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## Management of early blight of tomato *Solanum lycopersicum* Mill. Incited by *Alternaria solani* through salicylic acid *in vitro* and *in vivo*

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### Abstract

Study of Salicylic acid under *in vitro* condition resulted that SA at 200 ppm (68.45%) concentration was most effective in inhibiting mycelial growth followed by 150 ppm (46.98%) concentration. For activation of defense mechanism of plants, salicylic acid was evaluated through interaction between root dipping and foliar spray. Salicylic acid was proved to be most effective in treatment combination (root dip of 200 ppm and foliar spray of 150 ppm) against *Alternaria solani* and recorded minimum per cent disease intensity i.e., 17.41%, 20.43% at 45 DAS and 60 DAS, respectively.

**Keywords:** Defense mechanism, Concentration, *in vitro*, Inhibiting, Intensity, Salicylic Acid

### Introduction

Tomato (*Solanum lycopersicum* L., syn.= *lycopersicon esculentum* Mill.) belongs to the family *Solanaceae* and it is the second most important vegetable crop next to potato and first among processing crops. Important diseases incited by fungi are early blight of tomato caused by *Alternaria solani*, Late blight by *Phytophthora infestans*, Buck eye fruit rot by *Phytophthora parasitica*, leaf mould by *Cladosporium fulvum*, Septoria leaf blight by *Septoria lycopersici*, (Singh, 2009) [19]. Powdery mildew by *Oidiopsis taurica*, Fusarium wilt by *Fusarium oxysporum* f. sp. *lycopersici*, Verticillium wilt by *Verticillium albo-atrum*, Leaf spot by *Alternaria alternata* (Akhtar *et al.* 2004) [3]. Collar rot by *Sclerotium rolfsii*, storage rot of tomato by *Cladosporium oxysporium*, grey mould spot or ghost spot by *Botrytis cinerea*, anthracnose by *Colletotrichum phomoides*, rots by *Sclerotinia sclerotiorum*, Damping off by *Pythium* sp. and Bacterial disease are Bacterial spot by *Xanthomonas vesicatoria*, Bacterial wilt by *Ralstonia solanacearum*, (Yabuuchi *et al.* 1995) [20], Bacterial canker by *Clavibacter michiganensis* and Viral disease caused by Leaf curl Tomato Yellow leaf Curl virus (TYLCV), (Chatchawan-kanphanich *et al.* 1993) [7] Tomato mosaic caused by Tomato Mosaic Virus (Gibbs, 1977) [18] severe losses in tomato. Early blight caused by *Alternaria solani* is one of the most important and frequent occurring disease of the crop nation and worldwide (Jones *et al.* 1991).

*A. solani* is a soil inhabiting fungus and it can also come from other host through air and splashing rain. The germinating spores of *A. solani* penetrate susceptible tissue directly or through wounds and soon produce new conidia that are further spread by wind, splashing rain, etc. (Agrios, 2005) [2]. The disease can occur over a wide range of climatic condition, but it is most prominent in areas where received heavy dew deposition, heavy rainfall, humidity and fairly high temperatures 24-29 °C are most favourable for disease development (Peralta *et al.* 2005) [14]. The pathogen *Alternaria solani* belongs to phylum: Ascomycota, class: Deuteromycetes, order: Moniliales, family: Dematiaceae (Jones and Grout, 1986) [10]. According to morphological characters and phylogenetic analysis, *Alternaria solani* bear large, long- beaked and non catenated spores (Simmons, 2000) [17]. The mycelium consisted of septa, branched, light brown hyphae which turned darker with age. The conidiopores are short, 50-90 µm long and dark in colour. Conidia are 120-296 x 12-20 µm in size, beaked, muriform, dark colour and born singly. Conidia contained 5-10 transverse septa and 1-5 longitudinal septa (Singh, 1987) [18].

**Material and Methods**

**Effect of salicylic acid on early blight of tomato**

The test fungus was grown on PDA in which the desired quantity of one chemical was incorporated to obtain five different concentrations viz., 25, 50, 100, 150 and 200 ppm. Desired quantity of chemical was mixed thoroughly in 100 ml of PDA, just before pouring in sterilized petri plates and allowed to solidify. A mycelial disc of 5 mm diameter of the pathogen taken from a 7 day old culture with the help of sterilized cork borer was then placed at the centers of the Petri plate. The inoculated Petri plates were incubated at 25 ± 1 °C temperature in BOD. Four replications were maintained for each treatment. Colony diameter was measured after 7 days of inoculation. Per cent growth inhibition was calculated as per formula (Bliss, 1934) [6].

**Table 1:** Effect of salicylic acid on early blight of tomato

S. No.	Salicylic acid (ppm)
1.	25
2.	50
3.	100
4.	150
5.	200
6.	Control

**Activation of disease resistance through salicylic acid**

These Salicylic acid activator was tested by applying as Root dip as well as foliar spray in pots at 30 day old plant with four replication. Root dip as well as foliar spray of salicylic acid was given with following concentration.

**Table 2:** Activation of disease resistance through salicylic acid

S. No.	Root dip (ppm)	Spray of salicylic acid (ppm)
		0 50 100 150
1.	0	
2.	50	
3.	100	
4.	150	
5.	200	

PDI was recorded at 45 and 60 days after transplanting after spray using (0-5) disease rating scale of McKinney (1923) as follows.

Grade	Infection
0	No spot on leaves (Healthy)
1	0-10% leaf area covered
2	11-25% leaf area covered
3	26-50% leaf area covered
4	51-75% leaf area covered
5	76-100% Leaf area covered

Per cent disease intensity was calculated as per following formula:

$$\text{Per cent Disease Intensity} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves observed} \times \text{Maximum disease grade}} \times 100$$

**Experimental layout**

The experiment was laid out in Factorial Completely Randomized Design (FCRD) with twenty treatments and three replications (Fig 1.).

**Experimental details**

Number of treatments: Twenty

Number of replications: Three

**Treatment Details**

D = Root dipping

F= Foliar spray

D<sub>0</sub>= No root dipping

F<sub>0</sub>= No foliar spray

D<sub>1</sub>=Root dipping at 50 ppm

F<sub>1</sub>= Foliar spray at 50 ppm

D<sub>2</sub> = Root dipping at 100 ppm

F<sub>2</sub> = Foliar spray at 100 ppm

D<sub>3</sub> = Root dipping at 150 ppm

F<sub>3</sub> = Foliar spray at 150 ppm

D<sub>4</sub> = Root dipping at 200 ppm

R <sub>1</sub>				R <sub>2</sub>				R <sub>3</sub>			
D <sub>0</sub> F <sub>0</sub>	D <sub>0</sub> F <sub>1</sub>	D <sub>0</sub> F <sub>2</sub>	D <sub>0</sub> F <sub>3</sub>	D <sub>0</sub> F <sub>0</sub>	D <sub>0</sub> F <sub>1</sub>	D <sub>0</sub> F <sub>2</sub>	D <sub>0</sub> F <sub>3</sub>	D <sub>0</sub> F <sub>0</sub>	D <sub>0</sub> F <sub>1</sub>	D <sub>0</sub> F <sub>2</sub>	D <sub>0</sub> F <sub>3</sub>
D <sub>1</sub> F <sub>0</sub>	D <sub>1</sub> F <sub>1</sub>	D <sub>1</sub> F <sub>2</sub>	D <sub>1</sub> F <sub>3</sub>	D <sub>1</sub> F <sub>0</sub>	D <sub>1</sub> F <sub>1</sub>	D <sub>1</sub> F <sub>2</sub>	D <sub>1</sub> F <sub>3</sub>	D <sub>1</sub> F <sub>0</sub>	D <sub>1</sub> F <sub>1</sub>	D <sub>1</sub> F <sub>2</sub>	D <sub>1</sub> F <sub>3</sub>
D <sub>2</sub> F <sub>0</sub>	D <sub>2</sub> F <sub>1</sub>	D <sub>2</sub> F <sub>2</sub>	D <sub>2</sub> F <sub>3</sub>	D <sub>2</sub> F <sub>0</sub>	D <sub>2</sub> F <sub>1</sub>	D <sub>2</sub> F <sub>2</sub>	D <sub>2</sub> F <sub>3</sub>	D <sub>2</sub> F <sub>0</sub>	D <sub>2</sub> F <sub>1</sub>	D <sub>2</sub> F <sub>2</sub>	D <sub>2</sub> F <sub>3</sub>
D <sub>3</sub> F <sub>0</sub>	D <sub>3</sub> F <sub>1</sub>	D <sub>3</sub> F <sub>2</sub>	D <sub>3</sub> F <sub>3</sub>	D <sub>3</sub> F <sub>0</sub>	D <sub>3</sub> F <sub>1</sub>	D <sub>3</sub> F <sub>2</sub>	D <sub>3</sub> F <sub>3</sub>	D <sub>3</sub> F <sub>0</sub>	D <sub>3</sub> F <sub>1</sub>	D <sub>3</sub> F <sub>2</sub>	D <sub>3</sub> F <sub>3</sub>
D <sub>4</sub> F <sub>0</sub>	D <sub>4</sub> F <sub>1</sub>	D <sub>4</sub> F <sub>2</sub>	D <sub>4</sub> F <sub>3</sub>	D <sub>4</sub> F <sub>0</sub>	D <sub>4</sub> F <sub>1</sub>	D <sub>4</sub> F <sub>2</sub>	D <sub>4</sub> F <sub>3</sub>	D <sub>4</sub> F <sub>0</sub>	D <sub>4</sub> F <sub>1</sub>	D <sub>4</sub> F <sub>2</sub>	D <sub>4</sub> F <sub>3</sub>

**Fig 1:** Layout of pot experiment on activation of disease resistance in tomato through Salicylic acid

**Results and Discussion**

**Effect of salicylic acid on mycelial growth and sporulation of pathogen (in vitro)**

The efficacy of salicylic acid (SA) (Table 3. Fig. 2. and Plate 1) was tested *in vitro* at five levels of concentrations viz., 25 ppm, 50 ppm, 100 ppm, 150 ppm and 200 ppm concentrations against mycelial growth and sporulation of *Alternaria solani* on Potato Dextrose Agar (PDA) by poisoned food technique.

Among five concentrations viz., 25 ppm, 50 ppm, 100 ppm, 150 ppm and 200 ppm salicylic acid was found most effective in inhibiting mycelial growth (68.45%) of *Alternaria solani* at 200 ppm followed by 150 ppm (46.98%) over control (90.00 mm). Salicylic acid (SA) at 25 ppm (5.31%) was found least effective in inhibiting mycelial growth of *Alternaria solani* over control (90.00 mm).

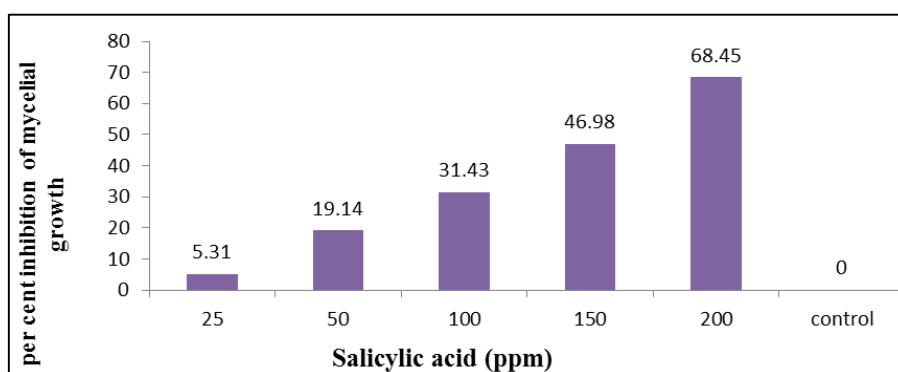
As we consider the results regarding with sporulation of pathogen Salicylic acid (SA) at 200 ppm which was most effective in inhibiting mycelial growth of the pathogen exhibited very poor sporulation (+) followed by Salicylic acid (SA) at 150 ppm poor sporulation under *in vitro* condition which showed poor sporulation (++). Salicylic acid (SA) at 25 ppm that was least effective in inhibiting mycelial growth of the pathogen exhibited better sporulation (+++++) as compared to control in which excellent sporulation (++++++) was occurred.

**Table 3:** Effect of Salicylic acid on mycelial growth inhibition and sporulation of pathogen (*in vitro*)

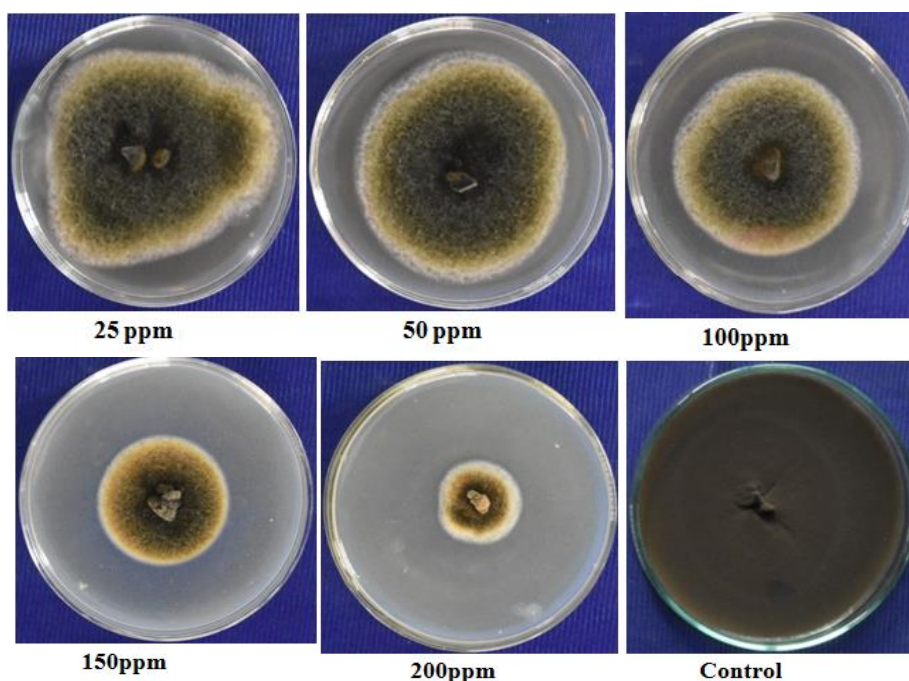
S. No.	Salicylic acid (ppm)	Percent inhibition of mycelial growth* ( <i>in vitro</i> )	Sporulation
1	25	5.31 (13.32)	+++++
2	50	19.14 (25.94)	++++
3	100	31.43 (34.10)	+++
4	150	46.98 (43.27)	++
5	200	68.45 (55.83)	+
6	Control	0.00 (0.00)	+++++++
	S.Em+	0.99	
	CD (P=5%)	3.05	

\*Average of four replications

Sporulation	Category
+	Very poor
++	Poor
+++	Moderate
++++	Good
+++++	Better
+++++++	Excellent



**Fig 2:** Effect of salicylic acid on mycelial growth of *Alternaria solani*



**Plate 1:** Efficacy of Salicylic acid at different concentrations against *Alternaria solani*

**Activation of disease resistance through salicylic acid (*in vivo*)**

**(A). Activation of disease resistance through salicylic acid at 45 DAS (*in vivo*)**

The effect of salicylic acid (SA) in activation of disease

resistance against early blight of tomato caused by (*Alternaria solani*) was studied by two different aspects viz., root dipping (0, 50,100,150,200 ppm) and foliar spray (0, 50, 100 150 ppm) under pot conditions and results were interpreted by interacting between root dip (D) and foliar spray (F). The

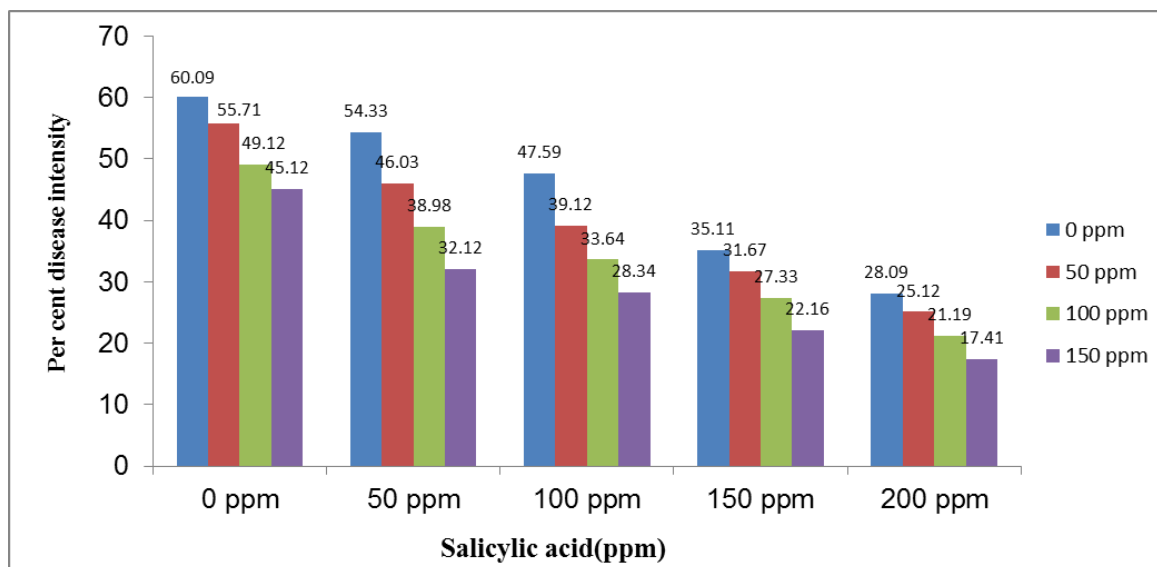
effect of interaction between root dip (D) and foliar spray (F) of salicylic acid (SA) on per cent disease intensity (PDI) at 45 days after sowing (DAS) was found to be significant. The data presented in Table 4. and Fig. 3. revealed that the minimum per cent disease intensity(17.41%) was recorded under the treatment combination root dip 200 ppm and foliar spray at 150 ppm (D4F3), which was significantly superior over rest of the treatment combinations except D3F3 (root dip at 150 ppm and foliar spray at 150 ppm) (22.16%),

D4F1(root dip at 200 ppm and foliar spray at 50 ppm) (25.12%) and D4F2 (root dip at 200 ppm and foliar spray at 100 ppm) (21.19%) which were found statistically at par to it. Salicylic acid was tested at five different concentrations viz., 25 ppm, 50 ppm, 100 ppm, 150 ppm and 200 ppm by poisoned food technique to inhibit the mycelial growth of *A. solani* under *in vitro* conditions. Salicylic acid gave maximum inhibition of mycelial growth at 200 ppm concentration followed by 150 ppm concentration.

**Table 4:** Activation of disease resistance through Salicylic acid at 45 DAS (*in vivo*)

S. No.	Root dip SA (ppm)	Foliar spray of SA ppm (45 Days) <i>in vivo</i> (per cent disease intensity*)				Mean
		0 ppm	50 ppm	100 ppm	150 ppm	
1	0 ppm	60.09 (51.30)	55.71 (48.28)	49.12 (44.50)	45.12 (42.20)	52.51 (46.43)
2	50 ppm	54.33 (47.47)	46.03 (42.72)	38.98 (38.63)	32.12 (34.52)	42.86 (40.86)
3	100 ppm	47.59 (43.51)	39.12 (38.720)	33.64 (35.45)	28.34 (32.16)	37.17 (37.52)
4	150 ppm	35.11 (36.33)	31.67 (34.25)	27.33 (31.52)	22.16 (28.04)	29.06 (32.96)
5	200 ppm	28.09 (32.52)	25.12 (30.08)	21.19 (27.41)	17.41 (24.66)	22.95 (28.59)
		45.04 (42.36)	39.53 (38.94)	34.05 (35.97)	29.03 (32.77)	
	Root dip		Foliar Spray		Root dip x Foliar spray	
S.Em±	0.46		0.41		0.93	
CD (p=5%)	1.29		1.15		2.58	

\*Average of three replications



**Fig 3:** Activation of disease resistance through salicylic acid (45 days)

**(B). Activation of disease resistance through salicylic acid at 60 DAS (*in vivo*)**

The effect of salicylic acid (SA) in activation of disease resistance against early blight of tomato caused by (*Alternaria solani*) was studied by two different aspects viz., root dipping (0, 50,100,150,200 ppm) and foliar spray (0, 50, 100 150 ppm) under pot conditions and results were interpreted by interacting between root dip (D) and foliar spray (F).The effect of interaction between root dip (D) and foliar spray (F) of salicylic acid on per cent disease intensity (PDI) at 60 days after sowing (DAS)was found to be significant. The data presented inTable 5. and Fig. 4. revealed that the minimum per cent disease intensity(20.43%) was recorded under the

treatment combination root dip 200 ppm and foliar spray at 150 ppm(D4F3), which was significantly superior over rest of the treatment combinations exceptD3F3(root dip at 150 ppm and foliar spray at 150 ppm) (26.20%), D4F1 (root dip at 200 ppm and foliar spray at 50 ppm) (28.03%) and D4F2(root dip at 200 ppm and foliar spray at 100 ppm) (23.22%) which were found statistically at par to it.

Under *in vivo* condition SA was tested by interaction between two different aspects viz., root dip (0ppm, 50ppm, 100ppm, 150ppm and 200 ppm) and foliar spray(0ppm,50ppm,100 ppm and 150 ppm) and resulted that treatment combination root dip at 200 ppm and foliar spray at 150 ppm showed minimum per cent disease intensity. These observations are in

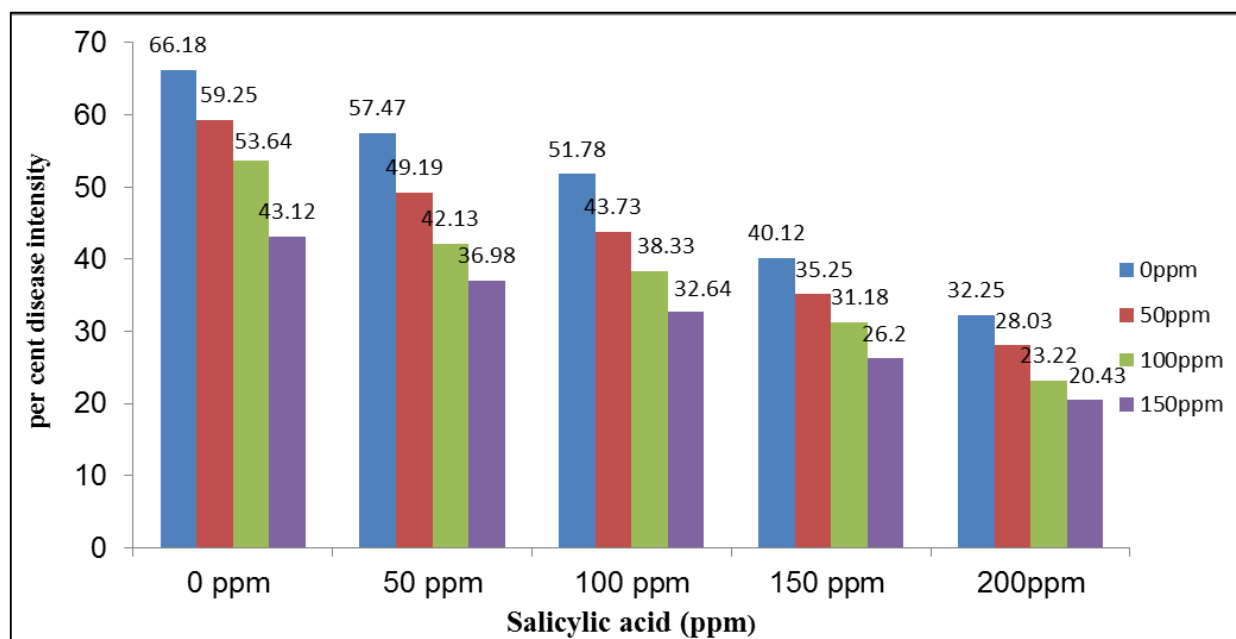
line with those recorded by Atik *et al.* (2013) [4], Abdalla (2014) [1], Raskin (1992) [15], Segarra *et al.* (2006) [16], Park *et al.* (2007) [13], Bari and Jones (2009) [5] and Mady (2009) [11]. Atik *et al.* (2013) [4] noted positive response of three systemic acquired resistance inducers (salicylic acid, Bion and beta amino butyric acid) against *Alternaria alternata*, causing

*Alternaria* leaf spot of tomato. Abdalla (2014) [1] noted enhanced systemic acquired resistance by salicylic acid and 2,6-dichloroisonicotinic acid and also proved that concentrations less than 500 ppm was able to induce the systemic acquired resistance in tomato and cucumber plants.

**Table 5:** Activation of disease resistance through Salicylic acid at 60 DAS (*in vivo*)

S. No.	Root dip SA(ppm)	Foliar spray of SA ppm (60 Days) <i>in vivo</i> (per cent disease intensity*)				Mean
		0 ppm	50 ppm	100 ppm	150 ppm	
1	0 ppm	66.18 (54.44)	59.25 (50.33)	53.64 (47.09)	43.12 (41.05)	55.54 (48.16)
2	50 ppm	57.47 (49.30)	49.19 (44.54)	42.13 (40.47)	36.98 (37.45)	46.44 (42.94)
3	100 ppm	51.78 (46.02)	43.73 (41.40)	38.33 (38.25)	32.64 (34.84)	41.62 (40.16)
4	150 ppm	40.12 (39.30)	35.25 (36.42)	31.18 (33.94)	26.20 (30.79)	33.18 (34.51)
5	200 ppm	32.25 (34.60)	28.03 (31.97)	23.22 (28.81)	20.43 (26.87)	25.98 (30.59)
		49.56 (44.71)	41.09 (40.34)	37.7 (37.88)	31.87 (34.33)	
	Root dip	Foliar Spray				Root dip x Foliar spray
S.Em±	0.39	0.35				0.78
CD(p=5%)	1.09	0.97				2.18

\*Average of three replications



**Fig 4:** Activation of disease resistance through salicylic acid (60 days)

## Conclusion

Under *in vitro* condition Salicylic acid was found most effective in per cent inhibition of mycelial growth at 200 ppm followed by 150 ppm. For activation of defense mechanism of tomato plants, salicylic acid (root dipping of 200 ppm and foliar spray of 150 ppm) was proved to be most effective in reducing disease intensity.

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