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# Antifungal potency of three plant extracts against *Rhizoctonia Solani* damping-off disease in tomato

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## Abstract

Tomato is economically important horticultural crop worldwide, especially in Pakistan. *Rhizoctonia solani* is a serious threat for tomato growing areas, responsible for considerable crop and yield losses. This study was designed to minimize these huge economic losses and to regain the attention of formers which was shifted towards other crops as compare to this profitable crop. Leaves extract of three medicinal plants, cinnamon (*Cinnamomum verum),* moringa (*Moringa oleifera*) and clove (*Syzygium aromaticum*) were investigated against *R. solani* causing damping off of tomato. Three different concentrations (1%, 2% and 3%) were used to check the efficacy of plant extracts. Three different trials were conducted as invtro under CRD, greenhouse and field under RCBD. Antifungal potency test showed that the maximum growth inhibition was observed at 3% concentration followed by 2 and 1% of each plant extracts. *In vitro* clove leave extract shown highest antifungal activity which causes complete mycelial inhibition 3% concentration. At 3% concentration efficacy of plants extracts was also examined on disease incidence as well as plant growth development in greenhouse and field experiment. Clove leave extract at 3% concentration recorded highly significant in disease reduction and other plant growth parameters. Thus, clove leaves are best choice for managing *R. solani* associated with damping off disease of tomato.

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## Introduction

Tomato (*Lycopersicon esculentum* L.) is a main vegetable and most commonly cultivated crop all over the world. It belongs to an important family solanaceae. It is native to the western South America. It contains tryptophan and tomatin (a main component of glucoalkaloid) which are very useful for human health.

It covers total area 45.5 lac ha with annual production 125.02 million tons worldwide (FAO, 2010). After potato crops it is considered as a second economically important crop in all over the world. Tomato is also a major crop of Pakistan with total production 530,000 tons/year. (Agriculture Statistics of Pakistan, 2011-12). It is used for different purposes such as fresh and processed foodstuffs (cooked foods, fresh salad, pickle, sauce, ketchup etc.) described (Gary and Tchamitchian, 2001).

Various species of *Fusarium, Verticillium, Pythium* and *Rhizoctonia* are responsible for the soil borne fungal diseases, wilt of flora and damping-off of seedlings (Kaprashvili, 1996; Lucas *et al.,* 1997).

Different types of symptoms are associated to damping off; that bring about the death of at least some seedlings in any particular population (Chana *et al.,* 1997). Symptoms start with round spots on seedlings, and then having stem abrasions at surface level. Seedling vigor is reduced because stems may additionally grow to be skinny and hard. Occasionally leaf spotting accompanies further symptoms, as ensures by a grey mildew boom on leaves and stems (Chana *et al.,* 1997).

Sabuquillo *et al.,* 2006 reported that "among the biotic stresses, plant pathogenic microorganisms are the extreme threat to crop production and environmental balance". *Rhizoctonia solani* is one of the most vigorous soil borne pathogens which increases both in refined and non-refined soils, causing infections in distinct vegetation together with bean, tomato and rice among others (Sneh *et al.,* 1991). *R. solani* is noticeably the important fungus responsible for causing root rot, black spot and dumping off disorders (Neha and Dawande, 2010). It lives inside the soil as sclerotia and does not produce asexual spores (Huang *et al.,* 2011). Damping-off of shoots is the utmost common disorder originated by *R. solani* (Moussa, 2002).

It has extensive host community and results in destruction to various crops, such as tomato, cucumber (Coa *et al.,* 2004), lawn grass (Parmeter *et al.,* 1969) and sugar beet (Sadeghi *et al.,* 2006).

The diseases might be controlled effectively by use of synthetic chemical substances; however, its use would be probably near the common sense of regular farming. Subsequently, exploring the different anti-fungal agents, in particular the extracts from different plants have advantages. Khare and Shukla, 1998 explored the capability of different plant extracts as antifungal substances towards various fungal diseases. Different plant produces like gums, oils, resins and important plant extracts were checked as bio-fungicidal mixtures and they also show organic activity *in vitro* and *in vivo* (Pawar and Thaker, 2006; Fawzi *et al.,* 2009). The objective of our findings was to determine the protective effect of plant extracts on damping off of tomato.

## Materials and methods

*Collection of Sample*

Diseased samples were collected on the basis of visual typical disease symptoms from the different localities of Faisalabad. Collected samples were preserved in a freezer at 4 $^{o}$C for 5-7 days. Investigation was done at the Department of Plant Pathology, University of Agriculture, Faisalabad, Punjab, Pakistan.

*Isolation and purification of pathogen*

Disease samples were surface sterilized with 5% $H_2O_2$ or bleach for 1 minute then washed three times with sterilized distilled water and plated on Potato Dextrose Agar (PDA) medium. Pathogens were isolated with the help of sterilized inoculating needle by newly growing tip and plated on PDA media. Inoculated plates were incubated at 25$^{o}$C± 2$^{o}$C for

emergence of colonies. To obtain pure cultures of the *R. solani* sub-culturing was done from single spore technique.

*Preparation of aqueous plant extracts*

Fresh leaves of cinnamon, moringa and clove were taken from different locations of Faisalabad. Leaves were washed carefully with sterilized distilled water and air dried at room temperature. 100 ml of sterile distilled water was added to each of 25g dried ground leaves.

The mixture was mechanically stirred for one hour at room temperature and filtered through four layers of chess cloth then centrifuged at 3500 rpm for 20 minute to remove coarse material. Soluble extract was then sterilized by passing through a Millipore filter (0.22 I pore size) using Seitz filter apparatus. Extracts 25.0% (w.v.) thus obtained and utilized for the experiments (Achimu & Scholsser, 1992).

*In vitro evaluation of antifungal activity of plants extracts against R. solani*

Antifungal activity was evaluated on tomato by using the food poisoning technique (Kumar *et al.,* 2008). Different concentrations (1, 2 and 3 %) of three medicinal plants were mixed with sterile potato dextrose agar medium (PDA) and then poured in sterile Petri dishes (90 mm diameter). Discs of 7 mm diameter of phytopathogenic fungi were cut from the periphery of 6 days old cultures and inoculated aseptically to the center of poured Petri dishes of treatment and incubated at 25 ± 2°C for 7 days. Fungal colony diameter of treatments was measured and percentage of mycelial inhibition was calculated using the following formula:

Percentage of mycelial inhibition = [C – T / C] x 100

Where, C and T are the growth diameter (mm) in control and treatment respectively.

*Greenhouse experiment*

Pots (12.5 cm in diam.) containing sterile soil (1kg /pot) were sown with four week-old tomato seedlings of the Castle Rock variety. The biocontrol agent's cinnamon, moringa and clove leaf extracts with 3 % dilution were added into potting soil (20 ml/pot). *R. solani* inoculum was added to the pots at 1% (w:w). Pots were distributed in greenhouse condition in compete randomized design with 4 replicates as following; RS, RS+Ci, RS+M, RS+Cl , RS+Ci+M+Cl, control (untreated), Ci alone, M alone, Cl alone and Ci+M+Cl.

Here: RS= *R. solani*

Ci= Cinnamon extract

M= Moringa extract

Cl= Clove extract

The plants carefully uprooted after two months of sowing for root rot severity according to the following scale.

0 = healthy roots; 1 = secondary roots are rotten; 2 = secondary root and part of taproot is rotten; 3 = taproot is rotten 4 = taproot and crown are rotten and 5=death of plant. The disease severity was calculated according to Mckinney formula (Mckinney, 1923).

*Field experiment*

The treatments were the same as described in greenhouse experiment. Four week-old seedlings were sown in rows followed by R×R distance 70 cm and P×P 25 cm respectively. The biocontrol agent's cinnamon, moringa and clove leaf extracts with 3 % dilution were added into the soil at 20 ml/plant during sowing. After two month of transplanting 6 plants of each row were carefully uprooted for determination of shoot length, root length, shoot weight, root weight, shoot lateral branches.

*Statistical Analysis*

The statistical test was performed by using SAS statistical software. Means were sorted out by using Fisher's least significant difference (LSD) procedure (Steel *et al.,* 1997).

**Results**

*Antifungal activity of plant extracts*

Data analysis showed that all tested plant extracts inhibited the mycelial growth of damping-off pathogen *in vitro*.

The clove plant leaf extract exhibited the highest antifungal activity against *R. solani* while moringa lowest one. The clove leaf extract gave the highest mycelial inhibition value (78.5, 96.4 and 100%) at 1, 2 and 3% concentration followed by cinnamon (29.3, 44.5 and 71.6%) and moringa plant leaf extract showed lowest (24.2, 40.8 and 67.6%) mycelial inhibition (Fig. 1).

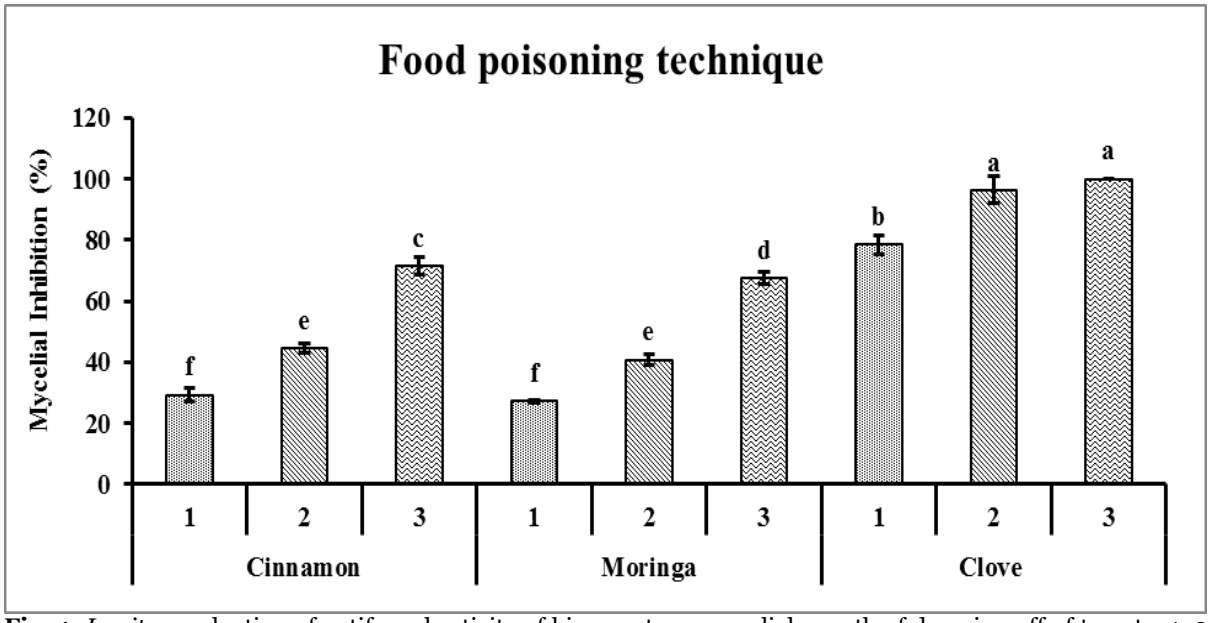


**Fig. 1.** *In vitro* evaluation of antifungal activity of bio-agents on mycelial growth of damping-off of tomato. 1, 2 and 3= % concentrations of plants extracts.

*Greenhouse experiment*

Results revealed that biocontrol agents used in this experiment induced significant reduction in disease incidence of damping-off of tomato, caused by *R. solani* under greenhouse conditions. The clove leaf extract reduced disease incidence (26.4%), cinnamon (36.5%), moringa (43.8%) and (16.33%) in combination of cinnamon, moringa and clove leaf extracts, compared with plants which were only inoculated with *R. solani* respectively (Fig. 2).

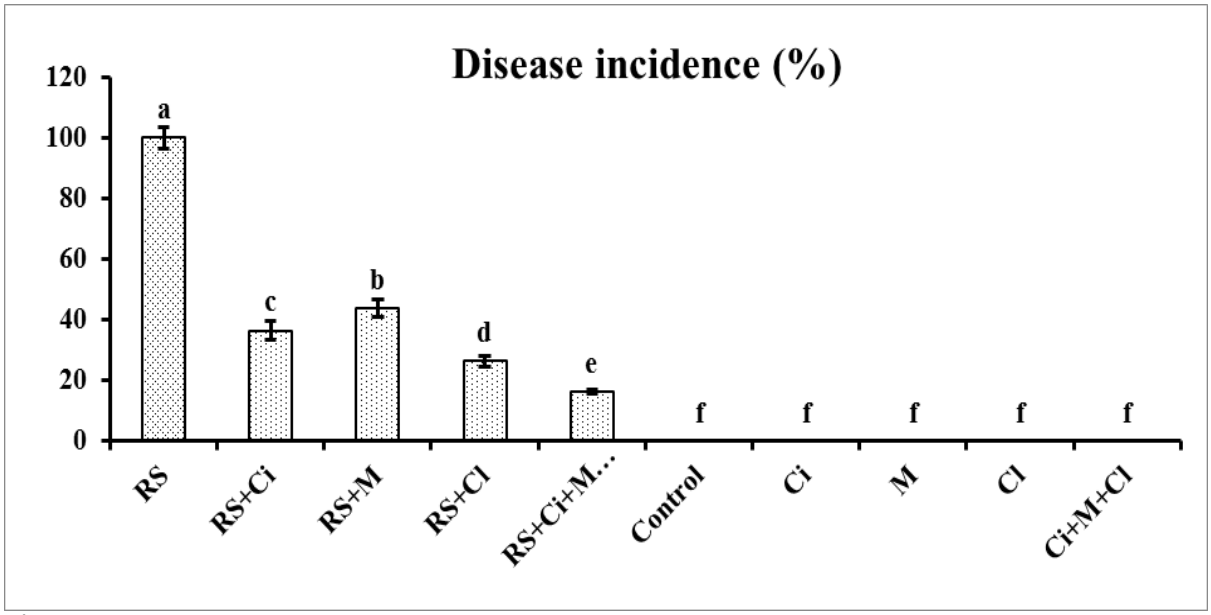


**Fig. 2.** Effect of different plant extracts on disease incidence Damping off of tomato under greenhouse conditions. RS= *R. solani,* Ci= Cinnamon, M= Moringa and Cl= Clove leaf extracts.

*In vivo effects of plant extracts*

*In vivo* effect of plant extracts against *R. solani* was investigated on tomato plant growth parameters shoot length, shoot weight, root length, root weight and shoot lateral branches (Fig. 3). In all treatments, there was a significant difference in growth parameters was observed. Shoot length, shoot weight, root length, root weight and shoot lateral branches were maximum (40 cm, 44.30 g, 16 cm, 8.10 g and 15 lateral branches) in combination of cinnamon, moringa and clove leaf extracts at 3% concentration followed by all other treatments while minimum shoot and root weight was recorded in *R. solani* alone treated plants. Direct relationship was observed between the shoot weight and shoot length. All bio-control agents were found to improved plant growth as significant increase shoot length, shoot weight, root length, root weight and shoot lateral branches as compared to plants treated with *R. solani* alone culture (Fig. 3).

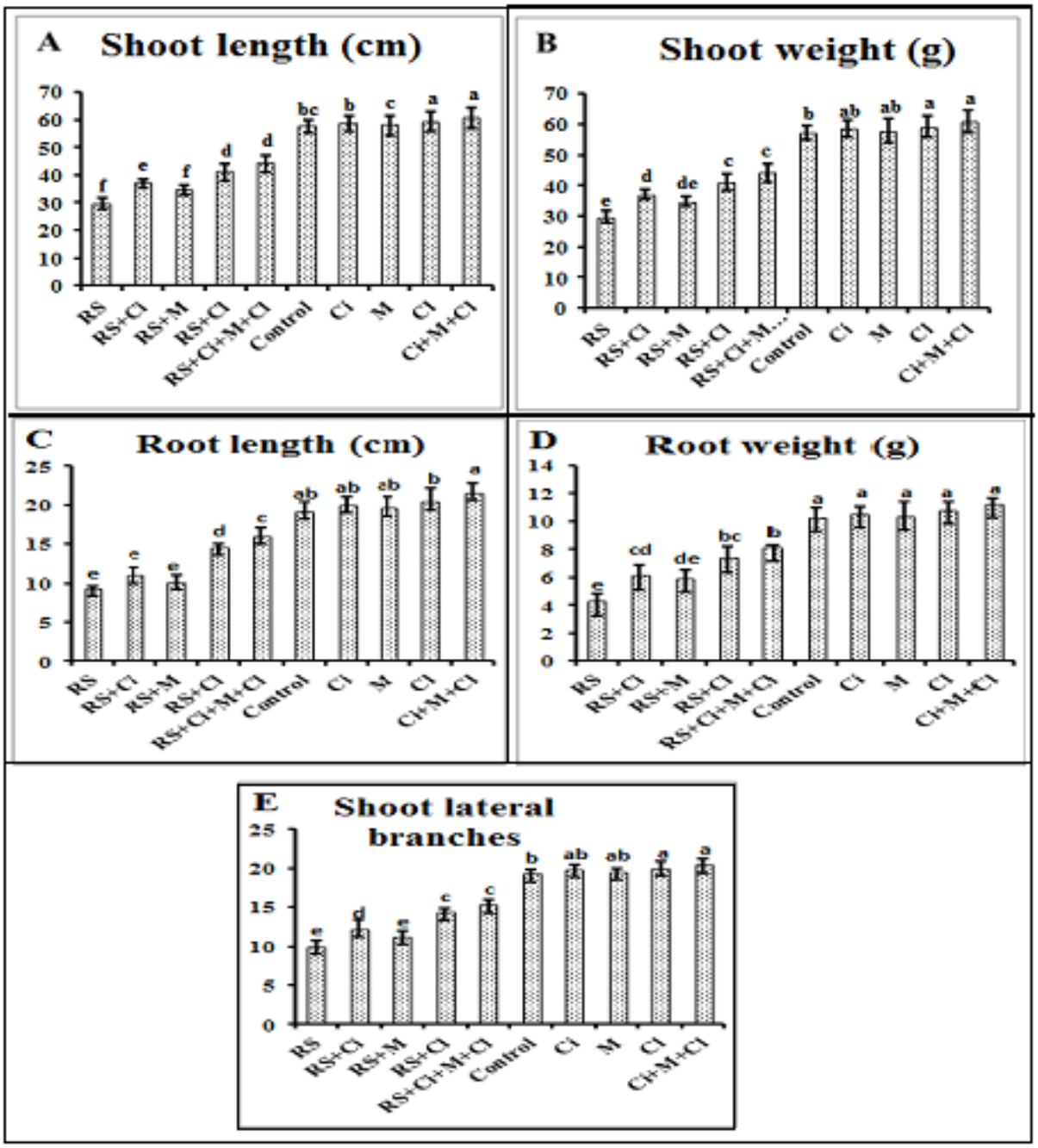


**Fig. 3.** Influence of different plant extracts under field conditions. RS= *R. solani,* Ci= Cinnamon, M= Moringa and Cl= Clove leaf extracts.

**Discussion**

The antifungal effect of plant extracts is mainly related to their chemical composition. Plant extracts as capability of antifungal substance has been explored towards several fungal diseases (Khare and Shukla, 1998). *In vitro* study the clove leaf extract gave significantly (P≤ 0.05) the maximum mycelial inhibition at 1, 2 and 3% concentration followed by cinnamon while moringa plant leaf extract showed minimum mycelial inhibition. The concentration is imperative because the persistence structure in damping-off pathogen of tomato (*R. solani*) is sclerotia described by (Lucas, 1975; Gopalachari, 1984; Agrios, 2005). Therefore, the concentration at which the sclerotia formations suppress should be considered the actual dosage in formulation for the management of *R. solani.* In present investigation maximum mycelial inhibition was observed at 3% concentration of plant extracts.

In greenhouse experiment it was shown that the tomato plants which treated with alone clove leaf extract significantly (P≤ 0.05) reduced disease incidence followed by other plants extract while combination of cinnamon, moringa and clove leaf extracts exhibited minimum disease incidence, compared with plants contaminated with *R. solani* alone. Islam and Faruq (2012) described that the clove, garlic and allamonda fresh leaf extract exhibited better results when compared to neem leaf extracts in decreasing the damping-off of tomato disease. Seema *et al.* (2011) evaluated that henna plant, betel, sweet scented geranium and false ashoka extracts have recorded significant antifungal activity against damping-off disease caused by *R. solani.*

Investigation in field experiment showed that the shoot length, shoot weight, root length, root weight and shoot lateral branches were maximum in combination of cinnamon, moringa and clover leaf extract followed by all other treatments as compared to *R. solani* alone treated plants. Numerous studies evaluated the inhibitory action of different plant extracts on different fungi, Ushiki *et al.* (1996) confirmed the suppression of various soil borne pathogens by using different plant extract; Ejechi *et al.* (1999) described that the pepper extracts against root rot fungi of tomato; Jasso de Rodriguez *et al.* (2005) studied the antifungal action of Aloevera plant extract against *R. solani.*

**Conclusion**

Only one variety Naqeeb had a resistance reaction to attack by the pathogen of *Rhizoctonia solani*. 6235, 6233, FS-2187, Sibarien show highly susceptible reaction with maximum disease incidence. So it is need to grow Naqeeb as a resistance source against damping off disease of tomato. Among five plant fungicides Success showed best result with the minimum disease reaction (46.77%).

It is recommended that if the disease spreads in epidemic form in the tomato field then former can use success at the rate of (25%) concentration at which showed significantly results against the damping off disease of tomato. Garlic reduced the damping of tomato disease significantly. Because use of the chemical management of disease pollute our environment continuously. So there is a need of antifungal potential of different plant extract.

It is also need of hour to search out particular effective compound (S) or element (S) present in different plant extract which should be employed for better managements of disease in future.

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