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Management of *Alternaria solani* in Tomato Using *Withania coagulans*- an Alternative to Chemical Control

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ABSTRACT

To reduce pesticide resistance in plant pathogens, alternate approaches are needed to be explored. In this study, dried powder of *Withania coagulans* was evaluated for the management of *Alternaria solani* causing early blight disease in tomato both *in vitro* and *in planta*. Different dried powder doses (5, 10, and 15% w/v) of different parts (succulent shoot, stem and leaves) of *W. coagulans* were tested against *in vitro* growth of *Alternaria solani* through food poisoned technique. Also, different dried powder doses (15 and 30 g kg^{-1} soil) of different parts (succulent shoot, stem and leaves) of *W. coagulans* were applied to soil at different application times [5, 10, and 15 Days Before Transplantation (DBT)] and were evaluated for the control of early blight in tomato plants under screen house conditions. Results from *in vitro* studies showed that the highest dose 15% (w/v) and succulent shoot plant part showed a maximum antifungal activity. Results from screen house experiment showed that the highest dose of 30 g kg^{-1} soil and longest application time of 15 DBT of succulent shoot dried powder suppressed the disease severity effectively in tomato plants (artificially inoculated with *Alternaria solani*) resulted in significantly higher root length, shoot length, and fresh biomass. Results of this study suggested that succulent shoot dried powder at 30 g kg^{-1} of soil applied at 15 DBT can be used for the management of tomato early blight disease under screen house conditions.

Keywords: Early blight, *Lycopersicon esculentum* Mill, Plant powder.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is an important vegetable crop used in the processed, fresh or preserved form and has potential health benefits. Because of the main source of vitamins and lycopene, tomatoes are considered a healthy food (Khan *et al*., 2019a). Tomatoes are grown worldwide over an area of 4.98 million hectares with an annual production of 130 million tons. China, USA, India, Turkey, Egypt and Italy are the principal producing countries, while in Pakistan its production is very low. In Pakistan, 163.3 thousand tons of tomato productions are obtained from an area of 19.8 thousand hectares (MINFAL, 2018). Multiple factors are responsible for reduction of tomato yield in Pakistan including pests and diseases. Tomato crop is vulnerable to different plant pathogens, among which the *Alternaria solani* is considered one of the most destructive pathogens of tomato causing early blight disease (Abada *et al*., 2008). It is air borne and survives mainly in soil or crop residue (Sreenivasulu *et al*., 2019). *A. solani* contains enzymes such as cellulases which degrade the host cell wall and also contain pectin methyl galacturonase, which facilitate host colonization (Shahbazi *et al*., 2011). Diseases affect crop production as they

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cause premature defoliation and result in heavy losses in production by reducing quality and quantity of fruit (Holm *et al.*, 2003). In Pakistan, early blight disease cause yield losses up to 15-100% (Azam and Shah, 2003; Akhtar *et al*., 2004).

The infection of *A. solani* can be controlled by chemicals, but the over-dose and continuous use of chemical pesticides lead to the emergence of pathogen resistance to pesticides and are hazardous to human, animal, and environmental health. For this reason, some alternative strategies for the control have been developed. Ongoing efforts have concentrated on growing durable, long term and compelling, biological control strategies for the control and management of plant disease. Innate plant items are an important source of obtaining agrochemicals that can be effectively used for controlling plant diseases (Kagale *et al*., 2004). It has been reported that plant products have the ability to decrease population of foliar as well as soil borne pathogens and control their infection. Moreover, they are considered to be the safest approach for controlling plant diseases and can be effectively used in integrated disease management programs (Bowers and Locke, 2004). Several plant extract have been observed to have antimicrobial activity under field conditions and *in-vitro* (Kagale *et al*., 2004). Soil amendment with plant powders including *Withania coagulans* have been successfully used for the management of soil borne pathogens (Najeeb *et al*., 2019). The *in vitro* antifungal activity of *W. coagulans* has been reported against *Trichoderma viridis*, *Aspergillus flavus*, *Fusarium laterifum*, *Aspergillus fumigatus*, *Candida albicans*, *Trichophyton mentogrophytes,* and *Microsporum canis* (Mughal *et al*., 2011; Sudhanshu *et al*., 2012).

The phytochemical screening of *Withania coagulans* extracts demonstrated the presence of different essential and auxiliary metabolites, for example, carbohydrates, proteins, amino acids, alkaloids, phenols, tannins, steroids, saponins and natural acids (Agarwal *et al*., 2014). Different species of *Withania* such as *W. coagulans* and *W. somnifera* have been evaluated *in vitro* and *in vivo* for the control of plant pathogenic bacteria (Khan *et al*., 2019b; Najeeb *et al*., 2019). The plants were also evaluated against human pathogenic bacteria, but its viability as far as effectiveness for plant pathogenic fungi, particularly early blight, has not been assessed yet.

The aim of this study was the evaluation of antifungal activity of *W. coagulans* powder for the management of early blight of tomato *in vitro* and *in planta*. Different doses of dried powders of stem, leaves and succulent shoot of *W. coagulans* were investigated for the antifungal activity. The study also was extended to observe the effect of different application times of dried powder on the management of *Alternaria solani* in tomato.

MATERIALS AND METHODS

Collection of Plant Material and Preparation of Dried Powder

Aboveground parts of the medicinal plant, *W. coagulans*, were collected from the dry-land areas of Darra Adam Khel and Kohat, KPK and authenticated by a weed botanist (Department of Botany, University of Peshawar, Pakistan). The parts (stem, leaves and succulent shoot) of the plant were separated, cleaned with water and shade dried at room temperature. When brittle-dry, each plant material was separately crushed to a fine powder and saved in air-tight plastic containers till use.

Source of Pathogen Culture and Inoculum Preparation

Commercial tomato fields of Charsadda district, KPK, Pakistan, were surveyed and tomato plants showing clear symptoms of early blight disease were collected. The plant surface was sterilized with 10% Clorox for 30 seconds. The tissue was rinsed with sterilized distilled water to remove the contaminants. The pathogen was isolated on Potato Dextrose Agar (PDA) medium. The sterilized tomato infected tissue was cut into small pieces. Four pieces were placed per plate and incubated at 25°C for two weeks. The culture was multiplied on PDA medium. Pathogen was identified based on cultural and morphobiometric characteristics (Figure 1) (Ellis, 1971; Barnet and Hunter, 1972). For the inoculum preparation, the fungal masses of previously raised culture of *A. solani* in each plate were flooded with 10 mL of sterilized water. The fungal growth along with the spores was scraped with spatula and the spore suspension was prepared. Spore suspension was adjusted to 4×10^4 spores mL^{-1}, using haemocytometer.

In vitro Antifungal Evaluation of W. coagulans Against Alternaria solani

The poisoned food technique was used to evaluate the effect of dried powder prepared by separately grinding parts (leaves, succulent shoot and stem) of *W. coagulans* for the *in-vitro* growth of *A. solani*. Powder suspensions, 5, 10 and 15% w/v were prepared, soaked in dark for 48 h, filtered and added to sterilized PDA medium (2 mL 100 mL^{-1} medium). The medium was poured in plates at 25 mL $plate^{-1}$ and a plug of the test fungus was placed at the center of each plate. The inoculated plates were incubated at 25°C for fourteen days. Fungal colonies grown on PDA without extracts were used as a negative control while colonies grown on fungicide-mixed with PDA were served as a positive control. For positive control treatment, Mancozeb stock solution was made by adding 2 g L^{-1} in sterilized distilled water and then 1 mL was added in 100 mL PDA medium just before pouring in petri dishes. Completely randomized design with 5 replications was used. The anti-fungal activity was evaluated by measuring the colony diameters of the fungus using a transparent plastic ruler in each petri plates. The data were subjected to statistical analysis.

In planta Antifungal Evaluation of *W. coagulans* Against *Alternaria solani* in Tomato

Experiment 1: Effect of powdered plant parts and doses

The effectiveness of two doses (15 and 30 g) of dried powders of each plant part i.e. stems, leaves and succulent shoots were tested for the management of *A. solani*, under screen house conditions. The dried powder was thoroughly blended with sterilized potted soil preceding transplantation. One-month old tomato seedlings were transplanted in pots (1 seedling pot^{-1}) each containing 1 kg sterilized soil. There were eight treatments comprising leaves, stem and succulent shoots powders each applied at 15 and 30 g dose. Mancozeb (2 g L^{-1}) at 20 mL $plant^{-1}$ was used as a positive control and un-treated group was kept as negative control. The experiment was arranged in a complete

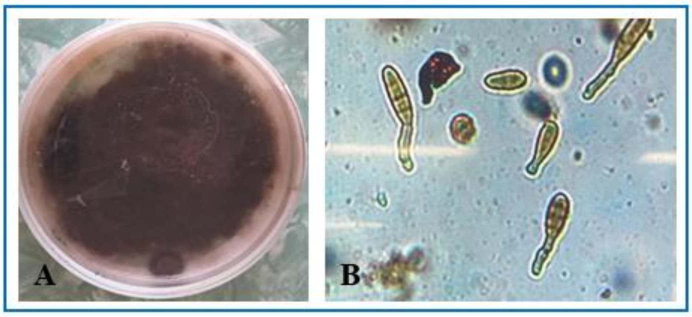

Figure 1. (A) Fresh culture of *A. solani* grown on PDA medium, and (B) Spores of *A. solani*.

randomized design with factorial arrangement with four replications. The plants were inoculated with the spore suspension *A. solani*, 30 days after transplantation. The inoculum suspension (50 mL plant^{-1}) was sprayed with the help of manual sprayer on tomato plants. The spray was done at evening time. Data were taken when the symptoms appeared on the inoculated tomato plants. Experiment was repeated once.

Experiment 2: Effect of application time and doses

To test the effect of time of application of the finely ground succulent shoot powder to soil on the management of early blight, dried powder (15 and 30 g kg^{-1}) of succulent shoot of *W. coagulans* was used at 5, 10, and 15 days before transplanting. Mancozeb (2 g L^{-1}) at 20 mL plant^{-1} was used as a positive control and un-treated group was kept as a negative control. The remaining conditions of the experiment were the same as for experiment 1. Experiment was repeated once.

Data Collection

The experiments were terminated 60 days post transplantation. Data was recorded on disease severity (%), fresh biomass (g), shoot length (cm) and root length (cm) of tomato plants. For disease severity, scale of Latha *et al*. (2009) was used. Different categories of the scale presented different rates of disease severity such as 0= No disease, 1= 1 to 5% leaf area infected, 2= 6 to 10% leaf area infected, 3= 11 to 25% leaf area infected, 5= 26 to 50% leaf area infected, 7= 51 to 75% leaf area infected, 9= > 76% of the leaf area infected. By using the formula of Song *et al*. (2004), the data were converted to percentage disease severity as follo9ws:

Disease severity (%)= $\sum T/N \times 100$.

Where, T= Number of infected leaves per plant and N= Total number of leaves per plant.

Statistical Analysis

All data were presented as mean±standard deviation in tabulated or graphical form. The data were subjected to one-way Analysis Of Variance (ANOVA). Analysis was done using software package Statistix 10.1. Completely Randomized Design (CRD) was used for *in vitro* data while CRD with factorial arrangement was used for *in planta* data. Significant differences among the treatments were presented with lower case lettering.

RESULTS

In vitro Antifungal Evaluation of W. coagulans Against Alternaria solani

Results showed that different plant parts and their concentrations of *W. coagulans* significantly affected the *in vitro* growth of *Alternaria solani*. Generally increased in dried powder concentration resulted in decreased colony diameter of the fungus. The highest concentrations (15 %) of all the plant parts (leaves, succulent shoot and stem) gave lower colony diameter. Among different plant parts, the dried powder of succulent shoot gave the maximum anti-fungal activity as compared to other parts. After the treatment of positive control (mancozeb), a minimum colony diameter of the fungus (3.0 cm) was noted with 15 % (w/v) succulent shoot powder followed by 3.8 cm with the same concentration of leaves powder. The negative control (SDW) exhibited the maximum colony diameter (Figures 2, and 3).

In planta Antifungal Evaluation of *W. coagulans* Against *Alternaria solani* in Tomato

Experiment 1: Effect of plant parts and doses

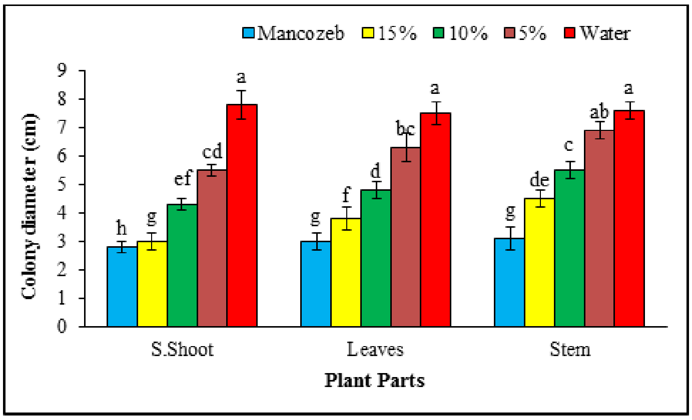

Figure 2. Effect of different concentrations of dried powders prepared from different parts of *W. coagulans* on radial growth (cm) of *A. solani*. Each value is a mean±SD replicates of 5 replicates. Bars represent standard deviation. Means followed by different letters differ significantly from each other at 5% level of probability.

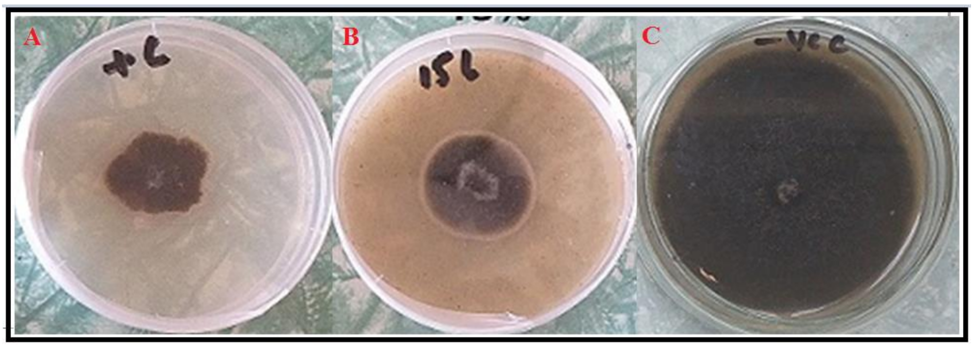

Figure 3. Comparison of colony diameter between best treatment and control. (A) Mancozeb (positive control), (B) 15% succulent shoot extract of *W. coagulans* (best treatment), and (C): Water (negative control).

Growth of inoculated tomato plants with *A. solani* was significantly affected by the application of different doses (0, 15 and 30 g) of dried powder of succulent shoot, leaves and stem of *W. coagulans*. Generally, succulent shoot powder with highest dose i.e. 30 g enhanced maximum plant growth as compared to other parts and doses. The highest dose of succulent shoot powder i.e. 30 g exhibited maximum root length (21.4 cm), shoot length (54.3 cm), fresh biomass (58.8 g) and lowest disease severity (26%) after positive control mancozeb. This treatment was followed by the same dose (30 g) of leaves powder that exhibited 19.3 cm, root length, 49.1 cm, and shoot length, 56.5 g fresh biomass and 40% disease severity (Tables 1, 2, and 3; Figure 4). The non-amended soil (negative control: 0 g) gave minimum root length, shoot length, fresh biomass and highest disease severity. Similar results of treatments were observed when the experiment was repeated. The succulent shoot powder and highest dose i.e. 30 g were superior over the other plant parts and doses.

Experiment 2: Effect of application time and doses

Different application times 5 DBT, 10 DBT and 15 DBT and doses of succulent shoot powder of *W. coagulans* significantly enhanced the growth parameters of inoculated tomato plants and caused reduction in disease severity. After positive control, the highest dose (30 g) and longest application time (15 DBT) gave maximum root length (19.3 cm), shoot length (45.6 cm) and fresh biomass (50.1 g) and minimum disease severity (22%). This was followed by the same dose (30 g) applied at

Table 1. Effect of *Withania coagulans* plant parts (dried powder) and doses on root length (cm) of inoculated tomato plants with *Alternaria solani*.[a]

Doses (g)	Plant parts		
	S. shoot	Leaves	Stem
		Experiment 1	
30	21.4±1.7 ab	19.3±1.6 b	14.1±1.5 cd
15	15.2±1.2 c	13.3±1.1 d	10.3±1.6 e
0	11.2±1.0 e	10.6±1.0 e	10.3±1.3 e
Mancozeb	23.4±2.1 a	24.1±2.3 a	23.1±1.9 a
		Experiment 2	
30	22.3±1.8 b	20.1±1.4 c	15.2±1.5 ef
15	18.1±1.4 cd	16.1±1.6 de	11.1±1.0 g
0	12.6±0.9 g	12.1±1.1 g	12.2±1.3 g
Mancozeb	26.2±2.3 a	25.3±1.8 a	25.3±2.3 a

[a] Each value is a mean of four replicates±standard deviation. Different lettering shows significant difference according to LSD test. Experiments I and II are the two same experiments repeated concurrently.

Table 2. Effect of *Withania coagulans* plant parts (dried powder) and doses on shoot length (cm) of inoculated tomato plants with *Alternaria solani*.[a]

Doses (g)	Plant Parts		
	S. shoot	Leaves	Stem
		Experiment 1	
30	54.3±3.4 ab	49.1±2.8 c	34.2±2.4 efg
15	41.3±3.1 d	37.3±3.6 de	29.3±2.8 fg
0	29.3±2.1 ef	30.2±2.3 fg	29.4±2.6 fg
Mancozeb	59.2±4.2 a	58.1±4.6 a	57.3±3.8 a
		Experiment **2**	
30	58.2±3.2 ab	54.5±3.2 b	34.5±2.5 cd
15	41.2±2.8 c	39.1±2.8 c	30.5±3.1 de
0	27.1±1.3 ef	28.5±2.1 ef	27.5±3.1 ef
Mancozeb	63.1±4.5 a	60.1±4.6 a	61.8±4.2 a

[a] The same note as under Table 1.

Table 3. Effect of *Withania coagulans* plant parts (dried powder) and doses on fresh biomass (g) of inoculated tomato plants with *Alternaria solani*.. [a]

Doses (g)	Plant Parts		
	S. shoot	Leaves	Stem
		Experiment 1	
30	58.8±3.7 ab	56.5±3.1 b	40.3±2.8 d
15	46.7±3.2 cd	38.7±2.6 d	27.7±2.8 e
0	29.7±1.8 e	30.1±2.1 e	28.8±2.1 e
Mancozeb	66.0±5.1 a	64.3±4.5 a	64.0±4.6 a
		Experiment 2	
30	56.2±4.1 ab	51.5±3.8 b	35.5±1.6 de
15	40.5±3.6 cd	35.5±3.1 de	24.2±2.1 f
0	23.5±1.8 f	25.1±2.3 ef	24.1±1.7 f
Mancozeb	60.5±5.2 a	61.1±4.7 a	59.5±4.7 a

[a] The same note as under Table 1.

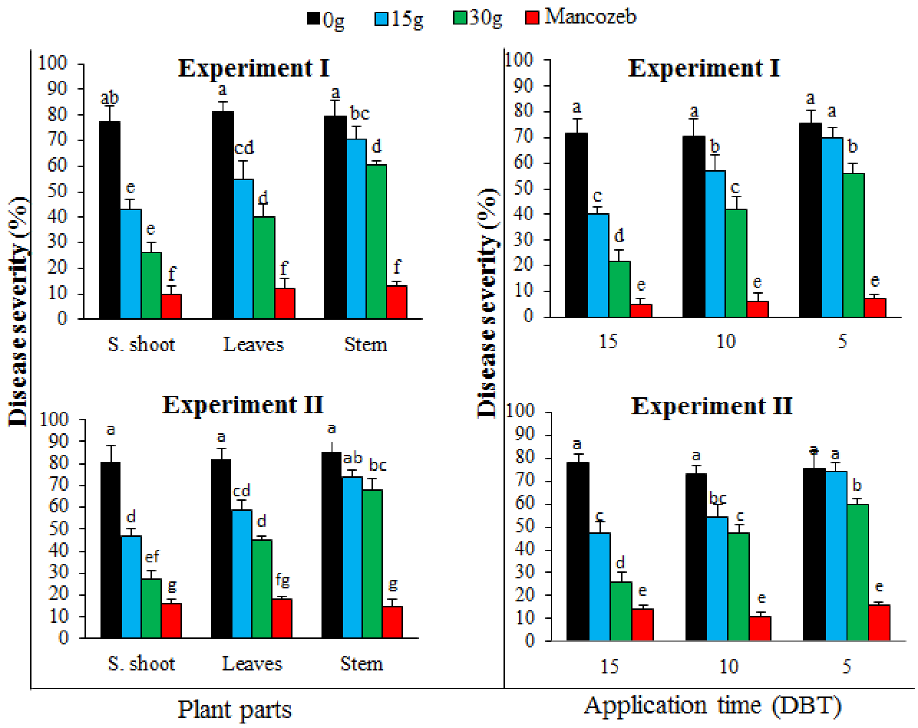

Figure 4. Effect of dried powder of *Withania coagulans* on disease severity of early blight in tomato plants artificially inoculated with *Alternaria solani.* (A) Plant parts (succulent shoot, leaves and stem) and their dried powder doses (0, 15, 30 g). (B) Application time (15, 10, 5 DBT) and dried powder doses (0, 15, 30 g) of succulent shoot dried powder. Each value is a mean of four replicates±standard deviation. Bars represent the standard deviation. Different lettering shows significant difference according to LSD test. DBT: Days Before Transplantation. Experiments I and II are the two same experiments repeated concurrently.

10 DBT giving root length (16.1 cm), shoot length (35.0 cm), fresh biomass (40.1 g) and disease severity (42%). he negative control with 0 g and shortest application time 5 DBT exhibited the lowest values for plant growth parameters and caused minimum reduction in disease severity (Tables 4, 5, and 6; Figure 4). The same trend was noticed when experiment was repeated with no modification.

DISCUSSION

Different practices are used to manage the early blight of tomato such as use of fungicides, biological control, and resistant varieties. Use of plants or plant products having antifungal activities is an ecofriendly approach to manage plant fungal disease. Different plants possess compounds that are antifungal, antibacterial, and anti-nematodes (Kareem *et al*., 2008). In this study, antifungal potential of *W. coagulans* was evaluated against *Alternaria solani*.

Results indicated that dried powder of *Withania coagulans* is effective in controlling *A. solani*. These results are in line with Mughal *et al*. (2011) who extracted different compounds from *W. coagulans* and used them against several fungal strains i.e. *T. viridis*, *A. flavus*, *F. laterifum*. The compounds showed significant results against fungi. The reduction in fungal growth by *W. coagulans* can be described base on the presence of anti-fungal metabolites. Several investigators reported that plants contain numerous groups of chemicals such as tannins, flavonoids, alkaloids and lactones (Kapoor, 2017).

Table 4. Effect of application time and doses of dried succulent shoot of *Withania coagulans* on root length (cm) of inoculated tomato plants with *Alternaria solani*.

Doses (g)	Application time (DBT)		
	15	10	5
	Experiment 1		
30	19.3±b	16.1±c	13.3±def
15	14.2±d	12.2±f	10.1±g
0	11.1±fg	10.0±g	11.3±fg
Mancozeb	22.1±a	23.2±a	22.2±a
	Experiment 2		
30	19.9±b	16.2±c	12.1±d
15	16.1±c	13.2±d	9.1±f
0	10.2±ef	11.3±de	9.3±f
Mancozeb	24.5±a	24.2±a	25.3±a

[a] The same note as under Table 1.

Table 5. Effect of application time and doses of dried succulent shoot of *Withania coagulans* on shoot length (cm) of inoculated tomato plants with *Alternaria solani*.[a]

Doses (g)	Application time (DBT)		
	15	10	5
	Experiment 1		
30	45.6±b	35.0±c	28.1±d
15	38.8±c	28.0±d	23.1±f
0	22.1±f	25.2±def	23.1±f
Mancozeb	49.6±ab	53.2±a	51.2±a
	Experiment 2		
30	46.1±b	32.1±de	25.2±f
15	37.2±c	28.1±ef	20.1±g
0	20.1±g	20.1±g	21.1±fg
Mancozeb	51.2±ab	54.2±a	54.1±a

[a] The same note as under Table 1.

Table 6. Effect of application time and doses of dried succulent shoot of *Withania coagulans* on fresh biomass (g) of inoculated tomato plants with *Alternaria solani*.[a]

Doses (g)	Application time (DBT)		
	15	10	5
	Experiment 1		
30	50.1±b	40.1±c	32.2±def
15	39.2±c	31.2±d	26.1±g
0	29.1±fg	28.1±e	28.1±fg
Mancozeb	53.3±ab	57.1±a	54.2±a
	Experiment 2		
30	50.1±ab	40.1±c	32.2±de
15	39.2±c	31.2±d	26.1±ef
0	29.1±d	28.1±de	28.1±e
Mancozeb	53.3±ab	57.1±a	54.2±ab

[a] The same note as under Table 1.

About 138 withanolides, 13 alkaloids and numerous sitoindosides have been extracted and reported from different parts of *Withania* species (Xu *et al*., 2011). *Withania coagulans* also contains other chemicals including cuscohygrine, pseudotropine, nicotine, somniferinine, choline, somniferine, tropine, withanine, and numerous antifungal alkaloids i.e. anhygrine and anaferine. Withanolides extracted from ethanolic extract of whole plant parts possessed antifungal and anti-bacterial properties (Gupta and Rana, 2007).

Application of *W. coaglans* dried powder to soil significantly improved growth of tomato plants by reducing disease severity caused by *A. solani*. Generally, succulent shoot part, longer application time, and highest dose caused a maximum reduction in disease severity and increase in plant growth as compared to the control. Application of plant powder to soil may induce systemic resistance in plants against pathogen. Several compounds from plants have been reported for the induction of resistance in tomato plants against pathogen (Walters *et al*., 2005). The best results of succulent shoot parts might be due to the presence of more compounds. The dried powders of *W. coagulans* also contain SAR elicitors, which activate the defense of plants and act as plant defense activators (Walters *et a*l., 2005). Various plant extracts have been reported to have SAR elicitors (Mitra and Paul, 2017; Hemalatha *et al*., 2008). Our results are in line with other investigations where *W. coaglans w*as used for the control of bacterial disease in tomato plants. For example, Najeeb *et al*. (2019) used dried powder of *W. coaglans* as soil application and managed bacterial wilt disease in tomato plants. Khan *et al*. (2019b) also managed *Ralstonia solanacearum* in tomato plants by soil application of another specie of *Withania* i.e. *W. somnifera*. The application of dried powders to soil also improved soil texture such as pH buffering, ion adsorption, and water holding capacity (Brady and Weil 1999). Improvement in soil texture by the application of dried powders results in improvement of plant growth parameters and healthier plants protect themselves more easily against pathogens. The best results of longer application time could be explained based on more decomposition of plant powder resulting in the release of more compounds as compared to shorter application time where fewer compounds might be released due to short time. The application time effect of soil application of plant powder was proved by many researchers. Different application times of dried powders of *Xanthium strumarium*, *W. somnifera* and *W. coagulans* applied as soil treatment were evaluated and found longer time as the best in term of improving plant growth and reducing disease severity (Khan *et al*., 2019b; Najeeb *et al*., 2019).

Withania coagulans is an evergreen plant that is easily available in large amounts and can be used as a part of IDM to control many plant diseases, especially early blight of tomato. These plants are easily affordable and can be used as powder, green manure, or extracts by poor farmers to control different diseases of tomato. Dried powders are directly applied to the fields by broadcasting method, which does not require big costs (Ji *et al*., 2007). *Withania coagulans* has a great potential to control plant diseases, therefore, further studies are required to isolate the anti-fungal or SAR inducer compounds from this plant.

CONCLUSIONS

In this study, the antifungal potential of *Withania coagulans* was evaluated against *Alternaria solani* both *in vitro* and *in planta*. Different doses of different plant parts applied through different application times were tested. It was found that succulent shoot dried powder at 30 g kg^{-1} soil applied at 15 days before transplantation exhibited maximum antifungal activity, suppressed early blight disease in tomato, which resulted in higher plant growth. Results of this study suggested that dried powder of *W. coagulans* could be used as an effective, eco-friendly, and economical alternative to chemical control for the management of *A. solani* in tomato.

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مدیریت ***Alternaria solani*** در گوجه فرنگی با استفاده از ***Withania coagulans*** : جایگزینی برای کنترل شیمیایی

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چکیده

به منظور کاهش مقاومت به آفتکش‌ها در پاتوژنهای گیاهی، رویکرد های جایگزینی مورد نیاز است که باید بررسی شود. در این مطالعه، پودر خشک شده گیاه Withania coagulans برای مدیریت Alternaria solani که منجر به ایجاد بیماری سوختگی زودرس در گوجه فرنگی (هم در درون شیشه و هم در گیاه) می شود ارزیابی شد. به این منظور، دوزهای مختلف پودر خشک شده (5٪، 10٪ و 15٪ وزنی حجمی W/V) قسمت‌های مختلف (شاخه ساکولنت، ساقه و برگ) با استفاده از روش مسمومیت غذایی W. coagulans بر علیه رشد درون شیشه‌ای *Alternaria solani* مورد آزمایش قرار گرفت. همچنین، برای کنترل سوختگی زودرس در بوته‌های گوجه فرنگی در شرایط زیر یک محفظه توری (screen house)، دوزهای مختلف پودر خشک شده (15 و 30 گرم بر کیلوگرم خاک) از قسمت های مختلف (شاخه ساکولنت، ساقه و برگ) W. coagulans در زمان های مختلف (5 روز قبل از نشاکاری (transplantation) (DBT)، 10 DBT و 15 DBT) به خاک افزوده شد و مورد ارزیابی

قرار گرفت. بر اساس نتایج حاصل از مطالعات آزمایشگاهی(درون شیشه ای)، تیمار بیشترین دوز 15٪ (وزنی/حجمی) و بخش ساکولنت شاخساره حداکثر فعالیت ضد قارچی را نشان داد. نتایج محفظه توری نشان داد که دوز 30 گرم بر کیلوگرم خاک همراه با تیمار DBT 15 بخش ساکولنت شاخساره پودر خشک ساکولنت شدت بیماری را به حد موثر در بوته های گوجه فرنگی (که به طور مصنوعی با Alternaria solani تلقیح شده بود) کنترل کرد و به طور معناداری طول ریشه، طول ساقه و زیست توده تازه بیشتر شد. از نتایج این مطالعه چنین بر میآید که برای مدیریت بیماری سوختگی زودرس گوجه فرنگی در شرایط زیر محفظه توری، می توان از پودر خشک ساکولنت با دوز 30 گرم بر کیلوگرم خاک که در DBT 15 اعمال شود استفاده کرد.