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Sensitivity of Fusarium spp. infecting tomato in Khyber Pakhtunkhwa to thiophanate methyl

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Abstract

Many constraints affect productivity and quality of tomato, the most important being an array of fungal and bacterial diseases. Fusarium wilt is present in all growing areas of KP, infecting tomato at different growth stages from vegetative growth up to fruit harvesting. This work aimed at determining the sensitivity of different pathogenic populations of *F. equiseti*, *F. graminearum*, *F. solani* and unknown *Fusarium* spp. isolates to thiophanate methyl an active ingredient in many broad spectrum fungicides used for Fusarium wilt of tomato. Fungal isolates were obtained from the diseased tomato collected in commercial fields at the Khyber Pakhtunkhwa, Pakistan. Twenty nine different isolates obtained from different areas were grown on PDA medium amended with increasing dosages and the effective concentration reducing the mycelial growth of isolates were determined. From the results it was observed that isolates of *F. equiseti* was most resistant to the thiophanate methyl even at highest concentration with average percent inhibition 68. 49% followed by average percent inhibition 72.48% for *F. solani*. However, most sensitive strain was *F. graminearum* with average percent inhibition 45.42% even at lowest concentration. We reported that thiophanate methyl is an effective fungicide against *F. equiseti*, *F. solani*, *F. graminearum* and unknown spp. of *Fusarium*, associated with tomato wilt in Khyber Pakhtunkhwa Pakistan, however due to resistance in some isolates of *F. equiseti* the fungicide should be used in combination with other broad spectrum fungicide for management of the disease, to avoid resistance build up in pathogen population against the fungicide.

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Introduction

Fusarium wilt of tomato (Massee, 1895) has been documented from all tomato growing areas around the world (Yeole *et al*., 2016). The disease is reported from more than 30 countries including; Austria, Argentina, New Zealand, Egypt, Turkey, UK, USA, Morocco, Surinam, Italy, India, France, Mexico, South Africa, Jamaica, Israel, Iraq, Netherland, Russia, Bermuda, Mauritius, Fiji, Bulgaria and probably present in many unreported sites where tomatoes are grown (Jones, 1981).

The causal organism of tomato wilt i.e. *Fusarium* spp. is a common soil-borne filamentous fungus belongs to Hyphomycetes, and is ranked among the top ten important plant pathogens in the world (Correll, 1991, Ramdial *et al*., 2016). The genus includes 300 phylogenetic species, with new species frequently being evolved (O'Donnell and Geiser, 2014, Benyephet, 1994, O'Donnell *et al*., 2004).

The pathogen resides in soil from season to season where it can thrive for extended period if the host is not available. Between crops, it endures in infected crop left over in the soil as mycelium or spores (Agrios, 2005). The pathogen is favored by acidic sandy soil while its growth is inhibited by alkaline soil (Gordon *et al*., 2018). The optimum temperature of 27 – 32 ^{0}C favors disease development, while the pathogen is disseminated through transplantation of infected seedlings, contaminated farm equipment and tomato stocks, infested soil as well as irrigation water (Ploetz, 2015). The fungus can easily be grown on artificial media such as PDA (Potato Dextrose Agar), PDB (Potato Dextrose Broth), and V8 (Vegetable 8 Juice), covering 9-10 cm diameter within a week and produces aerial mycelium with different pigmentations (Meletiadis*et al*., 2001). Some of the *Fusarium* spp. also produce mycotoxins such as fumonisin, trichothecene (Alexander *et al.,* 2009), zearalenone and deoxynivalenol (Häggblom, 2015) which contaminate agricultural products thus making them unsuitable for human as well as animal consumption (Woloshuk , 2013).

Many approaches have been adopted to control the pathogen. These include, molecular, biological cultural and chemical (Akbar *et al*., 2018). However, each has its limitation cost effectiveness, availability, and reliability. Besides many concerns about the use of chemical it is still a reliable and easily available method for management of many diseases. Researchers has worked and found that the use of different fungicides efficiently reduced Fusarium wilt from 90.96 to 9.30 % in plants examined after 15 days of inoculation (Biswas *et al*., 2012), and it is reported that tomato wilt can be controlled by less toxic and systemic fungicides and that pasteurization of infested soil with steam or fumigant helps in reducing the incidence of wilted plants (Ignjatov *et al*., 2015, Nel *et al*., 2007, Poddar *et al*., 2004).

Besides providing quick action aginst diseases, there have been concerns about the excessive use (Soria*et al*., 2012) and development of resistance in the pathogen due to continuous use of these fungicides (James *et al*., 2006). When fungicides of the same mode of action are applied repeatedly to fields, the fungal population can develop resistance or insensitivity to that particular fungicide mode of action. Application of fungicides with a single site mode of action can be overcome by fungal pathogens over time. They need to be updated at a regular interval for efficacy because they fail to manage the disease over time. Fungal pathogens evolve with time and overcome the effect caused by the fungicides and are rendered insensitive/tolerant or resistant to the fungicide.

Several lab based experiments are performed to determine whether a fungal pathogen is sensitive or has developed resistance against a fungicide. These lab based experiments are helpful in making successful recommendations for using a fungicide (Secor, 2012). Fungicide resistance assays are useful to determine if a fungal pathogen has developed resistance to a fungicide used to manage the disease it causes. Laboratory assays are used to determine loss

of sensitivity, or resistance, to a fungicide and can explain fungicide failures and for developing successful fungicide recommendations in the field.

The objective of this study was to assess fungicide resistance/sensitivity in *Fusarium* spp. populations associated with tomato wilt in Khyber Pakhtunkhwa Pakistan.

Material and methods

Isolation of the pathogen and culture maintenance

Disease plants samples showing typical symptoms of Fusarium wilt were collected from different tomato growing areas of KP and were brought to the laboratory of the Department of Plant Pathology, The University of Agriculture Peshawar. Samples were stored at 4°C in refrigerator for further processing.

From the disease samples leaves and secondary roots were cut off leaving only stem, hypocotyls, and main root. The samples were then cut into small pieces ($1cm^2$) and were surface sterilized by soaking in 10 % bleach (NaOCl) solution for 1 minute. The cut stem samples were washed with sterile distilled water thrice and blotted dry on paper towel. Potato dextrose medium (PDA) was prepared and was amended with 30mg/L streptomycin to inhibit bacterial growth. Surface sterilized samples were then placed on PDA medium in a 9cm petri plates. The plates were incubated at 28 °C for seven days for mycelial growth of the pathogen. The resultant fungal colonies were isolated and purified using single spore isolation method as described as by (Choi and Ho, 1999). A pure culture of each isolate was maintained at 4°C on PDA slants as a stock culture.

Morphological identification

For morphological identification and characterization, the culture of the isolates (n=30) including one reference strain of *Fusarium oxysporum* fsp. *lycopersici* (Fol) from Mid Florida Research and Education Centre (MREC), USA was grown using single- spore isolation method on PDA medium (Choi *et al*., 1999). Permanent slides for each isolate were prepared and examined for mycelial characteristics, spore shape, size and type using a confocal microscope (Leslie, 2008; Nelson, 1968).

Fungicide resistance assay of the isolates

A completely randomized experiment (CRD) with four replications was designed to determine the fungicide resistance of 29 *Fusarium* spp. isolates *in vitro*. A systemic fungicide 33365 with active ingredient thiophanate methyl was selected for resistance assay. Three different doses of fungicides were formulated that is 10mg/100ml (10%), 50mg/100ml (50%), and 100mg/100ml (100%) along with control (0%). After autoclaving and cooling to 48°C, PDA medium was amended with above mentioned concentration of thiophanate methyl and was poured in 90mm petri dishes in laminar flow unit (LFU) under aseptic condition. From the pure culture of the isolates grown on PDA at 28°C for 7 days, a 5cm culture plug was excised from the edge of the colony and placed upside down at the center of plates amended with different concentrations of thiophanate methyl, with concentrations of 0% fungicides as a control. The plates were incubated at 30°C for 7 days under 12 hour's light and dark condition. Later colony diameters on fungicide amended and non-amended control plates were measured and used to determine resistance to the thiophanate methyl (Xu, 2016). Four replicate plates were used for each concentration and the experiment was repeated three times. For each isolate, the average radial colony growth was used to calculate the percent inhibition of mycelial growth. The percentage of fungal growth inhibition was calculated according to the formula (Pandey *et al.,* 1982).

Growth inhibition% = [(growth in the control–growth in the sample)/growth in the control] × 100.

Statistical analysis

Data was statistically analyzed using STATISTIX software (8.1) with completely randomized design (CRD). Inhibition of radial mycelial growth was examined using analysis of variance (ANOVA). Differences between the means values were tested by using Fischer's least significant difference (LSD) at

0.05 probability level. Each experiment was performed in triplicate.

Results and discussion

Morphological and Cultural characteristics of Fusarium species identified

Twenty nine different isolates of *Fusarium* spp. were recovered from different tomato growing areas of Khyber-Pakhtunkhwa and identified into four species. Based on the morphology of their colonies using the *Fusarium* synoptic keys for species identification (Leslie and Summerell, 2006, Nelson *et al*., 1983).

The *Fusarium* species identified were *F. equiseti* (19 isolates), *F. solani* (2 isolates), *F. graminearum* (4 isolates) and unknown *Fusarium*spp (4 isolates). Macroscopic and microscopic characteristics of all the isolates are summarized in Table 1.

Table 1. Cultural characteristics of *Fusarium* spp. grown at 28°C for 7 days.

Isolate ID	*Fusarium* spp. identified	Colony color on upper and lower surface of the plate	Macroconidia septation
Pak-1	*F. equiseti*	Whitish Brown/Brown	3-5
Pak-2	*F. equiseti*	Whitish /Brown	3-5
Pak-3	*F. equiseti*	Whitish /Brown	3-5
Pak-4	*F. equiseti*	Whitish/ Brown	3-5
Pak-5	*F. equiseti*	Whitish/reddish Brown	3-5
Pak-6	*F. equiseti*	White/orange brown	3-5
Pak-7	*Unidentified*	Yellowish white/Yellow	3-6
Pak-8	*Unidentified*	Yellowish white/Yellow	3-6
Pak-9	*F. equiseti*	Whitish /Brown	3-5
Pak-10	*F. graminearum*	White/Pink-orange	4-6
Pak-11	*F. equiseti*	Whitish /brown	3-5
Pak-12	*F. equiseti*	White/Brown	3-5
Pak13	*Unidentified*	Olive green/ Brown	4
Pak-14	*F. graminearum*	White/Brown	4-6
Pak-15	*F. equiseti*	White/Whitish Brown	3-5
Pak-16	*F. equiseti*	Whitish /Brown	3-5
Pak-17	*F. equiseti*	Whitish /Brown	3-5
Pak-18	*F. equiseti*	Whitish Brown/Brown	3-5
Pak-19	*F. graminearum*	Whitish /Reddish brown	4-6
Pak-20	*F. equiseti*	Whitish Brown/Creamy	3-5
Pak-21	*F. solani*	brown/creamy	3-7
Pak-22	*F. equiseti*	White/Brown	3-5
Pak-23	*F. equiseti*	Brown/Reddish Brown	3-5
Pak-24	*F. equiseti*	Whitish Brown/Brown	3-5
Pak-25	*F. equiseti*	Whitish /Brown	3-5
Pak-26	*Unidentified*	White/Dark Pink	3-6
Pak-27	*F. graminearum*	Whitish /Reddish brown	4-6
Pak-28	*F. equiseti*	White/Dark Brown	3-5
Pak-29	*F. solani*	Brown / Creamy	3-7
FL-15	*F. oxysporum*	White/Brown	3-5

F. equiseti grown on PDA showed more robust conidia and red pigment in freshly isolated cultures. The color of colonies of 7 days old cultures on PDA varied from whitish, beige to creamy, tinged with yellow or peach, while the reverse was whitish to pale yellow with 3-5 septate macroconidia. Microconidia were absent and chlamydospores were produced in hyphae, frequently forming chains or clusters (Fig. 1).

Table 2. Effective concentration of thiophanate methyl that reduces the mycelial growth of *Fusarium equiseti* isolate associated with tomato wilt disease in KPK Pakistan.

Isolate ID	0 %	10% (cm)	% Inhibition	50% (cm)	% Inhibition	100% (cm)	% Inhibition
Pak-1	86.74±3.73^{a}	48.89±2.00^{b}	43.64	26.85±0.84^{c}	69.04	5.95±0.01^{d}	93.12
Pak-2	73.21±3.96^{a}	57.45±1.89^{b}	12.57	46.28±1.44^{c}	29.57	12.20±0.58^{d}	81.44
Pak-3	40.22±3.16^{a}	36.49±0.41^{ab}	3.24	32.60±1.01^{b}	13.58	8.83±1.51^{c}	76.58
Pak-4	86.70±1.75^{a}	67.34±2.63^{b}	22.33	55.39±1.72^{c}	36.11	16.31±1.55^{d}	81.18
Pak-5	72.04±3.90^{a}	54.12±193^{b}	9.09	44.03±1.37^{c}	26.05	21.26±1.33^{d}	64.29
Pak-6	85.32±0.68^{a}	36.67±2.55^{b}	57.02	18.36±0.57^{c}	78.48	14.85±1.91^{c}	83.18
Pak-9	56.74±3.00^{a}	55.50±2.23^{a}	2.19	44.97±1.54^{b}	20.74	28.73±1.81^{c}	49.37
Pak-11	84.34±2.37^{a}	80.63±2.79^{a}	1.47	70.81±2.24^{b}	13.48	55.57±3.63^{c}	32.10
Pak-12	86.26±2.23^{a}	51.10±1.19^{b}	40.76	42.18±1.36^{c}	51.10	30.38±3.12^{d}	64.78
Pak-15	85.85±2.85^{a}	85.39±1.08^{a}	0.54	74.16±2.38^{b}	13.63	22.94±1.35^{c}	73.29
Pak-16	86.81±1.81^{a}	70.57±1.68^{b}	18.71	53.89±1.67^{c}	37.92	20.66±1.17^{d}	76.21
Pak-17	85.17±1.81^{a}	71.24±1.43^{b}	16.35	68.22±2.14^{b}	19.91	20.96±1.32^{c}	75.38
Pak-18	82.78±2.43^{a}	56.90±1.58^{b}	29.12	52.85±1.69^{b}	34.16	20.84±1.12^{c}	74.04
Pak-20	85.63±2.09^{a}	53.37±2.14^{b}	37.68	42.81±1.35^{c}	50.00	27.26±0.40^{d}	68.16
Pak-22	85.83±2.11^{a}	83.96±1.90^{a}	2.18	71.48±2.35^{b}	16.72	19.92±3.35^{c}	76.79
Pak-23	51.71±5.13^{a}	31.79±2.40^{b}	11.00	20.05±0.63^{c}	43.87	16.19±1.02^{c}	54.67
Pak-24	50.94±2.04^{a}	33.78±1.47^{b}	26.46	25.16±0.81^{c}	45.24	17.09±0.72^{d}	62.78
Pak-25	68.59±4.37^{a}	43.35±2.24^{b}	36.79	34.14±1.07^{c}	50.23	26.70±0.73^{d}	61.08
Pak-28	29.22±2.53^{a}	28.92±0.85^{a}	1.03	26.53±0.84^{a}	9.19	13.77±1.29^{b}	52.86
Average percent inhibition			19.59%		34.68%		68.49%

Data were statistically analyzed and the small alphabetical letters (a, b, c…) in the same row shows the significant differences (P<0.05) among treatments. Each experiment was performed in triplicate.

Colonies of *F. solani* formed on PDA (Fisher *et al*., 1982) showed typical white to creamy mycelium and production of green pigments on PDA medium. The microconidia are oval, reniform, elongated oval to sometimes obovoid with a truncate base, and septate into 3–7 (Fig. 2). *F. graminearum* produced sub-globose to ovoid, blackish perithecia 150-250µm in diameter in culture. F. graminearum colors change in a very consistent and predictable pattern (Cambaza*et al*., 2018). The macroconidia are hyaline, canoe-shaped spores usually with 4-6 septate (Fig. 3).

Four unknown *Fusarium* spp. were identified producing different colony colors on PDA medium (White/Dark Pink, Yellowish white/Yellow and White/Dark Pink) at 28^{o}C for 11 days under 12 hours light and dark condition (Fig. 4). Based on morphological features of micro and macroconidia, *F. equiseti* was the most prevalent specie of *Fusarium* in the region followed by *F. graminearum,* and *F. solani*. Our results were supported by previous studies, where different *Fusarium* spp. including *F. oxysporum, F. verticillioides* (Rozlianah Sariah, 2006), *F. redolens, F. proliferatum*, *F. equiseti* and *F. solani* have been found associated with tomato wilt (Chehri, 2016, Chehri*et al*., 2011, Edel-Hermann *et al*., 2012, Edel-Hermann *et al*., 2012, Murad *et al*., 2016).

Table 3. Effective concentration of thiophanate methyl that reduces the mycelial growth of *Fusarium graminearum* isolates associated with tomato wilt disease in KPK Pakistan.

Isolates ID	0 %	10% (cm)	% Inhibition	50% (cm)	% Inhibition	100% (cm)	% Inhibition
Pak-10	88.52±0.43a	51.66±2.49b	41.64	38.35±1.26c	56.67	31.33±2.56d	64.61
Pak-14	65.04±3.54a	31.68±2.34b	42.18	19.49±0.60c	64.44	8.75±1.36d	84.04
Pak-19	87.31±2.01a	46.18±2.13b	47.10	24.21±0.75c	72.27	10.97±2.77d	87.44
Pak-27	83.59±0.82a	41.17±2.53b	50.75	29.84±0.98c	64.30	5.77±0.28d	93.10
Average percent inhibition			45.42%		64.42%		82.30%

Data were statistically analyzed and the small alphabetical letters (a, b, c…) in the same row shows the significant differences (P<0.05) among treatments. Each experiment was performed in triplicate.

Table 1. Effective concentration of thiophanate methyl that reduces the mycelial growth of *Fusariumsolani* isolates associated with tomato wilt disease in KP Pakistan.

Isolate ID	0%	10% (cm)	% Inhibition	50% (cm)	% Inhibition	100% (cm)	% Inhibition
Pak-21	47.92±0.99^a	28.46±0.28^b	40.62	17.94±0.62^c	62.57	16.42±2.33^d	65.75
Pak-29	59.55±1.13^a	39.23±1.47^b	34.13	33.46±1.08^c	43.82	12.38±1.30^d	79.22
Average percent inhibition			37.37%		53.20%		72.48%

Data were statistically analyzed and the small alphabetical letters (a, b, c…) in the same row shows the significant differences (P<0.05) among treatments. Each experiment was performed in triplicate.

Sensitivity of mycelial growth of Fusarium equiseti tothiophanate methyl

Thiophanate methyl was found to reduce the colony diameter of all isolates of *F. equiseti* (Fig. 5 and supplementary Fig. 1) after 7 days of incubation at 28^{o}C. However, at low concentration (10%), six isolates (Pak-3, 9, 11, 15, 22 and 28) were found to be resistant with less than 5% inhibition to the thiophanate methyl, whereas three isolates (Pak-1, 6 and 12) were found to be more sensitive with more than 40% inhibition.

Table 2. Effective concentration of thiophanate methyl that reduces the mycelial growth of Unknown *Fusarium* species associated with tomato wilt disease in KPK Pakistan.

Isolate ID	0%	10% (cm)	% Inhibition	50% (cm)	% Inhibition	100% (cm)	% Inhibition
Pak-7	38.01±3.67^a	16.04±0.90^b	57.81	11.29±0.32^b	70. 03	10.79±0.36^b	71.62
Pak-8	37.21±2.03^a	35.80±2.16^a	3.79	26.00±0.82^b	30.12	23.92±1.72^b	35.72
Pak-13	67.81±2.93^a	39.47±2.58^b	41.80	28.76±0.90^c	57.58	6.99±0.23^d	89.69
Pak-26	84.57±1.35^a	25.81±1.99^b	69.49	16.65±0.52^c	80.31	4.70±0.45^d	94.44
Average percent inhibition			45.42%		64.42%		72.87%

Data were statistically analyzed and the small alphabetical letters (a, b, c…) in the same row shows the significant differences (P<0.05) among treatments. Each experiment was performed in triplicate.

Highest percent inhibition of the isolates at low concentration was recorded in isolate Pak-6 (57.02%) followed by isolate Pak-1 with percent inhibition of 43.64%, whereas lowest percent inhibition 0.54% was recorded in isolate Pak-15 (Table 2). At (50%) concentration of thiophanate methyl colony growth of all isolates were significantly reduced, except Pak-28 with percent reduction of 26.53%. Highest percent reduction 78.48% was recorded for Pak-6, whereas lowest percent inhibition 9.19 was recorded for Pak-28. At highest concentration (100%) of thiophanate methyl the colony diameter of all isolates were significantly reduced. Highest percent reduction 93.12% was recorded for Pak-1, followed by Pak-6

with percent reduction of 83.18%, whereas lowest percent reduction 32.10% was recorded for Pak-11 (Table 2). Thiophanate methyl tested in the current study significantly reduced the mycelial growth and conidial germination of *Fusarium* spp. isolates (responsible for Fusarium wilt of tomato). In fact, most of the isolates of *F. solani*, *F. equiseti*, *F. graminearum* are susceptible and are inhibited more than 50% at 50mg/100ml concentration, whereas some isolates of *F. equiseti* react differently. Previously thiophanate methyl has been used to control *Fusarium* diseases on numerous plants, including, tomato, wheat and it showed great capability to prevent *Fusarium* infection.

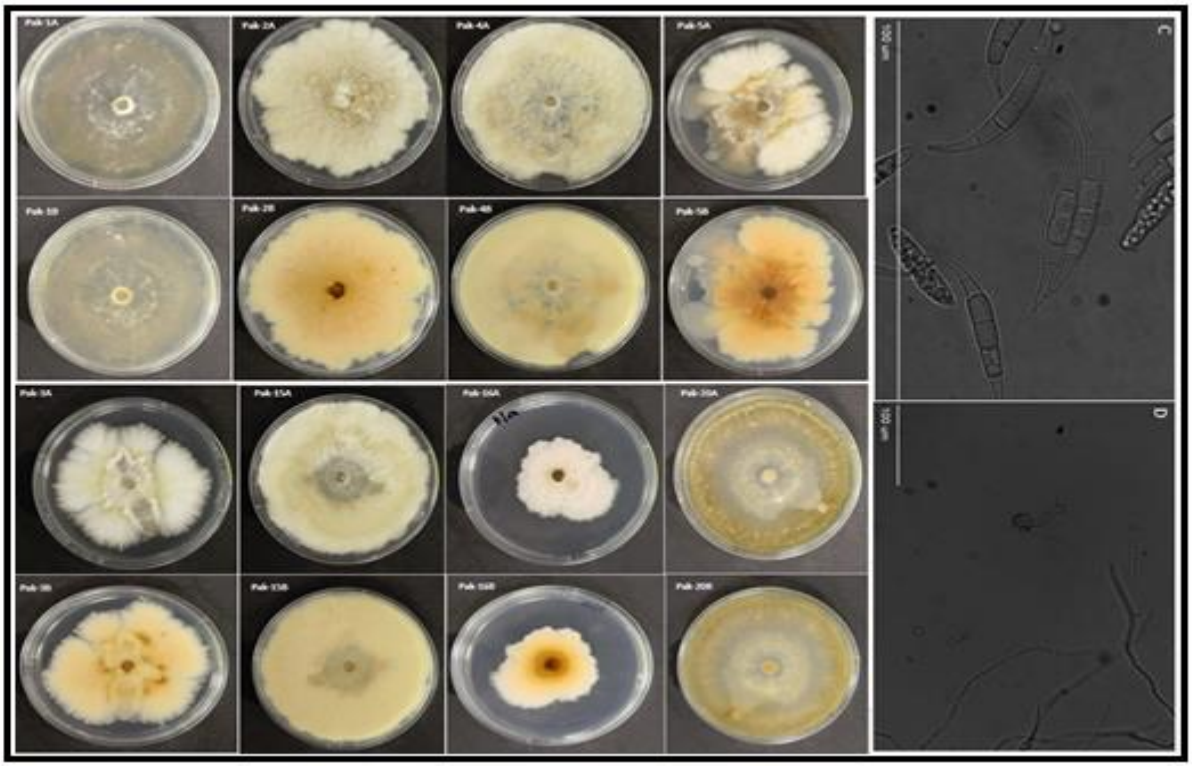

Fig. 1. *Fusarium equiseti* isolates growing on PDA medium, following incubation at 28°C for 7 days under 12 hours light and dark condition. A. Culture of the isolates on the upper side of the plate. B. Culture of the isolates on lower surface of the plate. C. Macroconidia. D. Germinating chlamydospores. (x40 and x60).

In another study thiophanate methyl was used in hydroponic system to control *Fusarium* wilt disease of tomato, the preventive effect was 87% after 5µg/ml thiophanate methyl was added to the liquid media for 2 weeks (Song *et al.*, 2004). However, our results also demonstrated that most of the isolates of *F. equiseti* were resistant to thiophanate methyl (Chen *et al.*, 2007, Brent *et al.*, 2007, O'Neill, 1995). Thiophanate methyl resistance was developed in many fungi after the fungicide had been used for several years (Zhou and Wang, 2001). The first thiophanate methyl resistant isolate of *F. graminearum* was detected from the field in Zhejiang Province of China (Chen and Zhou, 2009).

We showed that all *F. equiseti* isolates tested were slightly inhibited at low concentration and fungal growth inhibition was correlated to the increasing concentration of thiophanate methyl.

The results of this study showed that only four isolates showed more than 50% inhibition and almost all isolates showed more than 80% inhibition at 50 and 100mg/100ml concentration of thiophanate methyl, respectively. Although the resistance and sensitivity of the *F. equiseti* is not well documented but few studies have been shown that no significant difference was achieved to inhibit 50% colony growth at log effective concentration (Ramdial *et al.*, 2017).

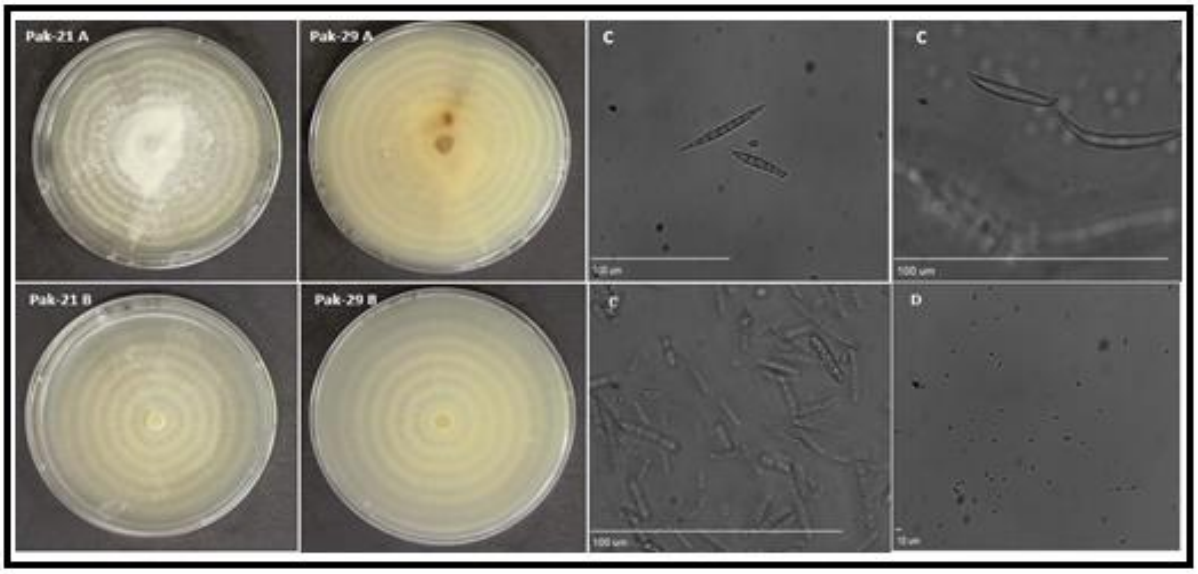

Fig. 2. *Fusarium solani* growing on PDA medium, following incubation at 28 ℃ for 7 days under 12 hours light and dark condition. A. Culture of the isolates on the upper side of the plate. B. Culture of the isolates on lower surface of the plate. C. Macroconidia. D. Microconidia. (x40 and x60).

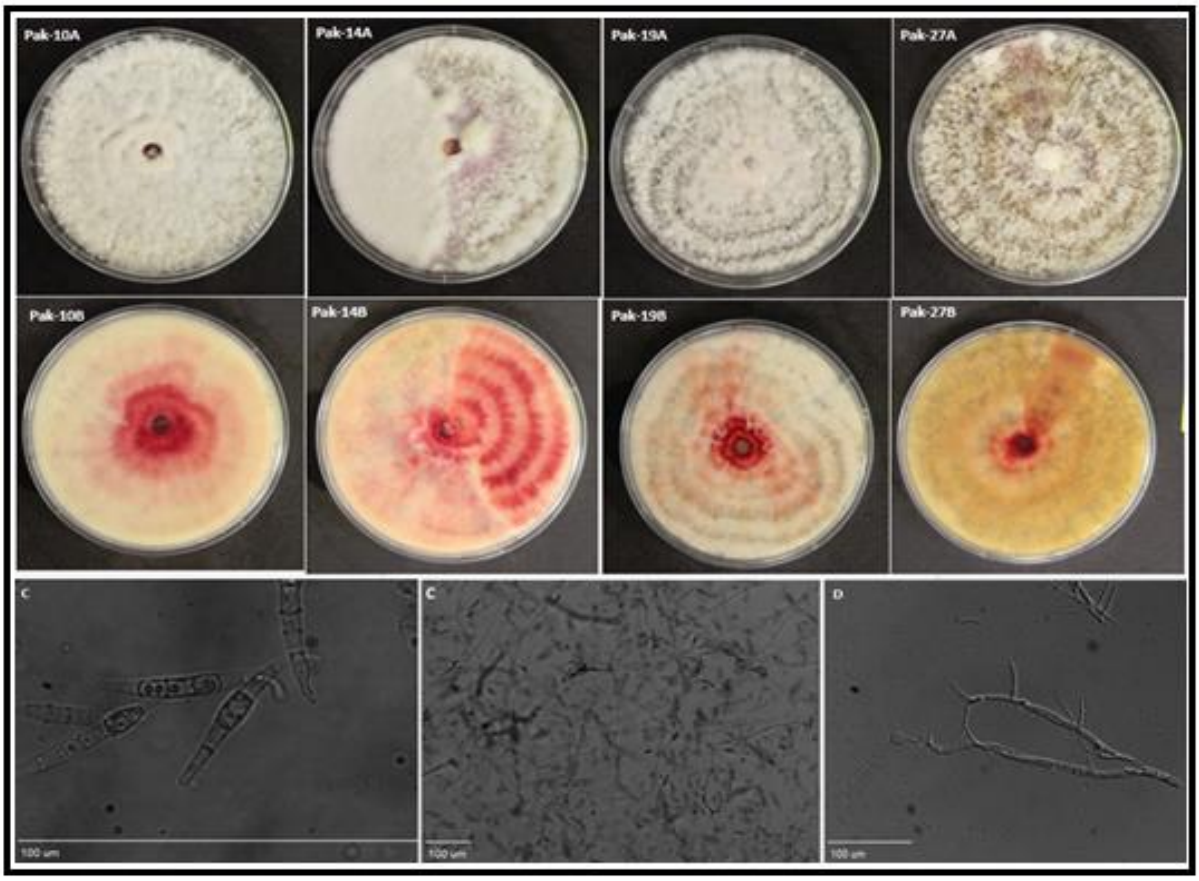

Fig. 3. *Fusarium graminearum* isolates growing on PDA medium, following incubation at 28℃ for 7 days under 12 hours light and dark condition. A. Culture of the isolates on the upper side of the plate. B. Culture of the isolates on lower surface of the plate. C. Macroconidia. D. Chlamydospores. (x40 and x60).

Five different fungicides were used *in vitro* to control *Fusarium* spp., thiophanate methyl at low and high doses completely inhibited the mycelial growth of the *Fusarium* spp. However, under *in vivo* condition 100% reduction in infection was achieved after the second spray of thiophanate methyl as compared to other fungicide (Khaskheli *et al*., 2007).

Effect of thiophanate methyl on isolates of Fusarium graminearum

Radial growth of all isolates significantly reduced after 7 days of incubation when treated with different concentration thiophanate methyl. All isolates were sensitive to the thiophanate methyl even at low concentration (Fig. 6 and supplementary Fig. 2).

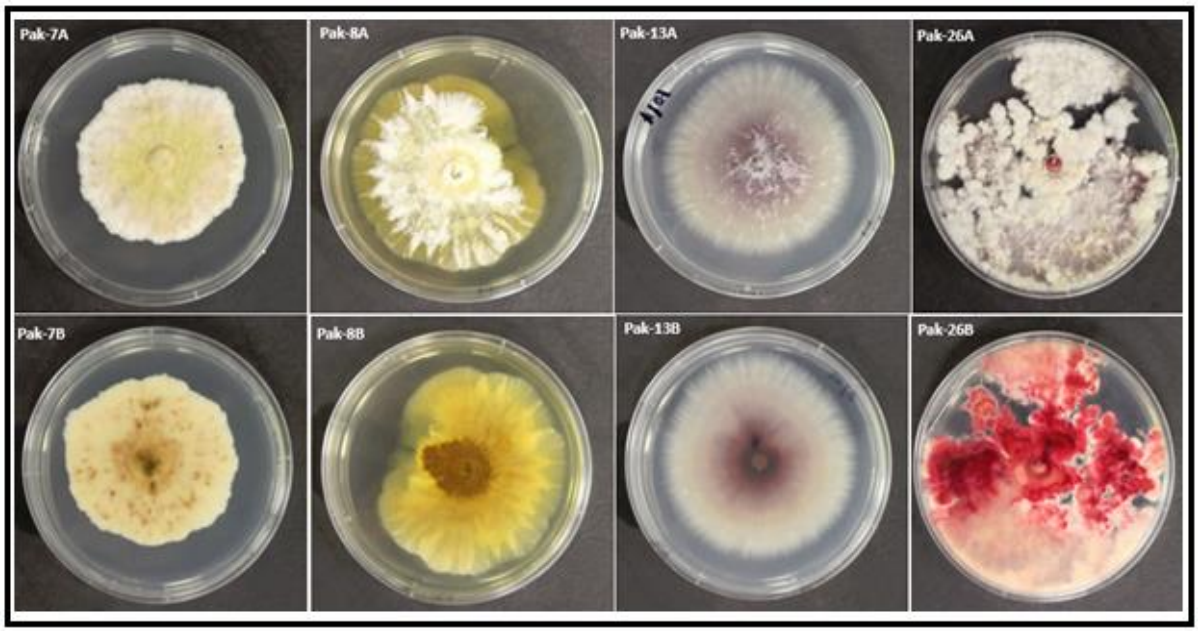

Fig. 4. Unidentified *Fusarium* spp. isolates growing on PDA medium, following incubation at 28°C for 7 days under 12 hours light and dark condition. A. Culture of the isolates on the upper side of the plate. B. Culture of the isolates on lower surface of the plate.

The highest percent inhibition 50.75% was recorded for isolate Pak-27 with colony diameter of 41.17mm followed by 47.10% for Pak-19 with colony diameter of 46.18mm, when treated with lowest concentration 10% of thiophanate methyl.

Highest percent inhibition 72.27% was recorded for isolate Pak-19 with colony diameter of 24.21mm followed by Pak-14 with percent inhibition of 64.44% with colony diameter of 19.49mm; whereas, lowest percent inhibition 56.67% was recorded for isolate Pak-10 with colony diameter of 38.35mm, when treated with 50% concentration of thiophanate methyl. Colony diameter of all isolates was significantly reduced when treated with (100%) concentration.

Highest percent inhibition 93.10% was recorded for isolate Pak-27 with colony diameter of 5.77mm followed by 87.44% with colony diameter of 10.97mm for isolate Pak-19, whereas lowest percent inhibition 64.61% was recorded in the isolate Pak-10 with colony diameter of 31.33mm (Table 3). The application of thiophanate methyl subsequently increased the sensitivity of *F. graminearum* at different concentration. The results demonstrated that more than 50% inhibition was achieved at 50mg/100ml concentration of thiophanate methyl.

Furthermore, thiophanate methyl was found to be more effective against *F. graminearum* to reduce the infection 48% under field conditions (Masiello *et al*., 2019). In another study 0.5mg/l Proline completely inhibit the fungal growth of *F. graminearum* and 0.5mg/l was chosen as an effective dose that lies between ED50 and ED90 (Schulz, 2015).

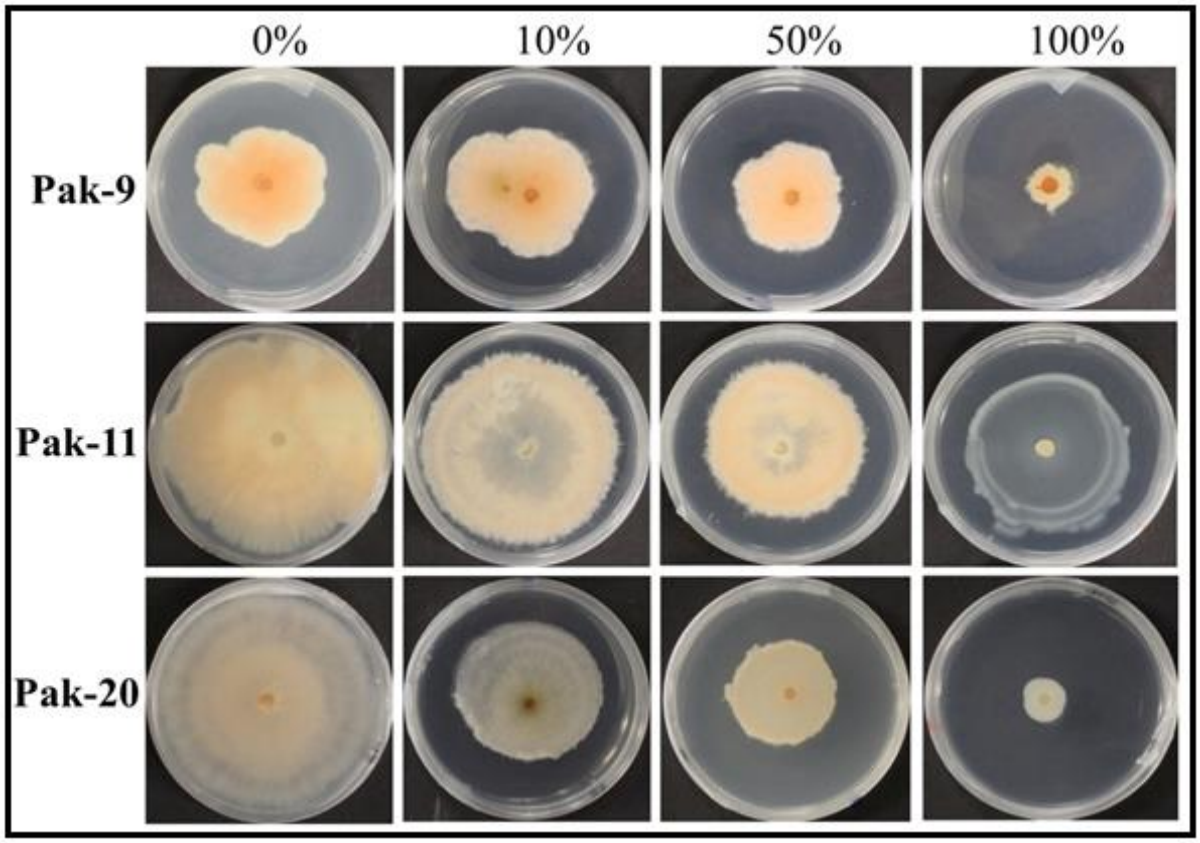

Fig. 5. Pictorial view of colony morphology and thiophanate methyl sensitivity of *Fusarium equiseti* isolates. All isolates were grown at 28°C for 7 days on PDA medium amended with different concentration of thiophanate methyl at 0%, 10%, 50% and 100%.

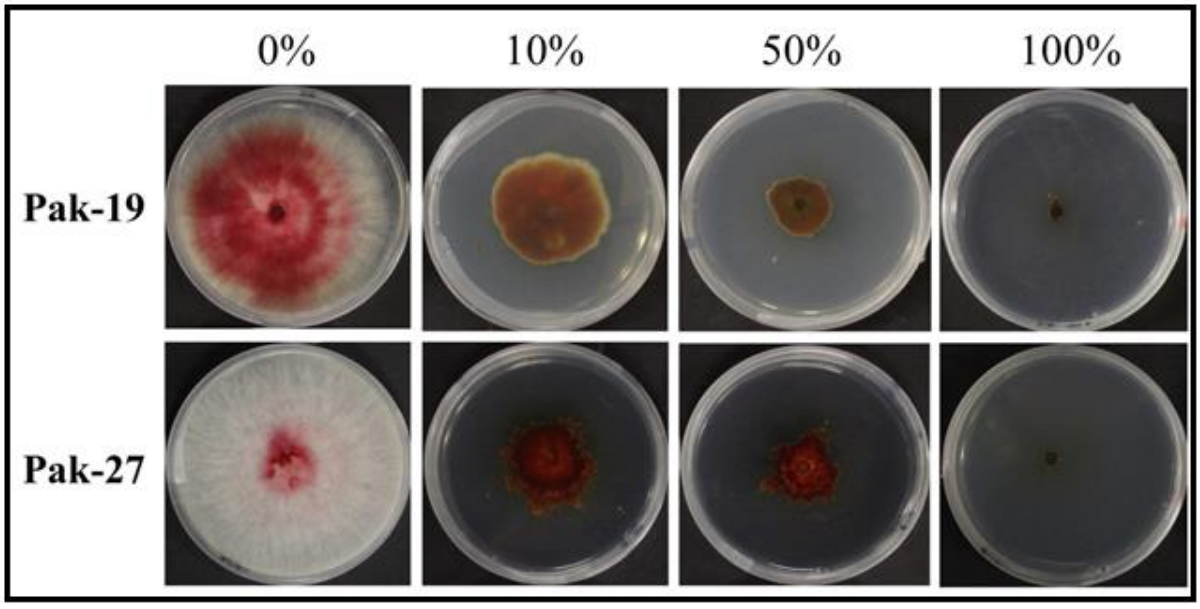

Fig. 6. Pictorial view of colony morphology and thiophanate methyl sensitivity of *Fusarium graminearum* isolates. All isolates were grown at 28°C for 7 days on PDA medium amended with different concentration of thiophanate methyl at 0%, 10%, 50% and 100%.

Our study results showed that all isolates of *F. graminearum* were sensitive to thiophanate methyl even at low concentration; however, in contrast the sensitivity of eight isolates of *F. graminearum* was conducted against different fungicides. The EC50 values were in ranged of 12.1 to 64.03 mg/l and all the isolates were found less sensitive to thiophanate methyl (Rekanović *et al*., 2011).

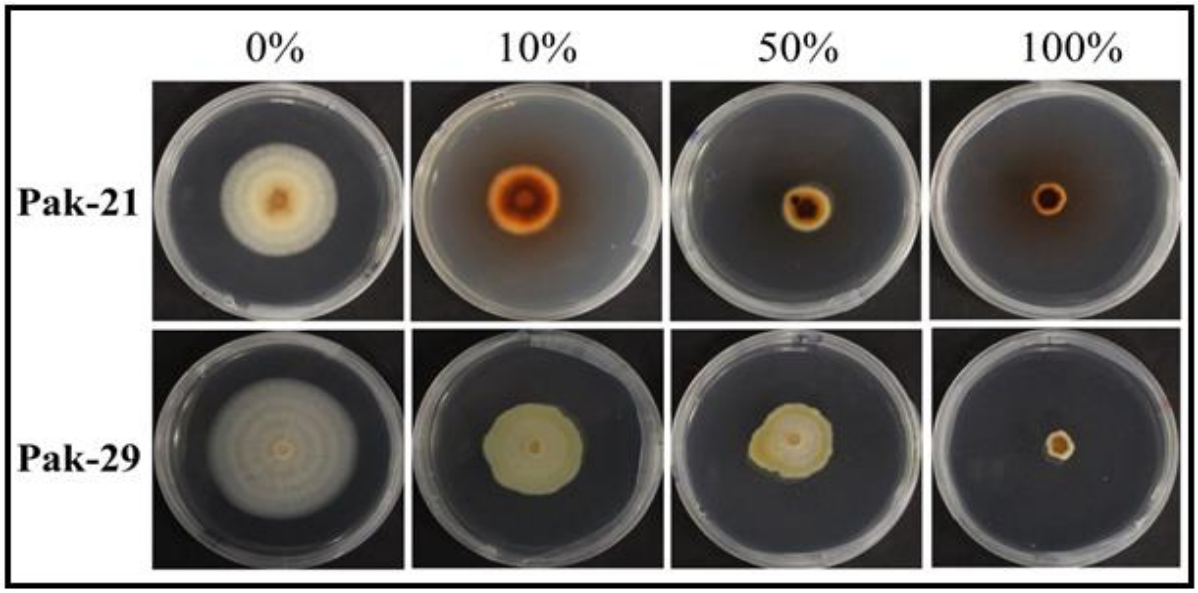

Fig. 7. Colony morphology and thiophanate methyl sensitivity of *Fusarium solani* isolates. All isolates were grown at 28°C for 7 days on PDA medium amended with different concentration of thiophanate methyl at 0%, 10%, 50% and 100%.

Effect of thiophanate methyl on isolates of Fusarium solani

Radial growth of both isolates was reduced significantly with treated with different concentration of thiophanate methyl. Data were recorded after 7 days of incubation at 28°C. Both isolates were sensitive to the different concentration thiophanate methyl even at low concentration (Fig. 7).

The highest percent inhibition 40.62% was recorded for isolate Pak-21 with colony diameter of 28.46mm; whereas, lowest percent inhibition 34.13% was recorded for isolate Pak-29 with colony diameter of 39.23mm, when treated with lowest 10% concentration of thiophanate methyl. Highest percent inhibition 62.257% was recorded for isolate Pak-21 with colony diameter of 17.94mm, while lowest percent inhibition 43.82% with colony diameter of 33.46mm, when treated with 50% concentration of thiophanate methyl. Highest percent inhibition 79.22% was recorded for isolate Pak-29 with colony diameter of 43.82mm followed by 65.75% inhibition for isolate Pak-21 with colony diameter of 16.42mm, when treated with (100%) concentration (Table 4).

The fungicide thiophanate methyl at three different concentrations inhibited the mycelial growth of *F. solani*. Systemic fungicide proved to be most effective and showed complete suppression of at highest concentration. Among different fungicides, thiophanate methyl was found to be most effective in inhibiting the mycelial growth (80.1%) of *F. solani* (Madhavi and Bhattiprolu, 2011). *F. solani* colony growth was suppressed more than 70% at higher concentration of thiophanate methyl.

The interaction of *F. solani* isolates with thiophanate methyl was different; complete inhibition of colony growth was observed at doses higher than 500ppm (Daami-Remadi and El-Mahjoub, 2006).

In another study, seven different fungicides at three different concentrations (50, 100 and 150ppm) by poison food method and Bavistin was found to be most effective fungicide in inhibiting the radial growth of *F. solani* (Bhanumathi and Ravishankar, 2007).

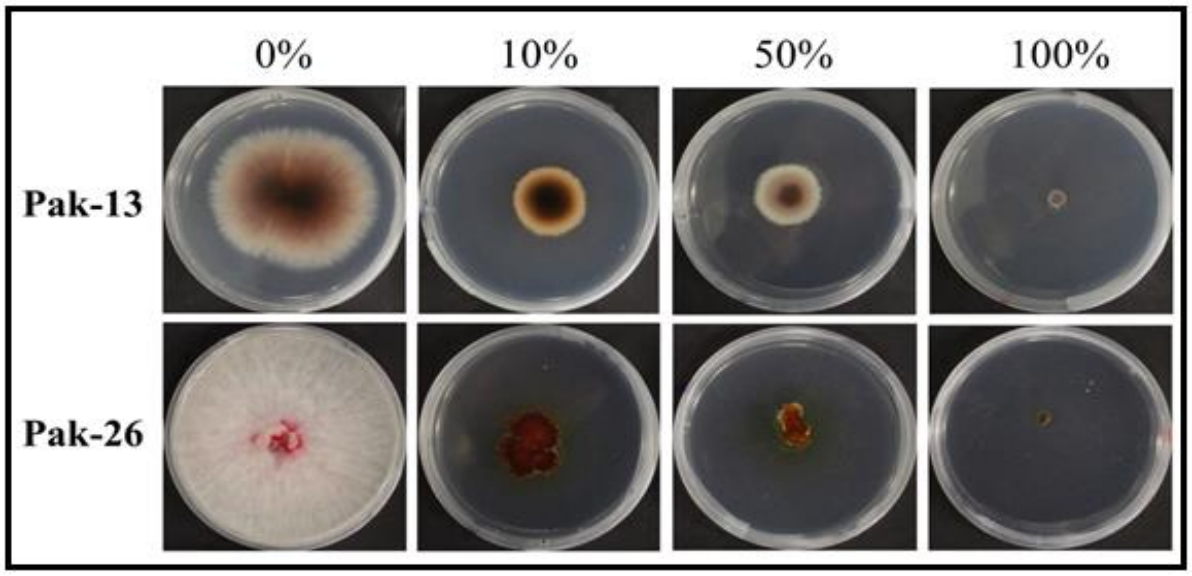

Fig. 8. Pictorial view of colony morphology and thiophanate methyl sensitivity of unknown *Fusarium* isolates. All isolates were grown at 28°C for 7 days on PDA medium amended with different concentration of thiophanate methyl at 0%, 10%, 50% and 100%.

Effect of Thiophanate methyl on isolates of unknown Fusarium species

Thiophanate methyl reduced the radial colony growth of all isolates significantly after 7 days of incubation when treated with (10%), whereas, the fungicide was least effective against the isolate Pak-8 (Fig. 8 and supplementary Fig. 3).

The highest percent inhibition 69.49% was recorded for isolate Pak-26 followed 57.81% for Pak-7. Highest percent reduction 80.31% was recorded for isolate Pak-26 with colony diameter of 16.65mm followed Pak-7 with percent inhibition of 70.03% with colony diameter of 11.29m; whereas, lowest percent inhibition 30.12% was recorded for isolate Pak-8 with colony diameter of 26.00mm, when treated with 50% concentration.

Colony diameters of all isolates were significantly reduced when treated with (100%) concentration of thiophanate methyl. Highest percent inhibition 94.44% was recorded for isolate Pak-26 with colony diameter of 4.70mm followed by 89.69% with colony diameter of 6.99mm for isolate Pak-13, whereas lowest percent inhibition 35.72% was recorded for isolate Pak-8 with colony diameter of 23.92mm (Table 5).

Conclusion

We reported that that thiophanate methyl is an effective fungicide against *F. equiseti*, *F. solani*, *F. graminearum* and unknown spp. of *Fusarium*, associated with tomato wilt in Khyber Pakhtunkhwa Pakistan and can be used as integrated disease management of the diseases.

Recommendation

Due to resistance in some isolates of *F. equiseti* the fungicide should be used in combination with other broad spectrum fungicide for management of the disease, to avoid future resistance build up in pathogen population against the fungicide.

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Supplementary Figures

Sensitivity of *Fusarium* spp. Infecting tomato in Khyber Pakhtunkhwa to Thiophanate Methyl.

Asma Akbar[1] and Shaukat Hussain[2].

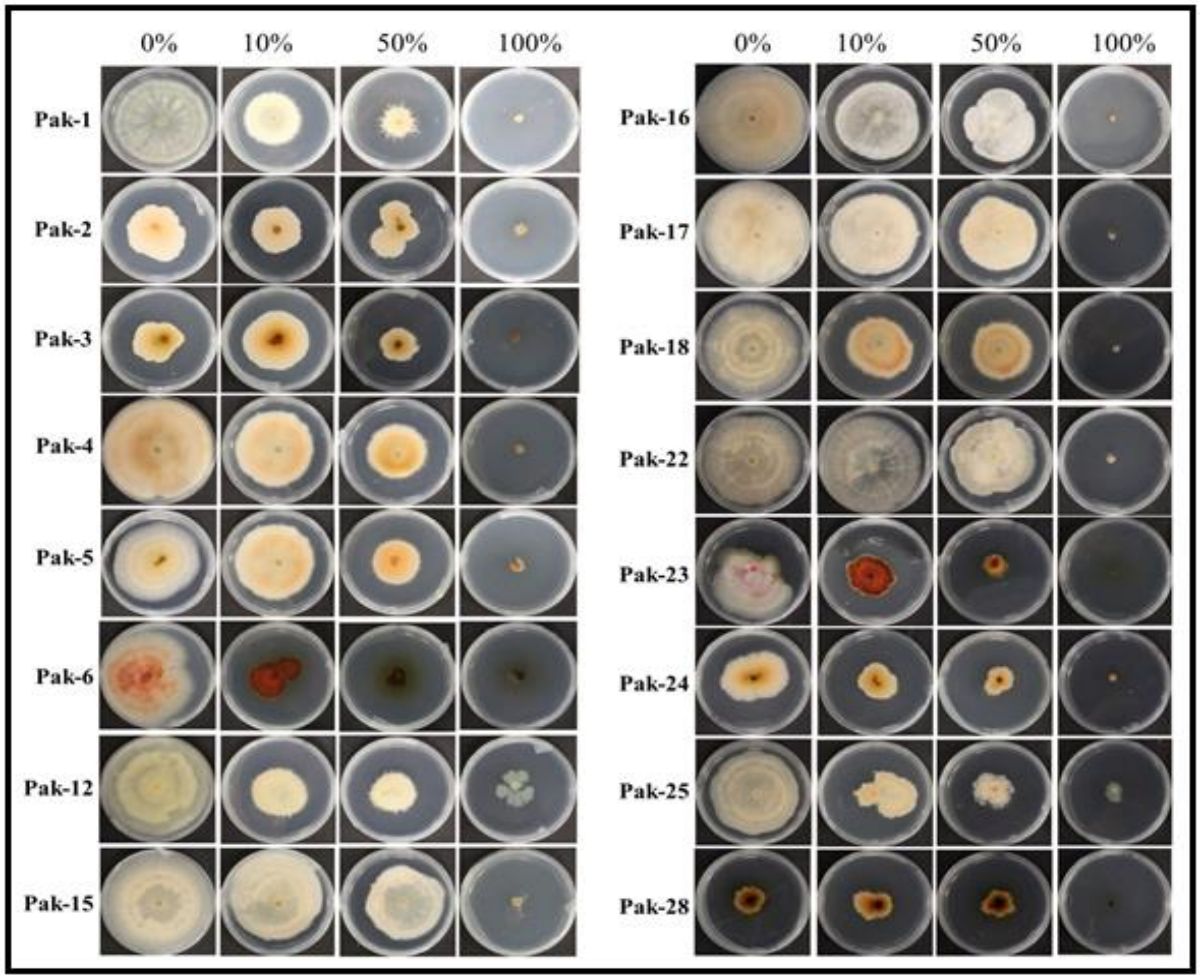

Fig. 1. Colony morphology and thiophanate methyl sensitivity of *Fusarium equiseti* isolates. All isolates were grown at 28°C for 7 days on PDA medium amended with different concentration of thiophanate methyl at 0%, 10%, 50% and 100%.

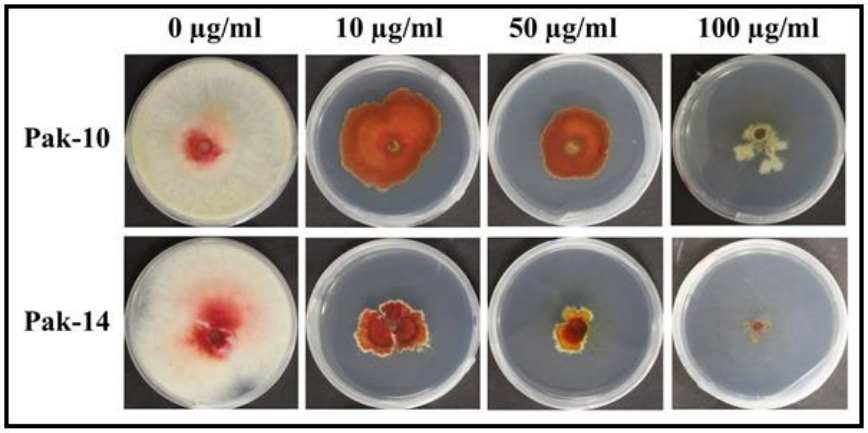

Fig. 2. Pictorial view of colony morphology and thiophanate methyl sensitivity of *Fusarium graminearum* isolates. All isolates were grown at 28°C for 7 days on PDA medium amended with different concentration of thiophanate methyl at 0%, 10%, 50% and 100%.

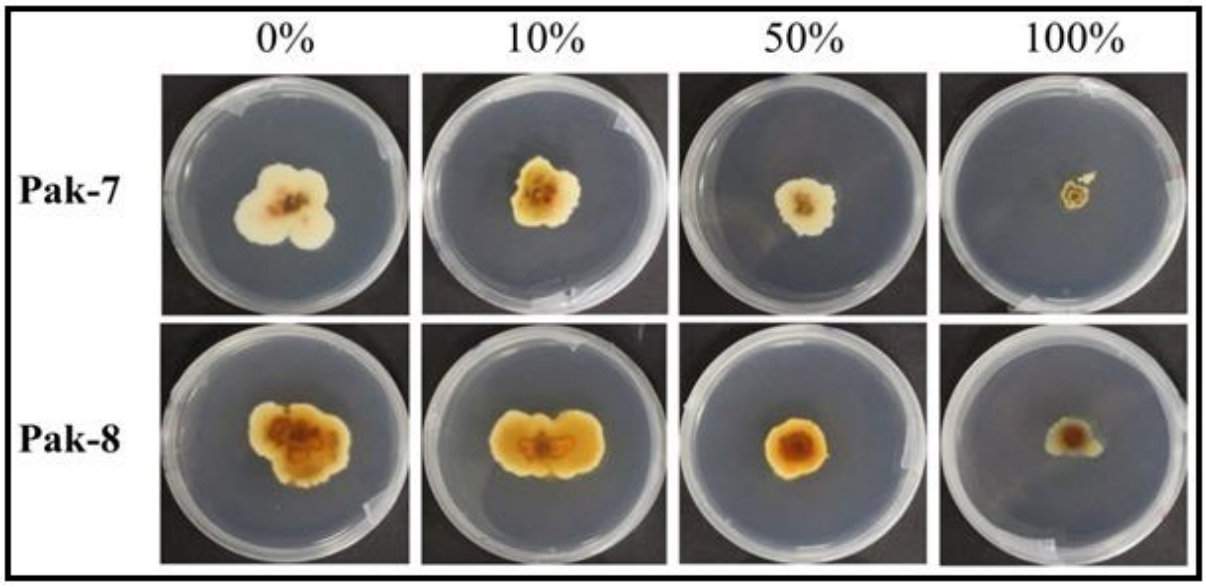

Fig. 3. Pictorial view of colony morphology and thiophanate methyl sensitivity of unknown *Fusarium* isolates. All isolates were grown at 28°C for 7 days on PDA medium amended with different concentration of thiophanate methyl at 0%, 10%, 50% and 100%.