativo, Score, 100 ppm of Hombre-excel and Dividend star (Fig. 1d).

Field experiment: All the used fungicides greatly reduced the disease development in chickpea plants under field conditions. The minimum disease incidences of 6-14% were observed in treated plants as compared to un-treated (control) plants with 80% disease incidence. Between various treatments, Nativo, Thiophanate-methyl, Carbendazim, Aliette, and Dividend star were appeared as highly effective (6-8% disease incidence). Maximum disease incidence after control were recorded in plants treated with Score (14%) followed by Hombre-excel (10%) (Fig. 2a). All fungicides remarkably checked the pathogen activity in treated plants as the significantly lowest pathogen infection was observed in fungicides treated plants (6-18%) as compared to the untreated plants (82%). The minimum pathogen infection recorded in plants treated with Thiophanate-methyl (6%), Carbendazim (7%), Aliette (7%), Dividend star (8%), Nativo (10%) and Hombre-excel (10%). Whereas, higher root infection after untreated plants were found in plants treated with Score (18%) (Fig. 2b).

The application of fungicides remarkably enhanced the growth of treated chickpea plants as compared to the untreated plants. In terms of root length it was highest in plants treated with Carbendazim (15cm) and Nativo (14cm) followed by plants treated with Thiophanate-methyl, Aliette, Dividend star, Hombre-excel and Score (14cm). The significantly lower root length was recorded in control plants (9cm) followed by plants treated with Score (12cm) (Fig. 2c). Similarly, minimum root weight ranging from 3.4-3.55g was recorded in plants treated with Hombre-excel, Thiophanate-methyl, Dividend star, Carbendazim and Nativo. The control plants produced significantly lowest root weight (1.025g) followed by plants treated with Score (2.97g) (Fig. 2d).

In terms of shoot length, the treated plants revealed significantly greater shoot length (29.52-31.92cm) as compared to the untreated plants (14.35cm) (Fig. 2e). Consequently, significantly maximum shoot weight was observed in fungicide treated plants as compared to the untreated plants. In treated plants shoot weight was ranging from 10.15-10.52cm, whereas in untreated plants shoot weight was only 2.35g (Fig. 2f).

The grain yield also increased by the application of fungicides as compared to that of untreated plants. The highest grain yield per plot recorded in plants treated with Nativo (4.025kg), Dividend star (3.92kg), Aliette (3.91kg), Thiophanate-methyl (3.88kg), Carbendazim (3.87kg) and Hombre-excel (3.82kg). The remarkable lower grain yield per plot was observed in untreated plants as they produced only (1.27kg). The Score appeared as a moderately effective fungicide gave 3.26kg grain yield (Fig. 2g).

Discussions

Chemical control based on the use of fungicides inspite of its all health hazards has proved to be the effective control strategy. Fourteen chemical fungicides with 1-10,000 ppm were screened against *Foc* under *In vitro* conditions. Out of fourteen fungicides only Carbendazim and Thiophanate-methyl were found as the most effective against the *Foc* at all concentrations. Other fungicides like Aliette, Nativo, Hombre-excel and Dividend star were moderately effective against *Foc*. Whereas, remaining tested fungicides were generally ineffective against the targeted pathogen. Generally, a positive co-relation observed between concentrations of the tested fungicides and inhibition of *Foc*. Higher doses of fungicides found to be more effective than their lower doses. There were several reports from elsewhere regarding *In vitro* evaluations of chemical fungicides against *F. oxysporum*. Our results are in conformity with those reported by Poddar *et al*., (2004), Song *et al*., (2004), Rajput *et al*., (2006), Khan *et al*., (2012), Mukhtar (2007), Sultana & Ghaffar (2010) and Ilyas *et al*., (1992).

The fungicides which efficiently inhibit the test fungus in *In vitro* study are supposed to be effective against the same pathogen in natural conditions. Based on the results of *In vitro* experiment, seven fungicides (Carbendazim, Thiophanate-methyl, Aliette, Nativo, Hombre-excel, Dividend star and Score) with three concentrations (10, 100 and 1000 ppm) were tested in greenhouse. Generally, all treatments check the activities of the inoculated fungus (*Foc*) and promote the

growth of chickpea plants. Although the higher concentrations of the few fungicides completely inhibited the pathogen as well as found to be phytotoxic and checked the plant growth. On over all bases, Carbendazim and Thiophanate-methyl followed by Aliette and Nativo were more effective in reducing the impact of pathogen as well as enhancing the plant growth. Except a few cases it was also noticed that higher concentration of chemical fungicides were more effective than lower ones. In addition, under field conditions seed dressing of all fungicides remarkably checked the disease development and subsequently increased the plant growth as well as grain yield as compared to untreated plants. Fungicides were found to be absorbed and translocated in seedlings persisting up to 12 days and protect the seedling in a field for 30 days or more evens through roots (Verma, 1976). The chickpea plants treated with fungicides gave 6-14% disease incidence of Fusarium wilt as compared to untreated plants, which have 80% incidence. Similarly, the application of fungicides (except Score) brought tremendous increment in plant growth as well as grain yield. The study revealed that the fungicide applications in chickpea plants were very effective, they have not suppressed the pathogen activities, but also increased the plant growth and grain yield (almost double than the control plants). Carbendazim seed treatment gave minimum disease incidence of Fusarium wilt and maximum grain yield (Kamdi *et al*., 2012). Similarly, Jimenez-Diaz & Trapero-Casas (1985), Subhani *et al*., (2011), Dwivedi & Updhyay (1988) reported that fungicide applications significantly increased the plant growth and yield, and also decreased Fusarium wilt incidence in chickpea plants.

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