

INDUCTION OF RESISTANCE IN TOMATO AGAINST EARLY BLIGHT DISEASE WITH NON-CONVENTIONAL CHEMICALS

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ABSTRACT

Early blight caused by *Alternaria solani* is one of the most common and destructive foliar diseases of tomato causing heavy yield losses wherever it is grown. Control of early blight disease has primarily been accomplished by the cultivation of resistant cultivars and application of chemical fungicides. Cultivation of resistant cultivars does not offer a practical method of control of early blight disease as many of the tomato breeding lines and cultivars with measurable level of resistance is less than sufficient under most early blight epidemic field conditions. Similarly, frequent and abusive use of fungicides brings about adverse effects. Hence, in the present investigation, attempts were made to explore the potential use of non-conventional chemicals to control early blight by inducing resistance in tomato. Foliar spray of aqueous solutions of nine non-conventional chemicals each at one concentration were tested against early blight of tomato along with mancozeb 2000 ppm as the standard fungicide. All chemicals except potassium bicarbonate 1000 ppm reduced the disease. The percent disease index was lowest (9.26) in fungicide check. Among the test chemicals, spray application of 1000 ppm di-potassium hydrogen orthophosphate gave the highest percent disease reduction over control (60.53) followed by 20 ppm potassium permanganate (53.95), 0.5% calcium nitrate (52.30) and 2000 ppm sodium bicarbonate (49.67). The number of days taken to express early blight symptoms was significantly higher when treated with calcium nitrate (5.00 days) followed by di-potassium hydrogen orthophosphate (4.67 days). The incubation period in mancozeb, fungicide check (5.67 days) was on par with calcium nitrate (5.00 days). Except potassium nitrate treatment (33.06) and potassium bicarbonate (28.75), all the test chemicals were superior to control in reducing the number of lesions per leaf. Induced resistance was expressed with increasing the incubation period and reducing the lesion numbers and percent disease index.

KEYWORDS: *Alternaria solani*, Early blight, Induced resistance, Tomato.

INTRODUCTION

Early blight of tomato (*Lycopersicon esculentum* Miller) caused by *Alternaria solani* (Ellis and Martin) Jones and Grout is one of the most common and destructive foliar diseases of tomato accounting for heavy yield losses varying from 25 to 100 percent (Sohi, 1984).

Control of early blight has been accomplished primarily by the application of chemical fungicides and using resistant cultivars (Spletzer and Enyedi, 1999). Many of the tomato breeding lines and cultivars with measurable level of resistance are late maturity or the level of resistance is less than sufficient under most early blight epidemic conditions (Maiero *et al.*, 2000). Further, breeding for resistance against early blight has little scope due to lack of single gene resistance, complex pattern of inheritance (Spletzer and Enyedi, 1999) and difficulties in the screening of plants for resistance and transferring of resistance genes across genotypes (Foolad *et al.*, 2000). Therefore cultivation of resistance tomato varieties does not offer a practical solution to control of early blight disease. Currently management of tomato early blight disease relies principally on foliar application of fungicides (Mills *et al.*, 2002). Frequent and abusive use of fungicides cause a number of problems such as adversely effects on the environment, consumers and non-target organisms (Pharand *et al.*, 2002) and emergence of resistant pathogens due to their high selection pressure (Yamaguchi, 1998)

The disadvantages associated with these measures have provided an incentive to develop more economical, environmentally sound and reliable methods of disease control. As an alternative to fungicide application, it may be possible to induce plant defense responses by exogenous application of certain chemicals. Treatments with various non-conventional chemical salts can induce resistance against a variety of pathogens and the effect of these chemicals have been mediated mostly through host plants (Bhattacharya and Roy, 1998).

The present investigation was undertaken to find out the possibility of induction of resistance in tomato plants against *A. solani* with foliar spray application of aqueous solutions of non-conventional chemicals such as sodium, potassium and calcium salts.

MATERIALS AND METHODS

Seed and plant materials

Seeds of tomato (*Lycopersicon esculentum* Miller) cultivar, Pusa Early Dwarf (PED); Ankur Seeds Limited, Nagpur-18, India were sown in plastic crates and 25-day old seedlings were transplanted in earthen pots (20 cm X 20 cm) containing non sterile red soil and farm yard manure (4:1 V/V). Plants were grown in a glasshouse under natural conditions of temperature and light. Plants were irrigated daily and fertilized weekly with a commercial preparation of NPK mixture (14:14:14).

Pathogen and preparation of inoculum

The pathogen was isolated from naturally infected tomato leaves showing typical early blight symptoms under aseptic conditions using tissue segment method (Aneja, 2000). The culture was purified by single spore isolation described by Tuite (1969) and identified as *A. solani* based on morphological characteristics described by Ellis (1971).

Since *A. solani* seldom produces conidia under laboratory conditions, a standard-mycelial spore suspension was prepared as previously described by Dhiman *et al.*, (1980) for mass inoculation of tomato plants. Seven day old culture of *A. solani* growing in three test tubes containing 5 ml of Czapek-Dox agar medium in each were collected and transferred to an motor operated electric blender containing 250 ml of distilled water containing 0.01% tween 20 (a kind of surfactant). Then the blender was run in alternate cycles of high and low speed for 3-4 minutes. The slicing and blending action of the blades accompanied by a strong centrifugation reduced the coarse fungal mats into bits transverse through a fine nozzle of an atomizer. The concentration of infective propagules (a mixture of spores and mycelial fragments) was adjusted to 10^4 per ml using haemocytometer.

Treatment with chemical salts

Nine salts of sodium, potassium and calcium, which were reported as inducers of phytoalexin and/or elicitors of resistance in different plant species were tested to determine their ability to induce resistance in tomato against *A. solani* by foliar application (table 1).

Table 1. Sodium, potassium and calcium salts applied as foliar sprays against *A. solani* on tomato grown in a glasshouse.

S.No	Treatments	Formula	Manufacturer	Concentration
1.	Sodium dihydrogen orthophosphate	$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	BDH Laboratory	50 mM
2.	Sodium bicarbonate	NaHCO_3	BDH Laboratory	2000 ppm
3.	Potassium dihydrogen orthophosphate	KH_2PO_4	Fluka	1000 ppm
4.	Di-potassium hydrogen orthophosphate	K_2HPO_4	Sigma	1000 ppm
5.	Potassium bicarbonate	KHCO_3	Fisher Scientific	1000 ppm
6.	Potassium permanganate	KMnO_4	Fluka	20 ppm
7.	Potassium nitrate	KNO_3	Fisher Scientific	1000 ppm
8.	Calcium nitrate (hydrated)	$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	Fisher Scientific	0.5%
9.	Calcium chloride (fused)	CaCl_2	Fisher Scientific	1000 ppm

Aqueous solution of each chemical prepared with 0.01% tween 20 solution was sprayed on foliage of 30-day old tomato plants until runoff.

Plants sprayed with mancozeb (2000 ppm) served as the fungicide check whereas, plants treated with distilled water containing 0.01% tween 20 served as the control.

Challenge inoculation

Five days after spraying with chemicals, plants were challenge inoculated with a mycelial spore suspension of *A. solani* (10^4 infective propagules per ml) followed by incubation in a moist chamber for 48 hours in the dark. Two days after challenge inoculation, plants were transferred to glasshouse bench and observed for disease development.

Assessment of induced resistance

Induced resistance was determined by the incubation period, number of characteristic lesions per leaf and area of lesions. The level of resistance was determined relative to the symptoms on control plants treated with water. The disease severity of early blight was recorded using a 0-4 scale (Rajagopal and Vidyasekaran, 1982; Devanathan and Ramanujam, 1995) seven days after challenge inoculation.

- 0 = Infection free or low infection
- 1 = Trace to 25% of leaf area blighted
- 2 = 26-50% of leaf area blighted
- 3 = 51-76% of leaf area blighted
- 4 = 76-100% of leaf area blighted

Percent Disease Index (PDI) was computed by using the following formula given by Rajagopal and Vidyasekaran (1982).

$$\text{PDI} = \frac{\text{Sum of numerical ratings} \times 100}{\text{Total number of leaves assessed} \times \text{Maximum rating}}$$

Experimental Design and Statistical analysis

The experimental design was a completely randomized design (CRD) with 3 replications. Data was analyzed statistically using ANOVA and mean separation were carried out using the *minitab* statistical software. When necessary, appropriate transformations of the values were performed to normalize data prior to performing ANOVA. All data expressed as percentage were angular (arcsine) transformed before analysis.

RESULTS AND DISCUSSION

Resistance can be induced systemically in apparently susceptible plants against a variety of pathogens by various non-conventional chemicals (Bhattacharya and Roy, 1998). In the present investigation, direct toxicity of the chemicals to the pathogen *A. solani* did not seem to explain the reduction of disease intensity since most of the effective chemicals were tested at rather low concentrations. A preliminary study revealed that foliar spray of tested concentrations of each chemical showed no phytotoxic effect on tomato foliage.

The results presented in table 2 showed that incubation period (number of days taken to express characteristic symptoms) varied from 3.0 days in control to 5.67 days in fungicide check. Among the test chemical salts, the incubation period was significantly higher in Ca (NO₃)₂.4H₂O (5.00 days) followed by K₂HPO₄ (4.67 days), KMnO₄ (4.11 days) and CaCl₂ (4.11 days) over control.

Significant differences were observed among treatments with regards to lesion number per leaf and it varied from 31.92 in control to 4.58 in fungicide check. Among the test chemicals, the number of lesions varied from 13.89 in K₂HPO₄ to 33.06 in KNO₃. All the test chemicals except KNO₃ and KHCO₃ were significantly superior over control in reducing the number of lesions per leaf by inducing resistance. Lowest number of lesions was recorded in K₂HPO₄ (13.89) followed by Ca (NO₃)₂.4H₂O (18.36) and KMnO₄ (20.97).

Experimental evidences suggest that all the chemicals tested except KHCO₃ significantly reduced percent disease index over control. The percent disease index varied from 9.26 in fungicide check to 70.37 in untreated control. Among the test chemical salts, K₂HPO₄ showed highest percent disease reduction of 60.53 followed by KMnO₄ (53.95), Ca (NO₃)₂.4H₂O (52.30) and NaHCO₃ (49.67). However these values were on par with each other. Several workers have confirmed the effectiveness of these chemicals in different host pathogen systems. Gottstein and Kuc (1989) demonstrated that solutions of K₃PO₄, K₂HPO₄, Na₃PO₄ and NaH₂PO₄ sprayed on cucumber plants induced systemic resistance to anthracnose caused by *Colletotrichum lagenarium*. Even though spraying of NaH₂PO₄ on cucumber plants demonstrated effective control against cucumber anthracnose, significantly low present disease reduction (17.11) was observed in tomato plants during the present investigation. Reuveni *et al.*, (1994) had previously reported that phosphate salts induced systemic acquired resistance (SAR) in cucumber, broad bean and maize. Calcium sequestration at the site of application of phosphates is thought to generate an endogenous SAR signal (Sticher *et al.*, 1997). In cucumber and tobacco, such SAR was accompanied by enhanced

activities of chitinase, β -1, 3-glucanase, peroxidase and polyphenoloxidase (Schneider and Ullrich, 1994).

Table 2. Effect of foliar spray of sodium, potassium and calcium salts against *A. solani* on incubation period, number of lesions and percent disease index.

S. No.	Chemicals/ Concentrations	Incubation period (days)	Number of lesions per leaf	Per cent disease index	Per cent disease reduction over control
1.	NaH ₂ PO ₄ .2H ₂ O – 50 mM	3.33	25.94	58.33 (49.80)	17.11 (24.34)
2.	NaHCO ₃ – 2000 ppm	3.56	22.78	35.42 (36.50)	49.67 (44.81)
3.	KH ₂ PO ₄ – 1000 ppm	3.78	22.50	43.52 (41.27)	38.16 (38.12)
4.	K ₂ HPO ₄ – 1000 ppm	4.67	13.89	27.78 (31.73)	60.53 (51.13)
5.	KHCO ₃ – 1000 ppm	3.44	28.75	67.59 (55.35)	4.82 (9.29)
6.	KMnO ₄ – 20 ppm	4.11	20.97	32.41 (34.67)	53.95 (47.28)
7.	KNO ₃ – 1000 ppm	3.11	33.06	62.04 (52.00)	11.84 (19.41)
8.	Ca(NO ₃) ₂ .4H ₂ O – 0.5%	5.00	18.36	33.56 (35.35)	52.30 (46.33)
9.	CaCl ₂ – 1000 ppm	4.11	23.14	53.70 (47.13)	23.68 (28.91)
10.	Mancozeb - 2000 ppm	5.67	4.58	9.26 (17.68)	86.84 (68.78)
11.	Control	3.00	31.92	70.37 (57.09)	--
	SEm \pm	0.333	1.569	1.713	2.976
	CD (P \leq 0.05)	0.977	4.604	5.026	8.731

Figures in parentheses are angular transformed values

Samiento *et al.*, (1999) showed that sodium bicarbonate (1%) was effective in controlling early blight of tomato under field condition while it had no effect on cucumber net spot caused by *Leandria momordicae*. In the present study, 2000 ppm NaHCO₃ reduced early blight by 49.67 % over control.

Results showed that KNO₃ was not much effective in inducing resistance in tomato against early blight. However Blachinski *et al.*, (1996) reported that the diameter of *A. solani* lesions on leaves sampled from tomato plants pretreated with KNO₃ was significantly smaller (56.5%) than those recorded on leaves sampled from untreated plants whereas KNO₃ did not affect the disease severity of cotton caused by *Alternaria maccrospora*.

The results of this study revealed that the foliar spray of $\text{Ca}(\text{NO}_3)_2$ showed significantly higher induction of resistance with 52.30% disease reduction over control. It is in agreement with Bhattacharya and Roy (1998) who evaluated twenty one non-conventional chemicals for induction of resistance in rice against sheath blight caused by *Rhizoctonia solani* and found that 10^{-2}M $\text{Ca}(\text{NO}_3)_2$ recorded 52% reduction in lesion size.

In the present investigation, KMnO_4 was proved to be the second best chemical, which induced resistance in tomato plants. Patel and Patel (1991) suggested treating tomato fruits with KMnO_4 to induce resistant against post harvest diseases. KMnO_4 has been suggested as a potent ethylene remover from atmosphere surrounding agricultural products leading to reduce disease (Elad, 1993).

CONCLUSIONS

Foliar application of aqueous suspensions of certain sodium, potassium and calcium salts can suppress the early blight by inducing resistance in tomato against *A. solani* under glasshouse condition. K_2HPO_4 , KMnO_4 , $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and NaHCO_3 can effectively be used to manage early blight disease.

Resistance inducing non-conventional chemicals provide novel benefits over fungicide applications such as their low cost, low toxicity, nutritional value, less chances of emergence of resistance pathogens, comparative safety to the environment and suitability in integrated disease management strategies. However before these inorganic non-conventional chemicals are recommended to farmers to be used in a commercial scale, the duration of efficacy of these chemicals have to be verified under field conditions.

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