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Integrated management of early blight of potato under Kashmir valley conditions

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Five non-systemic fungitoxicants viz., chlorothalonil 50 WP, mancozeb 75 WP, captan 50 WP, propineb 70 WP and copper oxychloride 50 WP at six concentrations (1000, 1500, 2000, 2500, 3000 and 3500 ppm) each and five systemic fungitoxicants viz., thiophenate methyl 70 WP, carbendazim 50 WP, hexaconazole 5 EC, fenarimol 12 EC and difenconazole 25 EC at six concentrations (100, 150, 200, 250, 300 and 350 ppm) each were evaluated in vitro against Alternaria solani (Ellis and Martin) Jones and Grout causing early blight of potato through poisoned food technique. Among non-systemic fungitoxicants mancozeb 75 WP, irrespective of concentration was most effective and inhibited a maximum mean mycelial growth inhibition of 75.46% over check, followed by propineb 70 WP, captan 50 WP, chlorothalonil 75 WP, and copper oxychloride 50 WP with mycelial growth inhibition of 68.09, 66.07, 58.89, and 57.81% respectively. Among systemic fungitoxicants hexaconazole 5 EC was most effective and exhibited a maximum mean mycelial growth inhibition of 84.19% over check. Under in vivo conditions seed treatment with mancozeb 75WP (0.3 %) + foliar spray with hexaconazole 5 EC (0.1%) + foliar spray with datura (50%) + foliar spray with Trichoderma harzianum (1 × 10^7 spore/ml) were highly effective in controlling the disease severity as compared to control.

Key words: Fungitoxicants, *Alternaria solani*, poisoned food technique, *Trichoderma harzianum*, datura, early blight, potato.

INTRODUCTION

Potato is one of the most important crops in the world and is planted in 18.2 million ha and a total yield reached 314.1 million ton (FAO, 2010). Potato is considered 'The King' in food staples and hardly any domestic kitchen is available which does not use it in one or the other form as it possesses all the attributes to be a potential food crop. Potato is the only non cereal food crop to commend such a high position in the world since being nutritious it can solve the problem of malnutrition and under nutrition if adopted as a major food crop. It has been recognized as a wholesome food and richest source of energy in most countries of the world where it forms important part of the human diet. Potato contains significant levels of phenolic compounds and vitamin C as potent antioxidants (Brown, 2005), which inactivate reactive oxygen species, reduce oxidative damage, lead to improved immune functions and reduce risk of cardiovascular diseases, cancer, cataract, diabetes and aging (Kour et al., 2004). Potato is highly remunerative and nutritive crop in Jammu and Kashmir particularly in high altitude cold and cold arid areas of Jammu and Kashmir (J&K) where it serves as a staple food. Early blight, caused by *Alternaria solani* (Ellis and Martin) Jones and Grout, is a serious disease of potatoes that

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occurs in most potato growing regions world-wide (Christ, 1990; Pelletier and Fry, 1990; Shtienberg et al., 1990; Vanderwalls et al., 2001). In recent years, increases in *A. solani* disease on potato foliage have been reported in various potato growing areas (Vloutoglou and Kalogerakis, 2000). Primary damage by early blight is attributed to premature defoliation of the potato plants, resulting in tuber yield reduction. Yield loss estimates resulting from foliar damage incited by early blight on potato vary by location, cropping season, cultivar, and the stage of potato maturity. In general, yield reductions of 20 to 30% have been reported in USA (Christ and Maczuga, 1989; Shtienberg et al., 1990). Early blight may also cause dry rot of tubers, reducing both the quantity and quality of marketable tubers (Nnodu et al., 1982). Environmental factors such as temperature, wetness duration and relative humidity (moisture) affect the development of early blight on potatoes (Adams and Stevenson, 1990; Vloutoglou and Kalogerakis, 2000). Temperature increases *A. solani* infection and sporulation (Vloutoglou and Kalogerakis, 2000). Water in the form of high relative humidity, rainfall or dew accumulation can increase conidia germination and pathogen infection (Rotem, 2004). Alternating low and high humidity conditions have also been shown to favour disease development (Vanderwalls et al., 2001). Early blight is also enhanced through continuous potato production (Olanya et al., 2009). The young plants of potato show high resistance to early blight due to *A. solani* as compared to older ones (Bambawale, 1978). Within the same plant, the lower leaves which are physiologically different from middle and top ones (Dowley et al., 1975) are more susceptible to certain pathogens with resistance increasing in an acropetal direction. Potato early blight symptoms firstoccur on the lower senescing leaves, which become chlorotic and abscise prematurely. Excessive defoliation may lead to death of the plant and consequent yield loss.

Several effective pesticides have been recommended against this pathogen but they not considered a long-term solution, due to concerns of expense, exposure risks and the hazards of its residues. Moreover, the development of resistance of pathogenic fungi towards synthetic pesticides is a great problem that can affect significantly the efficacy of chemical fungicides. Thus, to find safe, efficacious and environmentally friendly fungicides considered as a source of major concern (Mdee et al., 2009). Presently, the search for natural products with novel uses, particularly related to pest management is very important task. The use of plant extracts has been shown to be eco-friendly and effective against many plant pathogens (Latha et al., 2009; Moslem and El-Kholie, 2009; Satish et al., 2009; Duru and Onyedineke, 2010; Yanar et al., 2011; Talibi et al., 2012).

Trichoderma harzianum Rifai, *Trichoderma viride* Pers. Ex Gray are important biocontrol agents (BCAs) of plant pathogens. These BCAs were used for the control of soil borne, foliar and post harvest diseases in various crops in the field, in commercial green house and storage depots (Abeysinghe, 2009; Jegathambigai, 2010). *Trichoderma* work against fungal phytopathogens either indirectly by competing for nutrients and space, modifying environmental conditions, promoting plant growth and plant defense mechanism and antibiosis, or directly through mechanisms such as mycoparasitism (Shakeri and Foster, 2007; Reino et al., 2008).

Under Kashmir conditions, early blight of potato caused by *A. solani* is posing a great threat for its cultivation. The systemic study on potato early blight has not been conducted so far under Kashmir conditions. Therefore keeping in view the devastating nature of disease a detailed investigation was undertaken both under *in vitro* and *in vivo* conditions to devise an integrated management programme of the disease.

MATERIALS AND METHODS

In vitro evaluation of fungitoxicants

Five non-systemic fungitoxicants (chlorothalonil 50 WP, mancozeb 75 WP, captan 50 WP, propineb 70 WP and copper oxychloride 50 WP at six concentrations (1000, 1500, 2000, 2500, 3000 and 3500 ppm) each and five systemic fungitoxicants (thiophenate methyl 70 WP, carbendazim 50 WP, hexaconazole 5 EC, fenarimol 12 EC and difenconazole 25 EC) at six concentrations (100, 150, 200, 250, 300 and 350 ppm) each were evaluated *in vitro* against the test pathogen by poisoned food method (Nene and Thapliyal, 1993). Fifty milliliter of basal medium (potato dextrose broth) was poured in 150 ml conical flasks, plugged with non-absorbent cotton and autoclaved at 15 lbs pressure for 15 min. After cooling the medium, a known quantity of fungitoxicant as per treatment was incorporated into each flask, except control. Each treatment was replicated thrice in Complete Randomized Block Design (CRD). The flasks were then inoculated with 5 mm dia mycelial discs cut from actively growing fungus culture. The flasks were incubated at 25±2°C for 15 days. After incubation, the medium containing the mycelial growth of the fungus was filtered through previously weighed Whatman filter paper No. 41. The mycelial mat on filter paper was over dried at 60°C and weighed. The dry mycelial weight was calculated by subtracting weight of previously weighed filter paper from weight of filter paper with mycelial mat. Percent inhibition of mycelial growth was calculated using the formula of Vincent (1947):

$$\text{Percent inhibition} = \frac{C - T}{C} \times 100$$

Where, C = Weight of fungal colony in control (mg), and T = Weight of fungal colony in treatment (mg).

In vivo evaluation of biocontrol agents, botanical extracts and fungitoxicants

The fungitoxicants, biocontrol agents and botanical extracts were evaluated in *in-vivo* against the test pathogen. In case of biocontrol agents, a spore suspension of 1×10^7 cfu was used while plant/botanical extracts were used at 50% concentration. The experiment was laid out in Randomized Block Design with 11 treatments and 3 replications. The treatments consist of: T_1, Hexaconazole (F.S.); T_2, Mancozeb (F.S.); T_3, Datura (F.S.); T_4, *Trichoderma harzianum* (F.S.); T_5, Mancozeb (S.T.) + hexaconazole

Table 1. *In vitro* efficacy of non-systemic fungitoxicants in inhibiting the mycelial growth of *Alternaria solani*.

Fungi toxicants	Percent growth inhibition at different concentrations (ppm)*						
	1000	1500	2000	2500	3000	3500	Mean
Mancozeb 75 WP	53.30 (7.30)	58.70 (7.66)	68.61 (8.28)	80.16 (8.95)	92.03 (9.59)	100.00 (10.00)	75.46 (8.68)
Captan 50 WP	36.57 (6.04)	41.44 (6.43)	54.33 (7.37)	76.17 (8.72)	87.91 (9.37)	100.00 (10.00)	66.07 (8.12)
Copper oxychloride 50 WP	24.24 (4.92)	36.54 (6.04)	48.66 (6.97)	63.81 (7.98)	78.43 (8.85)	95.18 (9.75)	57.81 (7.60)
Propineb 70 WP	37.91 (6.15)	49.63 (7.04)	65.75 (8.10)	78.75 (8.87)	82.78 (9.09)	93.77 (9.68)	68.09 (8.25)
Chlorothalonil 75 WP	24.29 (4.92)	38.82 (6.23)	49.29 (7.02)	66.13 (8.13)	77.65 (8.81)	97.16 (9.85)	58.89 (7.67)
Mean	35.26 (6.02)	45.02 (6.70)	57.32 (7.57)	73.00 (8.54)	83.76 (9.15)	97.22 (9.86)	

$CD_{(p=0.05)}$: Fungi toxicant: 0.16; Concentration: 0.18; Fungi toxicant × concentration: 0.40; *Mean of three replications; *Figures in parentheses are square root transformed values

(F.S.) + Datura (F.S.) + *T. harzianum* (F.S.); T_6, Mancozeb (S.T.) + hexaconazole (F.S.) + *T. harzianum* (F.S.); T_7, Mancozeb (S.T.) + hexaconazole (F.S.) + Datura (F.S.); T_8, *T. harzianum* (S.T.) + Datura (F.S.); T_9, *T. harzianum* (S.T.) + Mancozeb (F.S.); T_{10}, *T. harzianum* (S.T.) + Hexacnazole (F.S.); T_{11}, Control; F.S., foliar spray; S.T. = seed treatment.

The first spray was given at the first initiation of disease symptoms and second 15 days later. Data on disease intensity was recorded 15 days after the last spray. In case of check, plants were sprayed with water. Per cent disease intensity (PDI) was calculated as per the following formulae given by FAO (Anonymous, 1967):

$$PDI = \frac{\sum(n \times v)}{N \times S} \times 100$$

Where, ∑= Summation; N = No. of leaves in each category; V = Numerical value of leaves observed; S = Maximum numerical value/grade. The data generated on various aspects of research were subjected to statistical analysis as per the methods described by Panse and Sukhatame (1978). The software used for analysis was 'Minitab'.

RESULTS AND DISCUSSION

Efficacy of fungitoxicants *in vitro*

The efficacy of different systemic and non-systemic fungitoxicant against *A. solani* was evaluated *in vitro* by poisoned food technique.

Effect of non-systemic fungitoxicants on mycelial growth

The data on *in vitro* efficacy of test fungitoxicants in inhibiting the mycelial growth of *A. solani* is presented in Table 1 and Figure 1. The test fungus *A. solani* was allowed to grow on fungicide-poisoned potato dextrose broth of each treatment concentration. After 15 days incubation at 25±2°C dry mycelial weight was recorded. An insight into the data revealed that the entire test fungi toxicants at all the concentrations tested significantly inhibited the mycelial growth of *A. solani* in comparison to check. Mancozeb 75 WP, irrespective of concentration was most effective and exhibited a maximum mean mycelial growth inhibition of 75.46% over check. This was followed by propineb 70 WP, captan 50 WP, chlorothalonil 75 WP and copper oxychloride 50 WP with mycelial growth inhibition of 68.09, 66.07, 58.89 and 57.81%, respectively. It was observed that with the increase in the concentration of each fungitoxicant there was significant decrease in the respective mycelial growth and accordingly maximum inhibition was observed at highest concentration (3500 ppm) than at lowest concentrations.

Data further revealed a significant interaction between fungitoxicant and concentration. Two fungitoxicants (mancozeb and captan) showed complete mycelial growth inhibition at 3500 ppm concentration while copper oxychloride, propineb and chlorothalonil exhibited only 95.18, 93.77 and 97.16% inhibition, respectively.

Among the fungitoxicants tested at 1000 and 1500 ppm concentration, mancozeb proved superior to all other test fungitoxicants with mycelial growth inhibition of 53.30 and 58.70%, respectively over check. At lowest concentration of 1000 ppm mancozeb, propineb and captan were superior to other test fungitoxicants exhibiting 53.30, 37.91 and 36.57% inhibition, respectively.

Efficacy of systemic fungitoxicants on mycelial growth

The data on *in vitro* efficacy of systemic fungitoxicants in inhibiting the mycelial growth

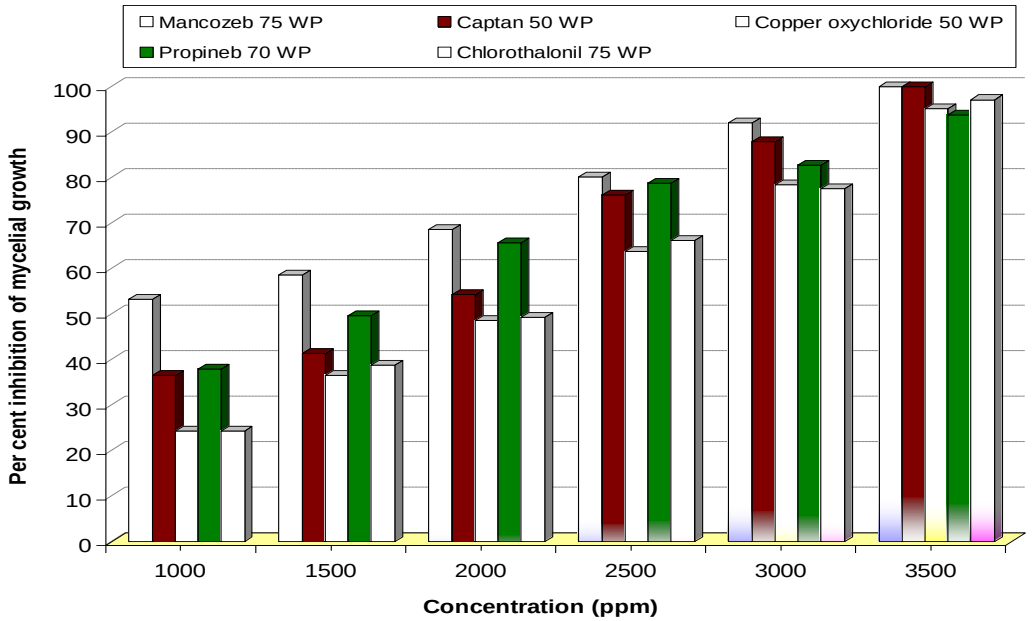

Figure 1. *In vitro* efficacy of contact fungi toxicants in inhibiting the mycelial growth of *A. solani***.**

of *A. solani* presented in Table 2 and Figure 2 revealed that all the test fungitoxicants at all the concentrations tested significantly inhibited the mycelial growth of *A. solani* in comparison to check. Hexaconazole at 5 EC, irrespective of concentration was most effective and exhibited a maximum mean mycelial growth inhibition of 84.19% over check. This was followed by difenconazole 25 EC which showed mean mycelial growth inhibition of 79.06% followed by fenarimol 12 EC (76.97%) and carbendazim 50 WP (65.05%). Thiophenate methyl 70 WP was less effective and inhibited mycelial growth by 53.15%. It was observed that with the increase in the concentration of each fungitoxicant there was a significant decrease in the respective mycelial growth and accordingly maximum inhibition was observed at highest concentration (350 ppm) than at lower concentrations.

Data further revealed a significant interaction between fungitoxicant and concentration. Four fungitoxicants (fenarimol, hexaconazole, difenconazole and carbendazim) showed complete mycelial growth inhibition at 350 ppm concentration. At lowest concentration of 100 ppm hexaconazole and difenconazole were superior to other test fungitoxicants exhibiting 56.51 and 50.18% inhibition, respectively. However, fenarimol and difenconazole were statistically at par with each other at all the concentrations tested over check. Lodha and Prasad (1975) reported that among different fungicides mancozeb was highly effective to check the colony growth of *A. solani* under *in vitro* conditions. Similar results regarding high efficacy of mancozeb against *A. solani* were also reported by other workers (Choulwar and Datar, 1994; Chethana et al., 2011). Deora et al. (2004) tested nine fungicides and observed that maximum disease control (> 50%) was obtained by Dithane M-45 followed by Kavach, and Tilt was less effective as compared to these fungicides to control the early blight disease. On the other hand, systemic fungicides were highly effective at much lower concentrations. Hexaconazole proved to be highly effective at much lower concentration and caused complete inhibition in fungal growth at 300 ppm concentration. High efficacy of hexaconazole in causing complete inhibition in growth of *Alternaria alternata* has already been demonstrated (Singh and Singh, 2006). Akbari and Parakhia (2007) also reported that systemic fungicides completely inhibited the mycelial growth of *A. alternata* even at a minimum concentration of 50 ppm. While non-systemic fungicides thiram and mancozeb gave cent percent inhibition of *A. alternata* at a minimum concentration of 500 ppm. Similar observations were also reported by Patel et al. (2005). Singh and Singh (2006) also studied the efficacy of different contact and systemic fungicides under *in vitro* conditions and reported that hexaconazole was most effective as it caused 100% growth inhibition even at lowest concentration of 250 ppm. The other fungicides like mancozeb, copper oxychloride, copper hydroxide, chlorothalonil, azoxystrobin and propineb were also effective and caused significant reduction in mycelial growth but at a much higher concentration. The results are also in agreement with Chethana et al. (2011) who reported that among systemic fungicides tested difenconazole at 0.1% showed 98.85% inhibition of the fungus while among non-systemic fungicides mancozeb at 0.3% was best in inhibiting the growth of *Alternaria porri* with 100% inhibition.

Table 2. *In vitro* efficacy of systemic fungitoxicants in inhibiting the mycelial growth of *A. solani*.

Fungi toxicants	Percent growth inhibition at different concentrations (ppm)*						
	100	150	200	250	300	350	Mean
Fenarimol 12 EC	48.91 (6.99)	60.90 (7.80)	74.14 (8.61)	82.55 (9.08)	95.32 (9.76)	100.00 (10.00)	76.97 (8.77)
Hexaconazole 5 EC	56.51 (7.51)	74.43 (8.62)	84.24 (9.17)	90.00 (9.48)	100.00 (10.00)	100.00 (10.00)	84.19 (9.17)
Difenconazole 25 EC	50.18 (7.08)	60.75 (7.79)	76.88 (8.76)	88.92 (9.42)	97.67 (9.88)	100.00 (10.00)	79.06 (8.89)
Carbendazim 50 WP	24.91 (4.99)	44.88 (6.69)	55.78 (7.46)	72.63 (8.52)	92.10 (9.59)	100.00 (10.00)	65.05 (8.06)
Thiophenate methyl 70 Wp	20.66 (4.54)	35.41 (5.95)	43.92 (6.62)	55.55 (7.45)	69.44 (8.33)	93.92 (9.69)	53.15 (7.29)
Mean	40.23 (6.34)	55.27 (7.43)	66.99 (8.18)	77.93 (8.82)	90.90 (9.53)	98.78 (9.93)	

$CD_{(p=0.05)}$; Fungitoxicant: 0.13; Concentration: 0.15; Fungitoxicant x concentration: 0.34; *Mean of three replications; *Figures in parentheses are square root transformed values.

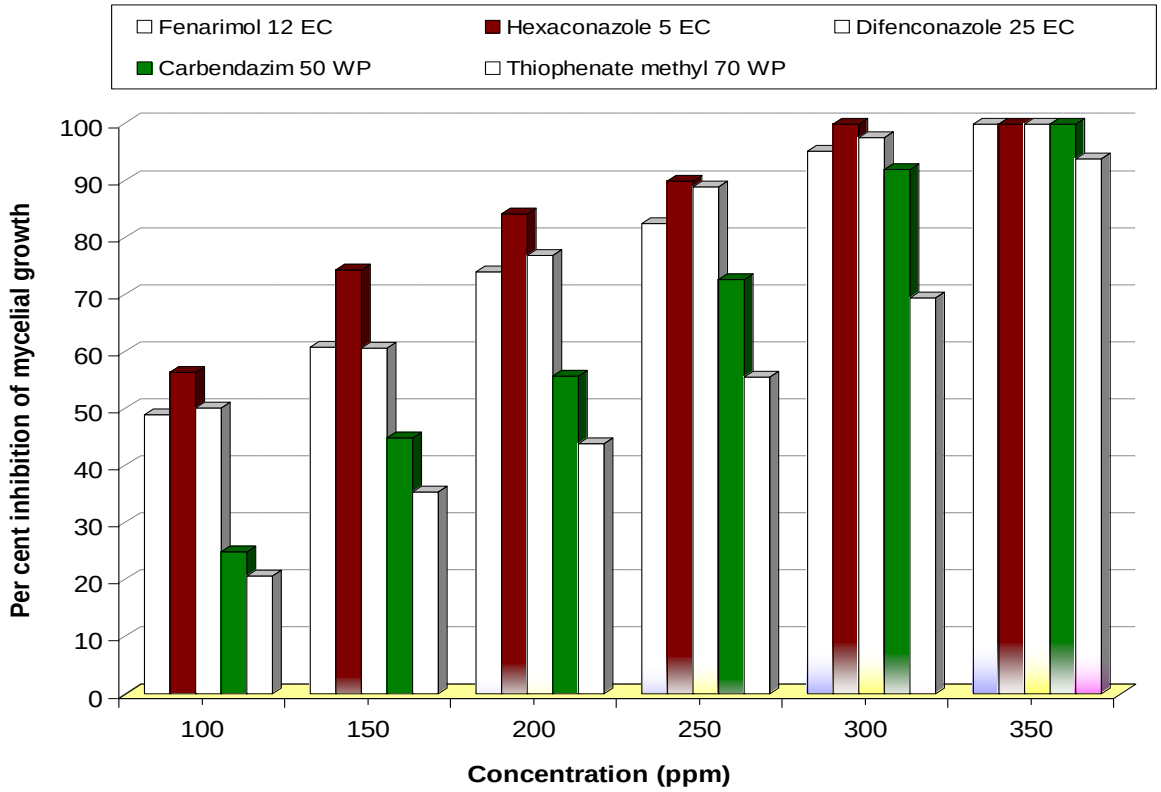

Figure 2. *In vitro* efficacy of systemic fungitoxicants in inhibiting the mycelial growth of *A. solani*.

Table 3. Integrated management of early blight of potato under field conditions.

Treatment		Disease intensity (%)		Pooled mean
		2008	2009	
T_1	Hexaconazole (F.S.)	12.06 (19.41)	14.37 (22.23)	13.21 (20.82)
T_2	Mancozeb (F.S.)	14.02 (21.97)	17.76 (24.91)	15.89 (23.44)
T_3	Datura (F.S.)	18.84 (25.70)	20.34 (26.79)	19.59 (26.24)
T_4	*Trichoderma harzianum* (F.S.)	17.85 (24.98)	19.56 (26.23)	18.70 (25.60)
T_5	Mancozeb (S.T.) + Hexaconazole (F.S.) + Datura (F.S.) + *T. harzianum* (F.S.)	4.91 (12.79)	6.37 (14.60)	5.64 (13.69)
T_6	Mancozeb (S.T.) + Hexaconazole (F.S.) + *T. harzianum* (F.S.)	8.70 (17.15)	9.70 (18.11)	9.20 (17.63)
T_7	Mancozeb (S.T.) + Hexaconazole (F.S.) + Datura (F.S.)	10.86 (19.23)	12.30 (20.50)	11.58 (19.86)
T_8	*T. harzianum* (S.T.) + Datura (F.S.)	15.17 (22.90)	18.76 (25.64)	16.96 (24.27)
T_9	*T. harzianum* (S.T.) + Mancozeb (F.S.)	12.18 (20.37)	15.45 (23.12)	13.81 (21.74)
T_{10}	*T. harzianum* (S.T.) + Hexaconazole (F.S.)	11.54 (19.83)	12.97 (21.08)	12.25 (20.45)
T_{11}	Control	28.00 (31.94)	34.38 (33.74)	15.27 (22.41)
Overall mean		14.01 (21.47)	16.54 (23.35)	
CD (p = 0.05)		1.88	3.22	

*Mean of three replications. Figures in parenthesis are arc sine transformed values; FS = Foliar spray; ST = seed treatment

Integrated disease management under field conditions

The antagonists, botanical extracts and fungitoxicants were assessed under field conditions against early blight disease of potato. The treatments comprised of *T. harzianum* at 1 × 10^7 spore/ml, datura ethanol extract at 50% and two fungitoxicants (mancozeb 75 WP at 0.3% and hexaconazole 5 EC at 0.1%). Observations on disease intensity were recorded 15 days after last spray.

It is evident from the data presented in Table 3 that during the year 2008, all the treatments significantly reduced the disease compared to control. However, the magnitude of reduction varied from treatment to treatment. The range of disease intensity in treatments varied from 4.91 to 18.84% in comparison with 28.00% recorded in check. Minimum disease intensity of 4.91% was observed in plants treated with mancozeb 75 WP (seed treatment) + hexaconazole 5 EC (foliar spray) + datura extract (foliar spray) + *T. harzianum* (foliar spray). This was followed by treatment mancozeb 75 WP (seed treatment) + hexaconazole 5 EC (foliar spray) + *T. harzianum* (foliar spray) with disease intensity of 8.70%. However, treatments T_7 mancozeb 75 WP (S.T) + hexaconazole 5 EC (FS) + datura (FS) and treatment T_{10} (*T. harzianum* (ST) + hexaconazole 5 EC (FS)] were statistically at par with other showing disease intensity of 10.86 and 11.54%, respectively. Treatments T_1 (hexaconazole 5 EC two foliar sprays) and T_9 (*T. harzianum* (ST) + hexaconazole 5 EC (FS)] with disease intensity of 12.06 and 12.18% were statistically at par with each other. Ethanol extract of datura at 50% (two sprays) was least effective in comparison to all other treatments and was statistically at par with *T. harzianum* at 1 × 10^7 spore/ml (two sprays) with disease intensity of 18.84 and 17.85% respectively over check (28.00%).

During the year 2009 the experiment was repeated and results (Table 3) revealed that all the treatments proved effective in reducing the disease intensity and were significantly superior over check. Least disease intensity of 6.37% was observed in treatment T_5 followed by treatment T_6 (9.70%). Among other treatments tested, T_7 and T_{10} did not show significant difference and were statistically at par with each other with disease intensity of 12.30 and 12.77%, respectively. Treatment T_3 (2 foliar sprays of datura ethanol extract at 50%) proved least effective in comparison to all other treatments but was significantly superior over check.

Two years pooled data (2008 and 2009) presented in Table 3 revealed significantly higher disease intensity of 16.54% during the year 2009, in comparison to 14.01% during the year 2008. All

the treatments were significantly superior over control in reducing the disease intensity. Among the treatments disease intensity ranged from 5.64 to 19.59% as against 31.19% in check. In the present study seed treatment with mancozeb 75 WP + foliar spray with hexaconazole 5 EC (0.1%) + foliar spray with datura (50%) + foliar spray with *T. harzianum* (1×10^7 spores/ml) was highly effective in controlling the disease severity as compared to control. Similar observations were also reported by (Verma and Gandhi, 2007; Phalisteen et al., 2008). Verma et al. (2008) reported that disease severity of *A. solani,* the causal agent of early blight of tomato could be significantly reduced with foliar spray of *Clerodendron aculeatum* leaf extract (15%) immediately after appearance of symptoms or foliar spray of *T. viride* (10^7 cfu/ml) followed by two sprays of mancozeb. Monica Sharma and Gupta (2003) reported that integration of efficacious seed treatment with biopesticides and biocontrol agents alone or in combination was effective in the management of root rot of French bean. In form of spray, plant extracts are used *in vivo* to control airborne plant pathogenic microbes. They act by inactivating or killing the pathogen spores as they land on plant surface (Leksomboon et al., 2001; Govindappa et al., 2011).These findings are also in agreement with Mishra and Gupta (2012) who reported that among 8 plant extracts, bioagents and fungicides evaluated in *in vitro* against purple blotch of onion, clove extracts of *Allium sativum* at 10% resulted in maximum inhibition of growth (58.05%) of *Alternaria porri* while among bioagents *T. viride* was effective in inhibition of growth (53.17%). Among fungicides mancozeb at 0.2% completely inhibited the growth of *A. porri.* Similar findings were also reported by (Raghavendra et al., 2009; Ramjegathesh et al., 2011; Zaker and Mosallenejad, 2011; Madhavi et al., 2012).

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