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In vitro evaluation of bioagents botanical and fungicides against *Fusarium oxysporum* f.sp. *baisilici*

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Abstract
The Holy Basil (*Ocimum sanctum* Linn.) commonly known as Tulsi, is an aromatic perennial plant, belongs to the family Lamiaceae. It is native to Indian subcontinent and wide spread as a cultivated plant throughout the south-east Asian tropics. Holy Basil is widely grown in India for medicinal as well as religious purposes. *In vitro* studies reveal that highest inhibition was observed in chemical fungicide Tebuconazole (91.81) followed by other fungicide Mancozeb (78.33) followed by bio-agents *Trichoderma viride* (74.82), *Trichoderma harzianum* (71.77) *and Pseudomonas fluorescence* (69.55) *and* one botanical Neem leaves (65.55) in respect to control. All the bio-agents produce toxic volatile metavolatile, which reduced the radial growth of the test fungus. That is *Fusarium oxysporum* f.sp.*baisilici* (7.50) Tebuconazole, Mancozeb (19.57) followed by bio-agents *Trichoderma viride* (22.62) *Trichoderma harzianum* (25.40) *and Pseudomonas fluorescence* (27.40) *and* one botanical Neem leaves (31.00).

Keywords: Tulsi, *Pseudomonas fluorescence*, Tebuconazole.

1. Introduction
The Holy Basil (*Ocimum sanctum* Linn.) commonly known as Tulsi, is an aromatic perennial plant, belongs to the family Lamiaceae. It is native to Indian subcontinent and wide spread as a cultivated plant throughout the south-east Asian tropics. Holy Basil is widely grown in India for medicinal as well as religious purposes. There are many species of Basil such as *Ocimum sanctum* L. (Tulsi), *Ocimum gratissimum* (Ram Tulsi), *Ocimum canum sims* (Dulal Tulsi), *Ocimum baisilicum* (Ban Tulsi), *Ocimum kilimandscharicum Gureke* (Camphor Basil) *and Ocimum americanum* Linn (Hoary Basil) among them holy Basil (*Ocimum sanctum*) has been well known for its therapeutic potential (Prakash and Gupta, 2005) [4]. The chemical constiuents present in *Ocimum sanctum* are Oleanolic acid, Ursolic acid, Rosmarinic acid, Eugenol, Carvacrol, Linalool and β-caryophyllene, β-elemene (Kothari *et al*. 2004) [2]
In several ancient system of medicine including Ayurveda, Greek Roman, Siddha and Unanni, *Ocimum sanctum* has vast number of therapeutic applications such as in cardiopathy, haemeopathy, leucoderma, asthma, bronchitis, catarrhal fever, otalgeia, hepatopathy, vomiting, lumbago, hiccups, ophthalmia, gastropathy, genitourinary disorders, ringworm, verminosis and skin diseases etc. It is commonly used in cough, cold and mild indigestion. Despite its therapeutic potential, holy Basil is susceptible to various disease including *Fusarium* wilt, Bacterial leaf spot, Dampping off, and Downy mildew, among them wilt caused by *Fusarium oxysporum* f. sp. *baisilici*, is most common and destructive disease of holy Basil. It becomes a

serious problem in commercial production of holy Basil worldwide thus management of disease is very important.

Materials and Methods

Efficacy of Bio-agents against *Fusarium oxysporum* f.sp. *baisilici* using dual culture method

Three bio-agents *Trichoderma viride*, *Trichoderma harzianum*, and *Pseudomonas fluorescens* were used against *Fusarium oxysporum* f.sp. *baisilici*. To test the antagonistic effect, culture of *T. viride* and *T. harzianum* was obtained from the department of plant pathology, N.D.U.A &T, Kumarganj, Faizabad (U.P.). The culture was maintained on PDA for further studies. Sub-culturing was done periodically to maintain the purity of culture. For *Pseudomonas fluorescens*, soil sample were collected around the rhizosphere of wilt affected from Medicinal and Aromatic Farm N.D.U.A. & T, Kumarganj Faizabad (U.P.) Isolate of *Pseudomonas fluorescens* were Isolated and identified using Bergey's manual of determinative Bacteriology. Bacterial colonies identified as *Pseudomonas fluorescens* were picked and pure culture of isolate was maintained. The potential culture of *Trichoderma viride*, *Trichoderma harzianum*, and *Pseudomonas fluorescens* was evaluated *in vitro*. Dual culture technique as described earlier was followed. Nine mm disc of fifteen days old fungal cultures were placed on PDA medium one cm away from the edge of the plate, separately. *Trichoderma spp*. (9 mm disc) was placed at opposite side of the Petri plate. Three replicated plates for each treatment was maintained and incubated at 25 ± 2 ^{0}C. Per cent inhibition over control was calculated as per the formula.

Efficacy of botanical against *Fusarium* wilt of Holy Basil using food poison technique

Efficacy of one plants extract *viz*. leaves of Neem, which were found most effective against *Fusarium oxysporum*. Fresh leaves were collected and washed thoroughly in clean water. Then the washed neem leaves were taken in 100 ml of sterilized water and heated at 8^{0}C for 10 minutes. The material was filtered through double layered muslin cloth. All the plant extract were tested at 5 per cent concentration under *in vitro* condition by using food poison technique to study the inhibitory effect of these botanicals on radial growth of *Fusarium oxysporum*. 10 ml plant extracts of stock solution were added to the 90.00 ml of sterilized cooled PDA medium. The flasks were thoroughly taken to get uniform mix of the extracts under aseptic condition before pouring it into the petri dishes.

Twenty ml medium was poured into each petri dish seven treatment having three replications were maintained control treatment was maintained by pouring PDA medium without plant extracts. Five mm discs of 7 days pure culture of *Fusarium oxysporum* f. sp. *baisilici* were cut with sterilized cork borer and placed in the center of plant extracts amended petri plates. These petri plates were incubated at 25 ± 2^{0}C. The observation was recorded on radial growth & per cent inhibition at 6 days of incubation in plant extracts amended petriplates as well as in control.

Efficacy of fungicide against *Fusarium* wilt of Holy Basil

Two fungicides namely Tebuconazole 250 EC @ 0.1% and Mancozeb 75 WP @ (0.1%) were used in food poison technique.

Table 1: Effect of bio-agents, botanical and fungicides on mycelial growth of *Fusarium. oxysporum* f.sp. *Baisilici In vitro*

Treatments	Radial mycelial growth in mm after 6 days	% Inhibition
T_1 *Trichoderma viride*	22.67	74.82 (59.93)
T_2 *Trichoderma harzianum*	25.40	71.77 (57.90)
T_3 *Pseudomonas fluorescence*	27.40	69.55 (56.50)
T_4 Neem leaves @ 5%	31.00	65.55 (54.08)
T_5 Tebuconazole 250 EC @ 0.1%	7.50	91.81 (73.47)
T_6 Mancozeb 75WP @ 0.1%	19.57	78.33 (62.27)
T_7 Control	90.00	0.00 (0.28)
C.V. (%)	2.26	
SEm±	0.74	
CD at 5%	2.20	

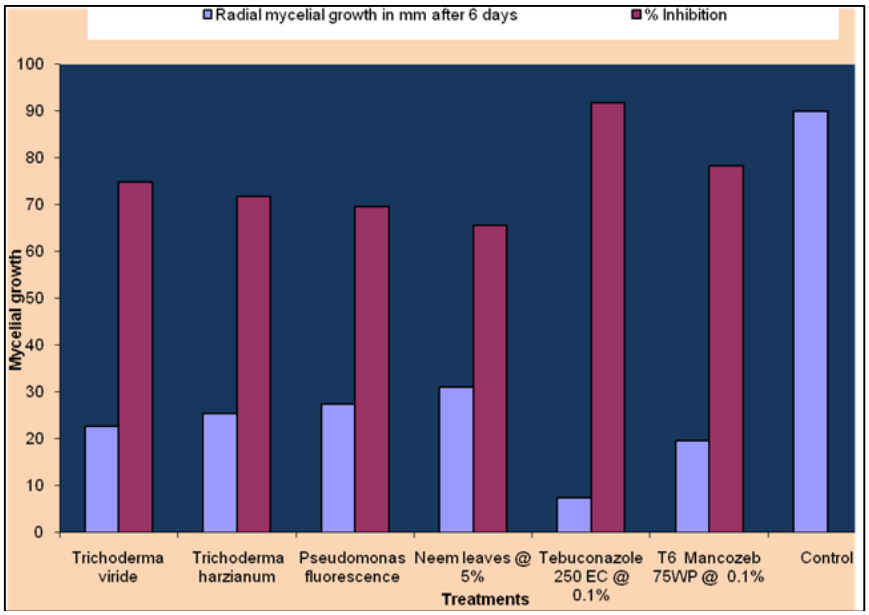

Fig 1: Effect of bio-agents, botanical and fungicides on mycelilal on my growth fusarium. oxysporum f.sp. baisilici *In vitro*

Results and Discussion

In vitro studies reveal that highest inhibition was observed in chemical fungicide Tebuconazole (91.81) followed by other fungicide Mancozeb (78.33) followed by bio-agents *Trichoderma viride* (74.82), *Trichoderma harzianum* (71.77) *and Pseudomonas fluorescence* (69.55) *and* one botanical Neem leaves (65.55) in respect to control. All the bio-agents produce toxic volatile metavolatile, which reduced the radial growth of the test fungus. That is *Fusarium oxysporum* f.sp.*baisilici* (7.50) Tebuconazole, Mancozeb (19.57) followed by bio-agents *Trichoderma viride* (22.62) *Trichoderma harzianum* (25.40) *and Pseudomonas fluorescence* (27.40) *and* one botanical Neem leaves (31.00) Sultana and Abdul (2013) [5]. Use fungicide microbiological antagonist and oil cakes *In vitro* and *In vivo* to control *Fusarium oxysporum* the cause of wilt of sunflower various worker like Moon *et al.* (1988) [3] farm that *Trichoderma viride and Trichoderma harzianum* arehighlyantagonistic to *Fusarium oxysporum*. and inhibited the growth of *Fusarium oxysporum* Xu *et al*. (1993) [6] reportedthat *Trichoderma* hyphal growth of *Fusarium oxysporum*. Similar results were also reported Jha, and Singh, (1997) [1] these finding are also in conformity with result reported.

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