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Evaluation of fungicides, biocontrol agents and plant extracts against early blight of potato caused by *Alternaria solani*

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Abstract
A study was conducted in the Department of Plant Pathology, College of Horticulture, Bharsar. During kharif season 2016 to control *Alternaria solani* (Ellis and Martin) Jones and Grout, causing early blight of potato. Ten different treatments were evaluated in vitro and in vivo condition. However, in vitro result revealed that the hexaconazole minimum radial growth (3.00mm) was observed at 3000 ppm concentration followed by mancozeb (3.16mm), Carbendazim (4.36 mm), *T. harzianum* (4.88mm) and neem oil (5.58mm). The maximum radial growth was observed in onion extract (5.75 mm). In vivo results revealed that hexaconazole (0.10%) was found most effective and recorded lowest disease intensity (12.61, 18.89 and 25.28%) with significantly increased yield (446.95 g/ plant) followed by mancozeb (0.25%) disease intensity (14.73, 21.70 and 27.95%) and yield (428.03 g/ plant), carbendazim (0.15%) disease intensity (17.13, 23.56 and 29.31%) and yield (416.97 g/ plant) at 45, 60 and 75 DAS. Results revealed that hexaconazole is highly effective against Alternaria solani in vitro and in vivo condition.

Keywords: Management, *Alternaria solani*, Potato, hexaconazole, mancozeb, Carbendazim, *T. harzianum,* neem oil

1. Introduction
The potato (Solanum tuberosum) is one of the most important vegetable crops in the world, belonging to the family solanaceae and is an important starchy food crop in both sub-tropical and temperate regions. Even in tropical regions it is widely grown during winter season. Potato (S. tuberosum) is a native of South America [1]. In India the potato has been cultivated since its introduction in the early part of the 17th century. In India potato is grown in almost all the states under diverse climatic conditions except Kerala and 82% of potatoes are grown in plains during the short winter days from October to March. Potato is the most popular crop in West Bengal next to cereals [2].

Potato plants are subjected to attack by numerous diseases wherever the crop is grown. Among them, early blight of potato caused by Alternaria solani is of major cause of concern in potato production at present. The causal organism is air and soil borne which cause disease on foliage (leaf blight), stem (collar rot) and tuber (tuber rot) and can result in severe damage during all stages of plant development spread by fungal spores [3, 4]. The disease causes losses to crop productivity in the field and to tuber quality in storage. Average annual yield loss of potato due to this disease was approximately 79% of the total production depending upon the nature of the disease, weather condition and type of variety grown [5].

Primary methods of controlling early blight include preventing long periods of wetness on the leaf surface, cultural scouting, sanitation, and development of the host plant resistance with the application of fungicides [6].

Regarding the management of early blight of tomato many workers had done lot of works based on the chemical control. Mancozeb was also effective in reducing the disease intensity and increase the yield of Pusa Ruby [7, 8, 9]. Patil *et al*. (2003) [11] reported that carbendazim was best fungicides to minimize the disease incidence and highest tuber yield while according to Datar and Mayee (1985), Fentin hydroxide and mancozeb were superior for the controlling the disease. Kumar *et al*. (2007) reported that hexaconazole (0.05%) and azoxytrobin (0.2%) was very effective in managing early blight of tomato. The wide and indiscriminate use of chemical fungicides has been the cause of development of resistance among plant pathogens,

leading to the occurrence of serious diseases. Due to this, there is an increasing interest to obtain alternative antimicrobial agents (bio control agents) and plant extracts for using in plant disease control systems. Plant products of recognized antimicrobial spectrum could appear in food conservation systems as main antimicrobial compounds or as adjuvant to improve the action of other antimicrobial compounds [12]. The development of such disease resistance to the pathogens and problems of environmental pollution due to excessive reliance on pesticides are the major causes today. Therefore, to avoid or minimize these problems, experiments have been conducted on management of early blight of potato by using the natural products such as plant extracts and bio control agents during 2016 crop seasons. The objectives and motive of present investigations were to evaluate the efficacy of selected fungicide, bio control, plant extract for minimizing the loss of crop and for increasing crop quality, quantity and productivity.

2. Materials and methods

Kufri Sutlej variety of potato susceptible to early blight disease. The diseased specimens of early blight were collected from potato growing areas of Tigaddu vegetable research block Bharsar. The infected tissues of leaves and stem showing typical symptoms of early blight and tuber rot were cut in to small pieces of 1-2 mm size. The surface sterilized with sodium hypochlorite solution (1%) for 2 min, rinsed thrice with sterile distilled water, blot dried and placed on PDA medium. Pathogen was identified following the cultural and morphobiometric characteristics criteria [13, 14]. Cultural characteristics were observed directly by pigmentation on medium and mycelial growth pattern on PDA plates.

2.1. Identification of pathogen: A. solani was isolated from infected potato leaves. Pure cultures of A. solani were maintained by sub culturing of pure culture and pathogenicity test was done for the conformation of the pathogen.

2.2. In vitro bioassay of different treatments: Pathogenicity test in artificial inoculation methods in vitro are commonly used to test the pathogenicity of a fungal species, as it is easy to control environmental conditions [15]. For confirmation of disease caused by the isolated pathogen Pathogenicity tests were conducted according to the Koch's postulates. Healthy host plants were thoroughly cleaned with sterilized distilled water. In vitro experiment was conducted by using ten treatments at different doses viz, 1000, 1500, 2000, 2500 and 3000 ppm with three replications and radial growth of pathogen was calculated after 72 hours and data was analyzed statistically design simple CRD. At six different concentrations (100, 150, 200, 250, 300 and 350 ppm) each were evaluated in vitro against the test pathogen by poisoned food method. 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 fungi toxicant 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 diameter mycelial discs cut from actively growing fungus culture. The flasks were incubated at 25±2°C for 15 days.

2.3. In vivo study: Field experiment was conducted on during Kharif session 2016 by using cultivated variety Kufri Sutlej with three replication, Plot size: 1.8 ×1.35 m, Spacing: 60 × 45cm after preparation of field sowing was done on date 20 March 2016 and after 45, 60 and 75 days after sowing recorded disease intensity.

Table 1

Treatments	Dose (%)
Hexaconazole	0.10
Mancozeb	0.25
Carbendazim	0.15
Trichoderma viride	0.35
Trichoderma harzianum	5
Pseudomonas fluorescens	5
Neem oil	5
Garlic extract	5
Onion extract	5
Check	00

Field experiment was conducted during crop session 2016. Three prophylactic sprays were given at 15 day interval.

Disease intensity: Diseased leaves will be categorized as per the scale given by Pandey & Pandey (2002).
Per cent disease intensity were calculated as per the following formula.

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

Table 2

Category	Grade/numerical value	Leaf area infected
I	0	Diseased tree
II	1	1-10
III	2	11-25
IV	3	26-50
V	4	51-75
VI	5	>76

Where,
Σ = Summation
n = No. of leaves in each category
v = Numerical value of leaves observed
N= Total No. of leaves examined
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) [18].

3. Results and discussion

Results revealed that all the treatments tested at 1000, 1500, 2000, 2500 and 3000 ppm concentrations), significantly inhibited radial growth of the test pathogen over untreated control. Among the treatment @ 3000 ppm there was minimum radial growth was observed in hexaconazole (3.00mm) followed by mancozeb (3.16mm) and Trichoderma harzianum (4.88 mm) recorded in Table (3).

3.1 Disease intensity result

All the treatments were found significantly effective in reducing the disease intensity when compared to Check. Minimum disease intensity was observed in hexaconazole (4.81, 10.51 and 18.26%) at 45, 60 and 75 DAS, respectively with maximum tuber yield (446.95 g/plant). It was found to be most effective followed by mancozeb (6.50, 13.70 and 22.00%) with (428.03 g/plant) and T. harzianum (9.91, 18.40

and 27.50%) with (388.66 g/plant) which were next best treatments. It can be concluded that hexaconazole was highly effective against early blight of potato. Among the treatments were not more effective in the garlic extract and onion extract to over control. Hexaconazole as best treatment against early blight of potato was also reported by Sadana and Didwania (2015) [19]. Ganie *et al*. (2013a) [20] among non-systemic fungitoxicants mancozeb 75 WP, irrespective of concentration was most effective followed by propineb 70 WP. Among systemic fungitoxicants hexaconazole 5 EC was most effective. 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 × 107 spore/ml) were highly effective in reducing the disease severity as compared to control.

The data on PDI of early blight was recorded periodically from 45 to 105 days after planting (DAP) with an interval of 15 days. It has been found that in all treatments PDI increased with age of the plants. Data on disease severity showed that all fungicide tested reduced the disease intensity significantly compared to control. From the table we also conclude that in all the treatments, there was increases in disease index from 45 to 105 DAP. However, the rate of increase in PDI was slow in case of newer fungicides treated plots compared to check treatments and control (Sahu *et al.*, 2013) [3, 13].

Ganie *et al.* (2013b) [22] evaluated five non-systemic fungitoxicants viz., chlorothalonil, mancozeb, captan, propineb and copper oxychloride and five systemic fungitoxicants viz., thiophanate methyl, carbendazim, hexaconazole, fenarimol and difenoconazole under *in vitro* conditions against *A. solani* and found that among non-systemic fungitoxicants mancozeb 75 WP was most effective and resulted in maximum mean mycelial growth inhibition of 75.46 per cent. Among systemic fungitoxicants, hexaconazole was most effective and exhibited a maximum mean mycelial growth inhibition of 84.19 per cent. Minimum disease intensity was also observed in hexaconazole with maximum plant height and Yield (kg/plot) Sudarshana *et al.* (2012) [23] and also hexaconazole was best treatment found It showed significant decrease in disease intensity compared to all other treatments including check.

Table 3: Effect of different treatments on radial growth of *Alternaria solani* at different ppm concentration after 72 hours.

S.N	Redial growth (mm) of *Alternaria solani* at different concentrations (ppm)					
	Treatment	**1000ppm**	**1500ppm**	**2000ppm**	**2500ppm**	**3000ppm**
T_1	Hexaconazole	4.28	4.08	3.88	3.46	3.00
T_2	Mancozeb	5.33	5.00	4.88	3.75	3.16
T_3	Carbendazim	5.53	5.30	5.11	4.80	4.36
T_4	*Trichoderma viride*	5.76	5.46	5.20	4.83	4.58
T_5	*Trichoderma harzianum*	6.16	6.00	5.35	5.00	4.88
T_6	*Pseudomonas fluorescens*	6.41	6.10	5.50	5.25	5.25
T_7	Neem oil	6.66	6.33	5.66	5.33	5.58
T_8	Garlic extract	6.66	6.58	6.00	5.58	5.66
T_9	Onion extract	7.30	7.00	6.21	6.25	5.75
T_{10}	Check/Control	15.16	15.16	15.16	15.50	15.03
	Mean	6.925	6.701	6.295	5.975	5.725
	SE.(d)	0.46	0.41	0.60	0.62	0.71
	C.D (0.05)	0.97	0.87	1.26	1.31	1.50

Table 4: Effect of different treatments on disease intensity at 45, 60 and 75 DAS

S.N	Treatments	Disease intensity			Tuber weight (g/plant)
		45 DAS	**60 DAS**	**75 DAS**	
T_1	Hexaconazole	4.81 (12.61)	10.51 (18.89)	18.26 (25.28)	446.95
T_2	Mancozeb	6.50 (14.73)	13.70 (21.70)	22.00 (27.95)	428.03
T_3	Carbendazim	8.70 (17.13)	16.00 (23.56)	24.00 (29.31)	416.97
T_4	*Trichoderma viride*	9.70 (18.12)	18.00 (25.08)	26.50 (30.96)	403.77
T_5	*Trichoderma harzianum*	9.91 (18.32)	18.40 (25.38)	27.50 (31.61)	388.66
T_6	*Pseudomonas fluorescens*	10.21 (18.61)	18.60 (25.53)	29.25 (32.72)	377.51
T_7	Neem oil	11.00 (19.35)	20.21 (26.70)	34.07 (35.69)	368.77
T_8	Garlic extract	11.50 (19.80)	20.44 (26.86)	35.50 (36.55)	357.18
T_9	Onion extract	12.50 (20.68)	21.50 (27.61)	39.25 (38.77)	345.47
T_{10}	Check	20.00 (26.55)	38.60 (38.39)	55.25 (47.99)	307.12
	S.E.(d)	0.14	0.07	0.04	1.14
	C.D. at 5%	0.297	0.157	0.089	2.42

*Mean of three replications; *Data in the parenthesis are angular transformed values.

4. Summary and conclusion

Treatments at 1000, 1500, 2000, 2500 and 3000 ppm concentrations), significantly inhibited radial growth of the test pathogen over untreated control. Among the treatment @ 3000 ppm there was minimum radial growth was observed in hexaconazole (3.00 mm) followed by mancozeb (3.16 mm) and Trichoderma harzianum (4.88 mm). Minimum disease intensity was observed in hexaconazole (4.81, 10.51 and 18.26%) at 45, 60 and 75 DAS, respectively with maximum tuber yield (446.95 g/plant). It was found to be most effective followed by mancozeb (6.50, 13.70 and 22.00%) with (428.03 g/plant) and T. harzianum (9.91, 18.40 and 27.50%) with (388.66 g/plant) which were next best treatments. It can be concluded that hexaconazole was highly effective against early blight of potato. Among the treatments were not more effective in the garlic extract and onion extract to over control.

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