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Efficacy of fungicides, botanicals and vitamins against early blight disease of Tomato

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Abstract

Early blight of tomato caused by *Alternaria solani* is one of the major production constraints to tomato production, causing significant yield reduction and resulting in a severe economic impact. The objective of the current study was to assess the efficacy of different fungicides, plant extracts, and vitamins in managing the disease under both *in-vitro* and in field conditions. Different doses of fungicides (0.05%, 0.1%, 0.25%) and plant extracts (5%, 10%, 15%) were used for i*n-vitro* evaluation employing the poisoned food technique. The results revealed that, among all the fungicides, (Antracol, Nativo 75%-WG, Topsin-M, Score, Ridomil gold, Champion) score (Difenoconazol) at 0.25% concentration and among all the botanicals (Ginger, Garlic, Kalonji, Ajwain) ginger at 15% concentration indicated maximum percentage inhibition of 62.85% and 48.48% respectively. However, thiamine among all the vitamins (Thiamine, Niacin, Pyridoxin) at 150mg/L concentration showed minimum (26%) disease incidence when evaluated under greenhouse conditions. While under field conditions score exhibited minimum disease incidence 15.19% among all the treatments. Overall findings revealed that weekly sprays of score at 0.25% concentration were found superlative for the management of early blight disease of tomato.

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Introduction

Tomato is the second most crucial solanaceous vegetable crop after potato. It is innate to South America and extensively cultivated in one hundred and forty countries of the globe with an annual consumption of 150 million tons (FAO, 2009). This crop ranks next to the potato and ranks foremost among the processing crops in the world acreage. It is a good source of antioxidants and commonly consumed in daily life (Capanoglu *et al.,* 2010). Besides that, it carries a balanced source of fundamentally imperative Vitamin A, C and E which are desirable to sustain good human strength (Olaniyi *et al.,* 2010).

In Pakistan, the average production of tomato is 11.78tons/ha which is significantly diminutive as compared to the other tomato producing countries such as USA (88.49tons/ha), China (61.78tons/ha), Egypt (45.27tons/ha), Turkey (37.53tons/ha) and India (22.93tons/ha) (FAO, 2009). Moreover, tomato crop is prone to various fungal, bacterial, viral and nematode diseases. Among all of them, the early blight of tomato, caused by *A. solani*, is one of the worst destructive and causes substantial drop in quantity and quality of tomato crop (Pawar *et al.,* 2016). Early blight of tomato is a prime disease of tropical and sub-tropical areas.

Alternaria solani is an air-borne, soil inhabiting pathogen that is liable for early blight, fruit and collar rot of tomato which is spread by fungal spores (Chaerani and Voorrips, 2006). The pathogen (*Alternaria solani)* causes infection on leaves, petioles, stems, twigs and fruits and in addition with lead to the defoliation, drying of twigs and early fruit drop that finally diminish the produce up to 78% (Pandey *et al.,* 2003). Characteristic bulls-eye form of the leaf spots with concentric rings of spores which are surrounded by a halo of chlorotic leaf area is the typical symptoms of this disease. When few spots are present, the leaves turn into yellow color and dry up (Vloutoglou and Kalogerakis, 2000). During fruiting period, when there are idealistic conditions of high humidity and temperature were prevailed (high rainfall, crowded plantation and extended time period of leaf moisture from dew) then plants are more vulnerable to the blight infection (Chaerani and Voorrips, 2006).

Primary approaches of controlling early blight comprise evading long periods of moisture on the leaf surface, sanitation, cultural scouting, use of plant extracts, vitamins and improvement of the host plant resistance along with the application of fungicides (Kumar *et al.,* 2008; Sahu *et al.,* 2013). Whereas, the definitive control of this disease is to be growing of resistant cultivars. However, the farmers in pursuance of attaining more yield are inclined to cultivate some high yielding but less resistant varieties.

The application of fungicides and botanicals can enhance the genetic potential and maximize the crop yield by diminishing the disease. The spore germination and penetration of pathogen were inhibited by the usage of preventive fungicides and botanicals. However, the consistent use of these chemicals made the pathogen able to get resistant against these fungicides and botanicals. So, the frequent application of fungicides at suitable dose and time interval is compulsory (Bartlett *et al.,* 2002; Nashwa and Abo-Elyousr, 2012). Vitamins are considered as bio-regulators, which in little application cause a significant influence on plant growth. In tomato plants, vitamin like thiamine proved to be effective in minimizing the early blight disease incidence.

To keep in view the former facts there is a dire exigency to manage the disease by implementing integrated approaches which diminish the yield reduction by controlling the pathogen efficiently. So, the current study was designed to ascertain the effectiveness of different treatments of fungicides (0.05%, 0.1%, 0.25%), plant extracts (5%, 10%, 15%) and vitamins (50mg/L, 100mg/L and 150mg/L) against early blight of tomato.

Materials and methods

In-vitro evaluation of fungicides, plant extracts and vitamins

Poisoned food technique was used to assess the efficacy of 6 fungicides (Antracol, Nativo 75%-WG, Topsin-M, Score, Ridomil gold, Champion) and four plant extracts Ginger (*Zingiber officinale*), Garlic (*Allium sativum*), Ajowan (*Carum copticum*), Kalonji (*Nigella sativa*) at different concentrations.

All the fungicides and botanicals were evaluated in the laboratory against *Alternaria solani* at different concentration: 0.05%, 0.1%, 0.25% and 5%, 10%, 15% respectively with 3 replications to each. Melted sterilized potato dextrose agar (PDA) was used as a nutrient medium, and prerequisite amount of each fungicide and botanical was poured separately. The fungicides and plant extracts were rigorously assorted by stirring, and nearby 15 milliliter poisoned medium was transferred to each of the ninty millimeter petri plates and permissible for solidification. The actively rising periphery of the culture of *Alternaria solani* was prudently cut via sterilized cork borer and shifted aseptically to the middle of each petri plate comprising the poisoned solid medium. In addition, the control was kept by growing the cultures on potato dextrose agar without fungicides and plant extracts. Besides, the petri plates were incubated at 25±1°C and the mycelial outward growth was recorded 3, 5 and 7 days after incubation. The percentage of mycelial radial growth inhibition (I) was recorded by using the following formula:

Inhibition of the mycelial radial growth (%)

$$= \frac{C - T}{C} \times 100$$

Where, C and T donated the average colony diameter of fungus (mm) in control and in antifungal treated potato dextrose agar medium, respectively.

While different vitamins such as Thiamine (B1), Niacin (B3) and Pyridoxin (B6) were evaluated in green house by using completely randomized design (CRD). The concentrations of these vitamins are 50mg/L, 100mg/L and 150mg/L with three replications each. Data of disease incidence was noted on weekly basis after the progress of disease.

Assessment of fungicides, plant extracts and vitamins into the field

The most effectual concentration of fungicides, plant extracts, and vitamins, evaluated in *in-vitro* experiments, were applied in the field using Randomized Complete Block Design (RCBD) in the research area of Plant Pathology, University of Agriculture, Faisalabad. Healthy tomato seedlings of Rio Grande and Long keeper were raised up in nursery beds and after three weeks the seedlings were shifted into the field with sixty centimeter inter and forty centimeter intra row spacing in plots computing 2 x 2 m. All the supplementary cultural and pest control methods were implemented to maintain the plants in good conditions.

As the pathogen of this disease were responsible to cause the damage at all stages of plant development, the vitamin at 150mg/L were evaluated as two consecutive sprays at an interval of 10 days and the data of disease incidence were recorded as the first observance of early blight in the field. While fungicide (Score) and plant extract (Ginger) at 0.25% and 15% concentrations were assessed as 3 successive sprays at an interval of fifteen days respectively. The tomato plants were sprayed instantly as the first manifestation of early blight was observed into the field (Farouk *et al.,* 2012). Data of disease incidence was noted on weekly basis after the expansion of disease by using this formula.

$$\text{Disease Incidence (\%)} = \frac{\text{No.of diseased plants}}{\text{Total no.of plants}} \times 100$$

Statistical analysis

The data was examined by using the Statistical Analysis System (SAS 9.3) (Sas, 2011). The data were subjected to analysis of variance (ANOVA) at 5% level of significance. Fisher's Least Significant Difference (LSD) test was used for statistical comparison among treatments.

Results and discussion

In- vitro assessment of fungicides, plant extracts and vitamins

The efficacy of different fungicides against *A. solani* was estimated *in-vitro* by poisoned food technique and the results revealed that difenoconazol was found expressively most efficacious and indicated maximum percentage inhibition that was 51.4, 60 and 62.8mm at 0.05%, 0.10% and 0.25% respectively (Table 1 and Fig. 2). While, Nativo was the second most effective fungicide followed by Ridomil Gold, Topsin-M, Antracol and Champion. The overall results exhibited that all the concentrations of fungicides inhibited the growth of *Alternaria solani* but Difenoconazol at 0.25% gave the best control over mycelial development of *Alternaria solani* after seven days of incubation at 24°C (Fig. 1).

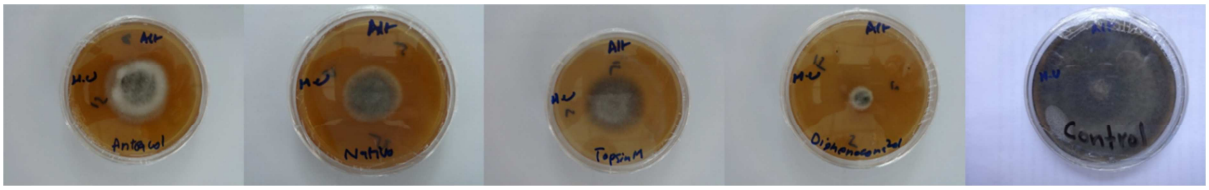

Fig. 1. Growth of *Alternaria solani* after 7 days.

In-vitro assessment of fungicides is a handy tool to access the maximum number of fungicides. Issiakhem and Bouznad (2010) exhibited the efficiency of score on the mycelial development of *Alternaria solani* and described that difenoconazol had a durable inhibition effect on *Alternaria solani* (89.00%) with a treatment of 0, 97 ppm and conidial germination was also sturdily reduced and reached up to 92% at 1.95 ppm.

Table 1. *In-vitro* evaluation of fungicides at different concentrations against *Alternaria solani* (mm).

Fungicides	Concentration (%)	After 3 days	After 5 days	After 7 days
Antracol	0.05	59.21^{M}	50.00^{R}	28.57^{Y}
	0.10	64.47^{HI}	55.55^{O}	37.14^{V}
	0.25	68.42^{EF}	61.11^{KL}	45.71^{T}
Nativo 75%-WG	0.05	64.47^{HI}	57.40^{N}	45.71^{T}
	0.10	69.73^{DE}	61.11^{KL}	51.42^{Q}
	0.25	73.68^{B}	68.51^{E}	60^{LM}
Topsin-M	0.05	61.84^{JK}	53.70^{P}	34.28^{W}
	0.10	65.78^{GH}	57.40^{N}	42.85^{U}
	0.25	71.05^{CD}	64.81^{H}	51.42^{Q}
Difenoconazol	0.05	67.10^{FG}	62.96^{J}	51.42^{Q}
	0.10	72.36^{BC}	68.51^{E}	60^{LM}
	0.25	76.31^{A}	70.370^{D}	62.85^{J}
Ridomil gold	0.05	63.15^{IJ}	55.55^{O}	42.85^{U}
	0.10	68.42^{EF}	59.25^{M}	48.57^{S}
	0.25	72.36^{BC}	66.66^{G}	57.14^{N}
Champion	0.05	55.26^{O}	44.44^{T}	20^{Z}
	0.10	56.57^{NO}	48.14^{S}	31.42^{X}
	0.25	65.78^{GH}	57.40^{N}	42.85^{U}
Control	0.05	0.000^{ZA}	0.000^{ZA}	0.000^{ZA}
	0.10	0.000^{ZA}	0.000^{ZA}	0.000^{ZA}
	0.25	0.000^{ZA}	0.000^{ZA}	0.000^{ZA}

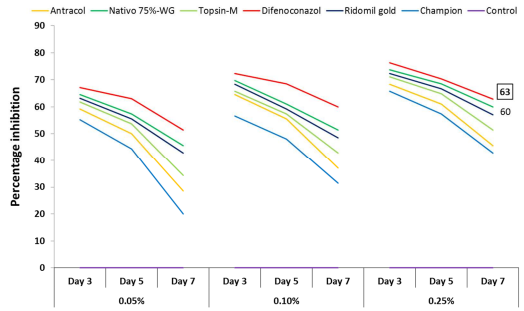

Fig. 2. *In-vitro* evaluation of fungicides at different concentrations against *Alternaria solani*.

Arunakumara (2006) described that, among contact fungicides, copper oxychloride and mancozeb were highly potent in inhibiting the progress of *Alternaria solani* when evaluated under laboratory studies using poison food technique. Among the systemic fungicides, metalaxyl MZ and propiconazole at all verified treatments were found to be immensely successful in inhibiting the development of *Alternaria solani*.

In case of plant extracts, ginger was found to be significantly most effective gave maximum percentage inhibition of 2.72, 3.7, 4.84 cm at 5%, 10%, and 15% respectively. While garlic was the second most effective extract followed by ajowan and kalonji at 5%, 10%, and 15% concentrations. The results revealed that all the concentrations of botanicals compressed the growth of *Alternaria solani*, however, the ginger application at 15% concentration revealed determined control over the mycelial development of *Alternaria solani* after seven days of incubation at 24°C (Table 2).

Table 2. Means of percent inhibition of *Alternaria solani* exhibited by various plant extracts at different concentrations under *in-vitro* studies.

Plant Extracts	Concentrations (%)	After 3 days	After 5 days	After 7 days
Ginger	5	37.0^{G}	31.0^{H}	27.00^{JK}
	10	48.1^{C}	41.3^{E}	37.00^{F}
	15	59.2^{A}	51.7^{B}	48.4^{C}
Ajowan	5	22.2^{M}	15^{P}	13.00^{P}
	10	29.6^{I}	20.6^{N}	17^{MN}
	15	37.0^{G}	31.0^{H}	27^{HI}
Garlic	5	25.9^{K}	24.00^{N}	20.00^{L}
	10	37.0^{G}	31.0^{H}	27.00^{HI}
	15	48.1^{C}	44.8^{D}	41.40^{E}
Kalonji	5	11.1^{Q}	9.00^{S}	6.00^{R}
	10	22.2^{M}	17.2^{O}	15.5^{P}
	15	29.6^{I}	27.5^{J}	21^{MN}
Control	5	0.00^{T}	0.00^{T}	0.00^{T}
	10	0.00^{T}	0.00^{T}	0.00^{T}
	15	0.00^{T}	0.00^{T}	0.00^{T}

It is noted that the dried root extracts of Sweet Flag at 5-10% concentration were most effective under *in-vitro* conditions and inhibit the mycelial growth and spore germination of *A. solani*. Extracts from garlic (5% bulb) and Mesquite (10% leaf) gave moderate levels of inhibition (Vadivel and Ebenezar, 2006). The plant extracts from twenty different non-host plant species minimize the incidence of early blight disease and inhibit the mycelial growth of *Alternaria solani* (Curtis *et al.,* 2004; Krebs *et al.,* 2006; Latha *et al.,* 2009). *In-vitro* studies revealed that extract of Zimmu plant, a hybrid of *Allium sepa* and *Allium sativum*, have the greater inhibitory effect to reduce the fungal hyphal growth of *A. solani* (Nashwa and Abo-Elyousr, 2012).

All vitamins (Thiamine, Pyridoxine, Niacin) inhibited the fungus growth and thiamine, at all concentrations (50mg/L, 100mg/L, 150mg/L), demonstrated minimum disease incidence and revealed most promising results that was 41%, 36% and 26% respectively followed by pyridoxine. Pyridoxine remained second most effective vitamin followed by niacin at 50mg/L, 100mg/L, and 150mg/L concentrations respectively (Table 3).

Table 3. *In-vitro* evaluation of vitamins at different concentrations against *Alternaria solani*.

Treatments	Disease incidence (%) at different concentrations		
	50mg/L	100mg/L	150mg/L
Thiamine	41^{E}	36^{G}	26^{I}
Pyridoxin	49^{C}	42^{E}	33.25^{H}
Niacin	55^{B}	47^{D}	39^{F}
Control	61.25^{A}	61.25^{A}	61.25^{A}

Maximum disease incidence was observed at 50mg/L concentration of all the treatments while minimum disease incidence was seen at 150mg/L concentration of thiamine and pyridoxine and that was 26.00 and 33.25 percent respectively. All the concentrations inhibited the growth of *Alternaria solani* although 150mg/L of thiamine gave minimum disease incidence over control (Fig. 3).

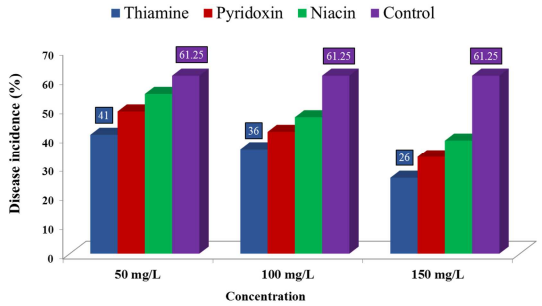

Fig. 3. *In-vitro* evaluation of vitamins at different concentrations against *Alternaria solani*.

In-vivo evaluation of fungicides, plant extracts and vitamins

Fungicide and plant extract were applied after the first symptom of disease appearance and the most effective concentration of fungicide and plant extract which was evaluated *in-vitro* experiments were applied in the field by using randomized complete block design (RCBD) and the results revealed that score gave minimum disease incidence (15%) among all the treatments (Fig. 4).

Fungal mycelial development can be inhibited by the application of score (0.2%) as it was confirmed by (Singh *et al.,* 2006; Chourasiya *et al.,* 2013). Serialized sprays of score (0.2%) and equivalent concentration of captafol has been found to be very auspicious in minimizing the infestation of *Alternaria solani* in tomato field (Babu *et al.,* 2001). Difenoconazol not only found friendly in managing the early blight incidence in field conditions but also gave remarkable findings in limiting the fungal colonial development during laboratory analyses conducted by (Prasad and Naik, 2003). Use of difenoconazol to control early blight in the field has been formerly recommended by different scientists (Ramakrishnan *et al.,* 1970; Stevenson, 1977). Recently, Gondal *et al.* (2012) published results suggested that score and mancozeb is still very active in the management of early blight disease of tomato plants.

Prasad and Naik (2003) assessed different plant extracts (onion bulb, garlic, Neem, Ocimum leaf extracts and Prosopis) in controlling the *Alternaria solani* pathogen by using the poisoned food technique. Prosopis leaf extracts and garlic bulb indicated the

most effective botanicals and inhibit the mycelial growth of *A. solani* at substantial level. It was observed that under natural and artificial inoculation situations the eucalyptus spp. leaf extract at 10 percent concentration efficaciously inhibited the mycelial growth of *A. solani*. Also, neem oils and garlic diminish the early blight disease progress on tomato when compared to the control (Wszelaki and Miller, 2005).

Moreover, ginger extracts reduced the early blight incidence and enhanced the harvestable yield of tomato fruits (*Patil et al.,* 2001). Aqueous extract of ginger helps in the suppression of mycelial growth of *A. solani* (Sharma *et al.,* 2007; Hassanein *et al.,* 2010). Ginger products such as ginger 60 EC and ginger oil limit the *A. solani* mycelial growth (Vadivel and Ebenezar, 2006). The degree of suppression enhanced with increasing in the concentration of ginger extract up to 20%. Curative application of ginger extracts suggests that it reduces the infection by 13-20%. Combined ginger spray along with application through irrigation reduces the disease severity up to 44% (Hassanein *et al.,* 2010).

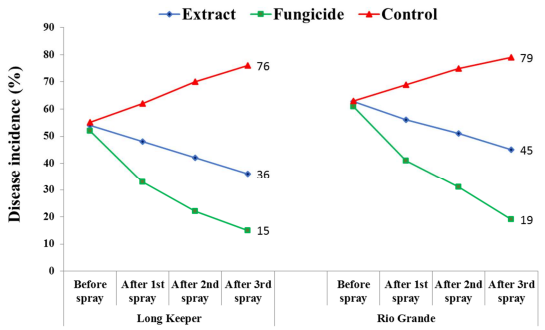

Fig. 4. Means of disease incidence revealed by fungicide and plant extract under in field conditions.

In case of vitamins, the results revealed that, the application of thiamine at 150mg/L concentration on long keeper variety, indicated minimum disease incidence after the third spray and showed most significant results that were 39% as compared to the control in which the disease incidence after the third spray was 68%. Hence, it inhibits the disease about 29%. However, in case of Riogrande variety, the disease incidence was 46% after the third spray as compared to the control in which the disease incidence, after the third spray, was 78% (Fig. 5). Thus, in case of Riogrande variety, thiamine at 150mg/L concentration inhibited the disease about 32% (Table 4).

Table 4. Effect of vitamin on disease incidence (%).

Treatment	Long Keeper				Rio Grande			
	Weeks after spray							
	Before spray	1st	2nd	3rd	Before spray	1st	2nd	3rd
Vitamin	0^{M}	17^{L}	29^{J}	39^{G}	0^{M}	20^{K}	33^{I}	46^{E}
Control	0^{M}	35^{H}	50^{D}	68^{B}	0^{M}	42^{F}	55^{C}	78^{A}

Vitamins are considered as bio-regulators, which in little application cause a significant influence on plant growth. In tomato plants, vitamin like thiamine proved to be effective in reducing early blight disease incidence. Foliar application of thiamine expressively increased tomato growth parameters including leaves per plant, leaf area per plant, no. of branches, shoot length, shoot fresh and dry weight. Besides that, some physiological aspects (N, P, K, total carbohydrate content as well as chlorophyll) in the shoot and also increased the tomato yield (Farouk *et al.,* 2012).

The results are agreed with -Hakimi and Alghalibi (2007) who reported that application of thiamine increased the membrane stability and growth rate. Application of Thiamine also counteracts entirely or partially the unfavorable effect of fungal infection (*Rhizoctonia solani* and *Fusarium solani*).

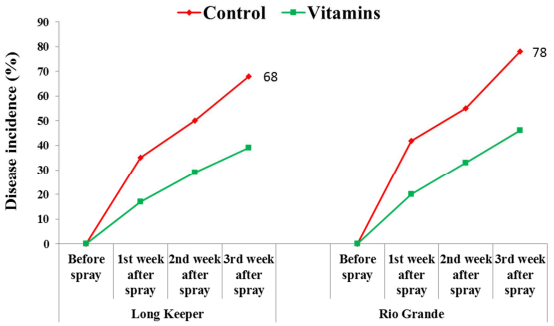

Fig. 5. Means of disease incidence revealed by vitamin under *in-vivo* conditions.

Nahed *et al.* (2010) reported that foliar application of Thiamine on *Thuja orientalis* plants promoted all morphological characters (root length, stem diameter, the dry and fresh weight of shoot and root, stem length), and increased N, P, K percentage, protein

and total soluble sugar. The effect of certain different vitamins like thiamine, ascorbic acid, biotin, nicotinic acid, inositol, riboflavin pyridoxine, was also studied against *Alternaria arachidis* which is the causal organism of *Alternaria* blight. The concentration of these vitamins used was 0.25%. It was noted that biotin, ascorbic acid, nicotinic acid, riboflavin, and pyridoxine were stimulatory while thiamine and inositol were inhibitory to the pathogen as compared to the control (Kamthane and Kareppa, 2011).

Conclusion

Our experiments concluded that the maximum percentage inhibition (62.85% and 48.48%) of *Alternaria solani* was attained by using the score and ginger at higher doses when applied under laboratory conditions while thiamine among all the vitamins (Thiamine, Niacin, Pyridoxin) at 150mg/L concentration showed minimum (26%) disease incidence when evaluated under greenhouse conditions. Under field situations score exhibited minimum disease incidence 15.19% among all the treatments. Overall findings discovered that weekly sprays of score at 0.25% concentration were found splendid for the management of early blight disease of tomato.

Authors' Contributions

HMUA and MMR wrote the article and corrected it. HMUA, AI, OY and Qurban Ali (QA) conducted the experimental work. HMUA, SA, MA and LA designed the study.

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