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Evaluation of inorganic chemicals against early blight of potato caused by *Alternaria solani* (Sorauer)

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Abstract
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. 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. An experiment was conducted in the Department of Plant Pathology, College of Agriculture, C S AUA & T, and Kanpur. During *Ravi* season 2015-16 to control. *Alternaria solani* (Sorauer), causing early blight of potato. Nine different treatments were evaluated in vitro condition. However, in vitro result revealed that the minimum radial growth of mycelium was found in salicylic acid treatment representing the value 1.5, 2.4, 3.6, 5.9, 10.0, 12.2 and 16.0 mm at 1, 2, 3, 4, 5, 6 and 7 days after inoculation, respectively against 7.4, 12.2, 16.3, 21.0, 25.6, 36.0 and 44.9 mm in case of control. Among the treatments, the maximum radial growth of mycelium was recorded in case of copper chloride treatment was showing 1.8, 2.5, 4.6, 8.3, 11.6, 16.2 and 21.2 mm radial growth of mycelium at 1, 2, 3, 4, 5, 6 and 7 days of inoculation. The maximum radial growth was observed in cupper chloride 6.8, 9.2, 11.6, 16.3, 19.1, 25.2 and 32.3. All, the tested inorganic chemicals were statistically significant in reducing the mycelial growth of pathogen as compared with control up to 7 days of inoculation.

Keywords: Inorganic chemicals, potato, *Alternaria solani*, salicylic acid and growth of mycelium

Introduction
The potato (*Solanum tuberosum* L.) is a starchy, tuber crop from the perennial nightshade and considered as a "King of Vegetable". The origins of the potato can be traced back to South American natives in 5000 B.C. in the highlands of the Peruvian Andes Mountains. Around 1570, the potato reached Europe with the returning Spanish explorers. There are more than 160 wild potato species, and most of them contain high levels of Alcaloids. The first edible potato are considered to have been cultivated 4000 years ago in Peru (www.wikipedia). The word "Potato" may refer either to the plant itself or to the edible tuber. It is the fourth most important food crop and represents a valuable source of nutrients in a balanced diet. The average nutritional value of 200g of potato is about, calories: 200 proteins: 4.6g, carbohydrate: 51g, fat: 0.2g, cholesterol free and good source of dietary fiber with 4-8g, niacin: 16mg, vitamin b6: 7mg, vitamin c: 26mg, iron: 2.75mg, iron: 2.75mg, magnesium: 55mg, thiamine: 22mg and pantothenic acid: 1.12g (USDA, 2014) [5]. Considering the importance of potato, Food and Agriculture Organization of United Nation has declared potato as "Food for future".
Potato plants are subjected to attack by numerous diseases wherever the crop is grown. Among them, early blight caused by *Alternaria solani* (Sorauer) is the most destructive disease of potato in the tropical and subtropical regions. The disease may appear from seedling stage to harvesting of crop and even storage condition as dry rot of tuber. The disease can be very destructive if left uncontrolled, often resulting in complete defoliation of plants. In contrast to the name, it rarely develops early, but usually appears on mature foliage (Rowe *et al*., 1993) [6]. Young and middle-aged plants have low susceptibility to infection being disease influenced by the crop age. Young plants are relatively resistant, but the susceptibility increases gradually and continuously from the initiation of tuber formation, so that mature plants are most susceptible to the disease (Johnson and Teng, 1990; Rotem, 1981; Shtienberg *et al.*, 1996) [8]. 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 [Sahu, *et al.* 2013; Datar, *et al., 1981*] [3]. 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 (Kumar, *et al*, 2007) [4]. 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 (Kumar and Srivastava., 2013; Maheswari., *et. el.,* 1991; Gondal., *at. el.,* 2012) [12]. Patil *et al*. (2003) [13] 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) [7] reported that hexaconazole (0.05%) and Azoxystrobin (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.

Materials and Methods

The present investigation based on laboratory Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur during 2015-2016. The procedure and techniques applied during the course of investigation were elucidated as below:

Isolation, purification and identification of *Alternaria solani*

Collection of infected plant sample

Potato plants with typical blight symptoms were first identified and then collected from Vegetable Research Farm, Chandra Shekhar Azad University of Agriculture and Technology, Kalyandurg (Kanpur). Infected leaf with concentric dark brown spots developing mainly in the leaf were taken from the field and washed in sterilized water. The sample was then placed in between two fold of sterilized blotter paper and preserved at 4-6 ^{0}C in refrigerator for further study. The samples were later used for isolation and purification of pathogen.

Preparation of culture media

The pathogen, *Alternaria solani* (Soraur) causes early blight of potato can properly grow on Potato Dextrose Agar Media. Therefore, preparation of PDA media is prerequisite for isolation of the fungus.

Requirements

Agar-Agar	-	20gm
Dextrose	-	20 gm
Peeled Potato	-	200 gm
Distilled water	-	1000 ml.

Along with conical flask, culture tubes, knife, muslin cloth, beaker, non-absorbent cotton, saucepan, heater, measuring cylinder and autoclave is required for preparation of PDA media.

Procedure

Exactly 200gm peeled and chopped potato slices were taken in a saucepan. About 500ml of water was added and was boiled for about 30 minute or until the slices were easily penetrated by glass rod. The content of saucepan was then filtered with a muslin cloth and potato slices were discarded. The filtrate was measured in a measuring cylinder and final volume made up to 1 liter by adding distilled water. It was again poured in saucepan and heated. 20gm dextrose was added slowly in hot water to dissolve it. At the same time 20 gm agar agar was added in boiled water (and mixed it properly). The solution was boiled for some time till it tends to solidify on cooling. The media was then ready and poured in conical flask of about 200 ml. in each. 10 ml of media was also poured in 10 culture tubes each. Both conical flask and culture tubes were plugged with none absorbent cotton and mouth was wrapped with butter paper and rubber band. Culture tubes were placed vertically in wire baskets. The media in flask and culture tubes were then autoclaved at 15 lb/inch^2 pressure (15psi) for 20 min at 121.6 °C.

Isolation of pathogen

A small piece of infected leaf from border of sporulating lesion along with some healthy green tissue was cut and dipped in mercuric chloride solution (0.1%) for 30 seconds followed by rinsed in sterilized distilled water thrice and dried off with sterilized filter paper. The tissue pieces were placed at the center of petri-plate which was previously filled with sterilized PDA media. The plates were then incubated at 18±1 ^{0}C in BOD. The Petri plates were observed daily at 24 hrs interval and noticed the presence of mycelium around the leave bits.

Purification of pathogen

As soon as the mycelia growth is noticed around the bits, the pathogen was purified by hyphal tip culture method. The mycelia bits of *A. solani* was removed from the margin of fungal colony and then transferred to another Petri-plate which was previous poured with sterilized Potato-Dextrose-Agar medium. After purification the pure culture of *A. solani* were sub cultured at monthly intervals and maintained on Potato-Dextrose-Agar slants under refrigeration at 6 to 8 ^{0}C for further studies.

Identification of *Alternaria solani*

The isolated pathogen was identified on the basis of its morphological, cultural characters and pathogenic behavior towards the host.

Collection and preparation of inorganic chemicals as inducer

The inorganic chemicals Table: 1) were collected from laboratory of the Department of Plant Pathology and purchases from local market. Different concentrations of inducers (Table: 1) were prepared by weighing required quantity of inducers separately and placed in conical flask separately by adding 100 ml of sterilized water for each conical flasks and shaken until they are dissolved completely to prepare the required concentration of solutions.

Table 1: List of inorganic chemicals

Treatment	Concentration
Salicylic acid (SA)	10mM
Calcium chloride ($CaCl_2$)	10mM
Hydrogen peroxide (HP)	10 ppm
Metalaxyl	0.1%
Di-potassium hydrogen Orthophosphate (DPHP)	0.2
Ferric chloride ($FeCl_3$)	5mM
Copper sulphate	10mM
Indole acetic acid (IAA)	1%
Copper chloride	10mM
Control-1	-

Effect of inorganic chemicals on mycelial growth of *Alternaria solani*

The efficacy of inorganic chemicals on mycelial growth of *A. solani* was tested *in vitro* condition using Poison food technique. The PDA media amended with inorganic chemicals at different concentration separately and poured in 90mm Petriplates. The plates were inoculated with mycelial discs of 0.5 cm diameter from the advancing edges of 7-day-old pure cultures of *A. solani.* Distilled water instead of inorganic chemicals was used in the control experiments. The petriplates were then incubated at a temperature of 25± 1°C. Four plates per treatment were used as replications. The diameter of the fungal colony was measured at every 24 hrs up to 7 days after inovulation using a meter rule along two diagonal lines drawn on the reverse side of each Petriplate.

Result and Discussion

The preliminary work on effect of inorganic chemicals on mycelial growth of isolated pathogen was conducted on petriplates by Poison Food Technique. The data presented in Table-2 showed that the minimum radial growth of mycelium was found in salicylic acid treatment representing the value 1.5, 2.4, 3.6, 5.9, 10.0, 12.2 and 16.0 mm at 1, 2, 3, 4, 5, 6 and 7 days after inoculation, respectively against 7.4, 12.2, 16.3, 21.0, 25.6, 36.0 and 44.9 mm in case of control. The calcium chloride treatment was showing 1.8, 2.5, 4.6, 8.3, 11.6, 16.2 and 21.2 mm radial growth of mycelium at 1, 2, 3, 4, 5, 6 and 7 days of inoculations. Among the treatments, the maximum radial growth of mycelium was recorded in case of Indole acetic acid treated plant. All tested inorganic chemicals were statistically significant in reducing the mycelial growth of pathogen as compared with control up to 7 days. Similar work reported by many researchers Chen *et al.,* (1999) also found that salicylic acid inhibited the mycelial growth and zoospore germination of *Pythium aphanidermatum*. Amal (2009) [15] has been found that inhibitory effect of various chemicals like DL-3-aminobutyric acid (BABA), 2, 6-dichloroisonicotinic acid (INA), Saver (Ai: salicylic acid), Syrup (nutrient supplement), and ASM on the mycelial growth *P. capsici.* He found that only Saver significantly reduced mycelial growth and sporangium production at concentrations of 100 mg mL^{-1} or higher. Kone *et al.,* (2009) [16] and Ziv and Zitter (1992) [18] also reported that Potassium and sodium bicarbonates have inhibitory effects against several pathogenic fungi. In addition to bicarbonates salts has been shown to have a profound inhibitory effect on several fungi and causes the collapse of hyphal walls and shrinkage of conidia (Punja and Grogan, 1982 and Ziv and Zitter, 1992) [18].

Table 2: Effect of inorganic chemicals on radial mycelial growth of *Alternaria solani in vitro*

Name of inorganic chemicals	Concentration of inorganic chemicals	Radial mycelial growth upto 7 days (in mm)							Per cent mycelial growth reduction over control after 7 days
		1	2	3	4	5	6	7	
SA	10 mM	1.5	2.4	3.6	5.9	10.0	12.2	16.0	64.36
$CaCl_2$	10mM	1.8	2.5	4.6	8.3	11.6	16.2	21.2	52.78
HP	10 ppm	2.9	4.2	5.0	10.5	14.0	18.4	22.6	49.66
Metalaxy	0.1%	3.8	5.7	7.1	11.5	15.2	19.2	25.7	42.76
DPHP	0.2%	4.4	6.9	9.0	12.3	15.9	20.0	26.3	41.42
$FeCl_3$	5mM	5.4	7.0	9.2	13.5	16.3	21.3	29.2	34.96
$CuSO_4$	10mM	5.6	7.1	10.0	14.3	17.1	21.9	30.3	32.51
IAA	1%	5.9	7.3	10.2	15.0	17.6	22.3	31.0	30.95
$CuCl_2$	10mM	6.8	9.2	11.6	16.3	19.1	25.2	32.3	28.06
Control-1	-	7.4	12.2	16.3	21.0	25.6	36.0	44.9	-
C.D.P=(0.05)		0.305	0.436	0.580	0.839	1.035	1.366	1.788	
S.E (m)		0.103	0.147	0.195	0.282	0.348	0.460	0.602	
S.E (d)		0.145	0.207	0.276	0.399	0.493	0.650	0.851	
C.V.		3.914	3.937	3.904	3.774	3.717	3.745	3.730	

Conclusion

Thus, the present finding of study revealed that the inorganic chemicals have ability to decrease radial growth. It can be concluded that salicylic acid was highly effective against early blight of potato.

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