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EVALUATION OF ANTIFUNGAL POTENTIAL OF PHYTOEXTRACTS AND CHEMICALS AGAINST ROOT ROT OF SOYBEAN CAUSED BY *RHIZOCTONIA SOLANI*

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ABSTRACT

Rhizoctonia solani is a soil-borne fungal pathogen that causes root rot diseases in various crops. The soybean crop is also susceptible to this pathogen, resulting in severe yield losses. Due to its soil-borne nature, controlling infestations with a single treatment is challenging. Therefore, the current study was designed to evaluate synthetic chemicals and phytoextracts as an integrated approach to manage soybean root rot caused by *R. solani* under laboratory and greenhouse conditions. Diseased samples were collected from the field area of the University of Agriculture, Faisalabad. The pathogen was isolated and identified as *Rhizoctonia solani* using available literature at the Phytopathology Laboratory, Department of Plant Pathology. Four fungicides (Tilt, Cabriotop, Amister top, and Champion) and phytoextracts (Garlic, Ginger, Turmeric, and Eucalyptus) were investigated at three different concentrations under *in vitro* conditions using the poisoned food technique. The findings revealed that significant inhibition of mycelial growth was observed with Tilt fungicide (4.5 mm) and Garlic extract (12.92 mm) at all concentrations. Subsequently, these two were further investigated under greenhouse conditions, both individually and in combination. Results indicated that the combination of Tilt + Garlic was highly effective against soybean root rot, with the lowest disease incidence of 4.52%, followed by the sole application of Tilt (13.35%) and Garlic (15.75%), respectively. Therefore, it was concluded that using more than one treatment in an integrated approach can be more effective in controlling soil-borne pathogens such as *R. solani*.

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INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is native to China and belongs to the Fabaceae family (Prasanthi et al., 2019). The worldwide area under soybean cultivation is 126.95 million metric hectares, with a production of 353.46 million tons. In Pakistan, the total area under soybean cultivation is 106 hectares with a production of 152 tons (FAO STAT 2020). The global oil production from soybean stands at 396.9 million tons (FAO STAT 2020). The demand for soybean oil is expected to rise in the coming years (Arias et al., 2022). The USA, Brazil, Argentina, China, and India are leading producers of soybean. The soybean crop has boosted the export earnings of the USA, Brazil, and Argentina. Their agricultural sectors have derived significant growth from this crop, as these countries account for 80% of the total production of soybean and have dominated the export market (Voora et al., 2020).

The nutritional value of soybean makes it a wholesome diet, containing 40% protein, 30% carbohydrates, and 20% oil (Arias et al., 2022). Essential trace elements like iron, manganese, copper, molybdenum, potassium, and vitamins such as vitamin K and isoflavones have been found in abundance (Kushwaha and Singh, 2022). Many food industries use soybean to produce value-added products, both non-fermented and fermented soy foods, which are widely consumed by humans. Non-fermented soybeans include fresh soybeans, dehydrated soybeans, bean sprouts, whole and defatted soybeans, soybean meal, soy milk and its products tofu, okra, and yuba. Fermented soy foods include miso, natto, tempeh, soy sauces, and fermented tofu. Soybean is also used as fodder for cattle (Chen et al., 2012).

Soybean is subject to multiple impediments, including viruses, bacteria, fungi, nematodes, and phytoplasma (Roth et al., 2020). *Rhizoctonia solani*, the most destructive soil-borne fungus causing root rot of soybean during early summer, poses a significant threat. As the soybean crop thrives at 29℃ (Siddiqui et al., 2012), it becomes vulnerable to fungal attacks. Reddish-brown and sunken lesions formed on the hypocotyl of plant seedlings transform into dry cankers, ultimately leading to wilting and the death of seedlings. This fungus can be controlled by seed dressing with fungicides; however, the use of fungicides comes at the expense of human and animal health. The Integrated Disease Management (IDM) system is environmentally safe, long-lasting, and sustainable. The combined use of chemical, cultural, and biological techniques is always helpful in better managing *R. solani* compared to their individual use. Therefore, it may be possible to control the rhizoctonia root rot disease of soybeans effectively and efficiently through the integrated use of pesticides, bio-control agents, and natural organic matter (Tahmidur et al., 2020).

Management through chemicals is an effective and quick way to control diseases when they occur in epidemic form. Chemical control involves the use of different fungicides, such as Topsin-M, Mancozeb, carbendazim, carboxin, thiophanate methyl, and ipconazole, to control root rot of soybean. Seed treatment with carbendazim has been reported to be the most effective against root rot of soybean (Ajayi and Bradley, 2018).

Phyto-extracts have proven to be very effective without posing much danger to living organisms nowadays. It has been investigated that phytoextracts such as garlic (*Allium sativum*), ginger (*Zingiber officinale*), and *Eucalyptus* spp. inhibit 100 percent growth of *R. solani*, followed by *Azadirachta indica*, *Pongamia pinnata*, *Hibiscus rosa-sinesis*, and *Ocimum sanctum* (Patole and Narute, 2011). Keeping the whole scenario in view, the current study was designed to manage the root rot disease of soybean by using chemical fungicides and phytoextracts.

MATERIALS AND METHODS

Sample collection, isolation and purification of fungus

Soybean infected roots were collected from Agronomy fields of University of Agriculture, Faisalabad, and brought to the laboratory for isolation and purification of associated pathogen. Diseased samples were rinsed with tap water to remove dust and were cut into small pieces (2-3 mm). Surface sterilization was carried out with 1% sodium hypochlorite for 30 seconds and subsequently rinsed twice with distilled water. After drying these samples on blotting paper, these were placed on PDA media and kept for incubation at 25±2℃ for 48 hours. Samples were observed on daily basis for mycelial growth. Concerned pathogen was purified by growing tip method. Morphological characters of the pathogen were studied under compound microscope by staining Lactophenol cotton blue. Whitish growth of mycelial colony with characteristic multinucleated hyphal cells of about 4-15µm wide were identified (Ogoshi, 1987). The hyphae were branched at the right angles.

Pathogenicity test

Pathogenicity of the said pathogen was confirmed by employing Koch's postulate. Healthy seedlings of soybean were grown in earthen pots (10×15 cm). Seedlings of 5 inches were pulled out carefully and rinsed to do away dirt. Moist filter paper was placed at bottom of the trays. Then seedlings were placed in trays (27×19 cm) and 6 mm disc of fungal inoculum was placed at basal part of root where it emerges near soil line. Trays were completely sealed and placed in pathogenicity box after wrapping completely. Symptoms were observed after 11 days following the literature by Eken and Demirci, (2004).

In vitro evaluation of plant extracts against *Rhizoctonia solani*

Powder of three plant extracts (garlic, ginger and turmeric) were purchased from local market of Faisalabad (Table 1). Powder of above mentioned plant parts were dissolved in distilled water and sieved using four layer of muslin cloth. Eucalyptus leaves were washed with tap water to remove debris. These were shade dried and then sundried for few days. Dried leaves were ground to fine powder. Extraction was carried out using cold extraction technique. Three concentrations (3, 5 and 7%) were made by adding 3, 5 and 7 ml of extracts in 100 ml of PDA medium. Poisoned food technique was used for *in vitro* experiment to check efficiency against isolated pathogen (Ramaiah and Garampalli, 2015). The 5 mm disc of mycelium was taken from seven days old pure culture with the help of cork borer and placed in the center of Petri plate containing treated PDA, while control treatment contained un-amended PDA media. Incubation was done at 25°C for 7 days. Data were recorded at 24 hours of interval till three days.

Table 1. List of phytoextracts, their antifungal compounds and mode of action.

Common name	Botanical name	Antifungal compound	Mode of action	References
Garlic	*Allium sativum*	S- allyl cysteine	Enzyme inhibition	(Rousta et al., 2020; Curtis et al., 2004)
Ginger	*Zingiber officinale*	6-Gingerole	Immunomodulatory action	(Rasheed, 2020; Al-Askar et al., 2014)
Eucalyptus	*Eucalyptus*	Gallic and ellagic acid	Spore suppression and mycelial growth inhibition	(Amakura et al., 2002; Abdelkhalek et al., 2020)
Turmeric	*Curcuma longa*	Curcumin	Antioxidant	(Amalraj et al., 2017; Prajapati et al., 2021)

In vitro evaluation of different fungicides against _R. soalni_ using poisoned food technique

Four fungicides Tilt (propiconazole), Amister top (azoxystrobin + difenoconazole), Cabrio top (pyraclostrobin) and champion (copper hydroxide) were used against pathogen through poisoned food technique (Table 2). All the chemicals were tested at three different concentrations (50 ppm, 100 ppm, 150 ppm). Specific quantity of fungicides was mixed in semi cooled PDA media for desired results and shaken well to mix chemicals. Hand gloves were used to work with poisonous chemicals. PDA media was poured into petri plates and kept for solidification. After solidification, 5 mm disc of fungus was taken from 7 days old culture plate and inoculated in center of petri plates containing amended PDA medium. Three replications were prepared for each treatment. PDA without chemical acted as control. All Petri plates were wrapped with wrapping tape and labeled with permanent marker. Incubation was carried out at 25±2°C. Radial growth was measured for every treatment at the interval of 24 h, 48 h and 72 h and compared with control.

Evaluation of most effective fungicide, plant extract and their combination against root rot of soybean under greenhouse conditions

Experiment was conducted on soybean plants grown in earthen pots (15×17 cm) at green house trial area of Plant Pathology at UAF. For artificial inoculation *R. solani* was obtained from cultural plate grown on PDA media. The suspension was prepared by adding 10 ml of distilled water into Petri plates. The upper mycelial surface was obtained by gently scratching, and the resulting mixture was filtered through muslin cloth. Distilled water was then added for dilution. Soil drenching method was used for inoculation of pathogen. After ten days of inoculation, root rot symptoms were established. Lesions of dark brown color appeared on hypocotyl portion of root and whole stem girdled followed by wilting. After the establishment of disease symptoms, the most effective fungicide, plant extract, and their combination were applied using the soil drenching method (Prescott et al., 1986). Their antifungal potential against the disease was then assessed. Control pots were treated with distilled water.

T1= Tilt (1.5%) + Garlic (7%)
T2= Tilt (1.5%)
T3= Garlic (7%)

Statistical Analysis

All the experiments were conducted under Completely Randomized Design (CRD). The data were analyzed by performing analysis of variance (ANOVA) following least significance difference (LSD) test at 0.05% significance level. All the data were analyzed using "Statistics 8.1" software.

Table 2. List of fungicides, their active ingredients and mode of action.

Trade name	Active ingredient	Mode of action	Reference
Tilt	Propiconazole 25%	Demethylation inhibitor, Inhibit sterol biosynthesis affecting fungal cell wall production	(Somani et al., 2019; Leng et al., 2012; Uppala and Zhou, 2018; El-Enany et al., 2019)
Amister top	Azoxystrobin 200 g/l+ difenoconazole 125g/l	Prevention of respiration and prevent ATP synthesis	(Liu et al., 2021; El-Enany et al., 2019)
Cabrio top	Metiram 55%+ Pyraclostrobin 5%	Blocks mitochondrial electron transport and inhibit fungus energy supply	(Emara et al., 2021; Ranjan et al., 2021)
Champion	Copper hydroxide 770g/ kg	Disruption of cellular proteins	(Sinisi et al., 2018; Phatak et al., 2020; Singh et al., 2019)

RESULTS

Evaluation of plant extracts against *Rhizoctonia solani* causing root rot of soybean under lab conditions

Garlic exhibited the maximum inhibitory effect on the mycelial growth of *R. solani* (12.92±0.64 mm), followed by Ginger (13.05±1.03 mm), Eucalyptus (13.32±1.30 mm), and Turmeric (14.92±1.02 mm) compared to the control (26±0.64 mm) (Table 3). The interaction between (T×C) revealed that Garlic demonstrated the highest control of mycelial growth of the pathogen at 7% (4.55±0.39 mm), 5% (5.19±0.85 mm), and 3% (6.31±1.30 mm), followed by Ginger, Eucalyptus, and Turmeric compared to the control (Table 4). The relationship amongbetween treatments and days (T×D) indicated that Garlic showed the minimum mycelial growth (3.85±0.84, 5.43±1.04, 6.78±0.85 mm) of *R. solani* at 24 h, 48 h, and 72 h, respectively. This was followed by Ginger (3.83±0.89, 5.20±1.05, 7.42±0.64 mm), Eucalyptus (5.15±1.04, 6.12±0.49, 7.34±1.32 mm), and Turmeric (7.37±1.95, 7.49±0.74, 7.51±0.74 mm) (Table 5).

Table 3. Evaluation of treatments against fungal growth of *Rhizoctonia solani.*

Phytoextracts	Mycelial growth (mm)
Garlic	12.92±0.64 d
Ginger	13.05±1.03 c
Eucalyptus	13.32±1.30 c
Turmeric	14.92±1.02 b
Control	26±0.64 a
LSD	0.76

Mean value in this column having similar letters do not differ significantly as determined by LSD test (P<0.05)

Table 4. Impact of interaction of treatment and concentrations (T×C) on fungal growth of *R. Solani* under lab conditions.

Phytoextracts	Mycelial growth (mm)		
	Concentration		
	3%	5%	7%
Garlic	6.31±1.30 ac	5.19±0.85 ab	4.55±0.39 c
Ginger	6.81±0.72 ac	4.78±0.58 ad	4.85±0.48 a
Eucalyptus	5.57±0.85 bc	5.61±0.63 b	7.43±0.87 ac
Turmeric	7.58±0.89 a	7.44±0.87 ab	7.36±0.04 ab
Control	18±0.83 b	18±0.78 c	18±0.98 c
LSD	0.67		

Mean value in each column having similar letters do not differ significantly as determined by LSD test (P<0.05)

Table 5. Impact of interaction between treatment and days (T×D) on fungal growth of *R. Solani* under lab condition.

Phytoextracts	Mycelial growth (mm)		
	Days		
	24 h	48 h	72 h
Garlic	3.85±0.84 b	5.43±1.04 c	6.78±0.85 ab
Ginger	3.83±0.89 c	5.20±1.05 c	7.42±0.64 c
Eucalyptus	5.15±1.04 ac	6.12±0.49 b	7.34±1.32 c
Turmeric	7.37±1.95 b	7.49±0.74 b	7.51±0.74 b
Control	18±0.74 b	18±0.74 b	18±0.49 b
LSD		0.80	

Mean value in each column having similar letters do not differ significantly as determined by LSD test (P<0.05)

Evaluation of chemicals against *R. solani* causing root rot of soybean under lab conditions

The maximum inhibitory effect on the mycelial growth of *R. solani* was demonstrated by Tilt (4.52±0.78 mm), followed by Amistar Top (7.98±0.39 mm), Cabrio Top (10.45±0.48 mm), and Champion (12.45±0.87 mm) (Table 6). The interaction between (T×C) revealed that Tilt exhibited the highest inhibition of mycelial growth of the pathogen at all concentrations, i.e., 50 ppm (5.43±0.75 mm), 100 ppm (4.5±1.10 mm), and 150 ppm (4±0.83 mm), while the maximum growth was recorded by Champion (6.3±0.74, 8±1.95, and 5.8±0.85) at all three concentrations (Table 7). The relationship between treatments and days (T×D) indicated that Tilt showed the minimum mycelial growth of *R. solani* at 24 h (2.52±0.84, 4.53±1.04, 6.52±0.85 mm), followed by Amistar Top (6.04±0.89, 8.00±1.05, and 9.92±0.64 mm), Cabrio Top (8.34±1.04, 10.52±0.49, and 12.49±1.32 mm), and Champion (10.49±1.95, 12.45±0.74, and 14.42±0.74 mm) respectively (Table 8).

Table 6. Average mycelial growth of *R. solani* under different chemical treatments.

Fungicides	Mycelial growth (mm)
Tilt	4.52±0.78 e
Amister	7.98±0.39 d
Cabrio	10.45±0.48 c
Champion	12.45±0.87 b
Control	21±0.04 a
LSD	0.56

Mean value in the column having similar letters do not differ significantly as determined by LSD test (P<0.05)

Table 7. Impact of interaction between treatment and concentrations (T×C) on fungal growth of *R. solani* under lab conditions.

Fungicides	Mycelial growth (mm)		
	Concentration		
	50 ppm	100 ppm	150 ppm
Tilt	5.43±0.75 ab	4.5±1.10 ab	4±0.83 b
Amister	5.5±0.29 bc	5±0.93 bc	4.8±1.02 cd
Cabrio	6±0.85 d	10.4±0.89 c	5.4±0.93 b
Champion	6.3±0.74 b	6±1.04 ac	5.8±0.85 bc
Control	8±1.04 cd	8±1.95 ab	8±0.98 c
LSD		0.75	

Mean value in each column having similar letters do not differ significantly as determined by LSD test (P<0.05)

Table 8. Impact of interaction between treatment and days (T×D) on fungal growth of *R. solani* under lab condition.

Treatment	Mycelial growth (mm)		
	Days		
	24 h	48 h	72 h
Tilt	2.52±0.84 b	4.53±1.04 c	6.52±0.85 ab
Amister	6.04±0.89 c	8.00±1.05 ac	9.92±0.64 c
Cabrio	8.34±1.04 ac	10.52±0.49 b	12.49±1.32 c
Champion	10.49±1.95 a	12.45±0.74 d	14.42±0.74 b
Control	21±0.74 b	21±0.74 b	21±0.49 c
LSD		0.70	

Mean value in each column having similar letters do not differ significantly as determined by LSD test (P<0.05)

Evaluation of most effective plant extract and most effective fungicides individually and in combinations against root rot of soybean under greenhouse conditions

The treatment Tilt + Garlic exhibited the minimum disease incidence (4.52±0.85%), followed by Tilt (13.35±0.58%) and plant extract Garlic (15.75±0.63%), as compared to the control (40±0.87%) (Table 9). Interactions between treatments and weeks revealed that Tilt + Garlic resulted in the lowest disease incidence after 7 days (2.52±0.58%), 14 days (4.53±0.58%), and 21 days (6.52±1.49%), followed by Tilt and Garlic, as compared to the control (Table 10).

Table 9. Average disease incidence (%) of most effective plant extract and chemical and their combination under greenhouse conditions.

Treatment	Disease Incidence (%)
Tilt+ Garlic	4.52±0.85 d
Tilt	13.35±0.58 c
Garlic	15.75±0.63 b
Control	40±0.87 a
LSD	0.79

Mean value in the column having similar letters do not differ significantly as determined by LSD test (P<0.05).

Table 10. Impact of interaction between treatment and days on disease incidence (%).

Treatment	Disease Incidence (%)		
	Weeks		
	Week 1	Week 2	Week 3
Tilt+ Garlic	2.52±0.58 d	4.53±0.58 ac	6.52±1.49 b
Tilt	11.85±0.72 ac	13.43±0.98 a	14.78±0.84 ac
Garlic	13.82±1.00 b	15.85±1.03 b	17.59±0.92 b
Control	40±0.07 bc	40±1.32 c	40±0.74 c
LSD		0.60	

Mean value in each column having similar letters do not differ significantly as determined by LSD test (P<0.05)

DISCUSSION

Soybean (*Glycine max* (L.) Merr.) is a native crop of China classified under the family Fabaceae, genus *Glycine*, and species *G. max* (Prasanthi et al., 2019). *Rhizoctonia* root rot is caused by *Rhizoctonia solani*, with symptoms including red-brown lesions on the hypocotyl of seedlings close to the soil line and wilting of plants. The pathogenic fungus survives winter in agricultural debris and soil as sclerotia and mycelia, attacking more plants in spring (Tsror, 2010). Management through chemicals is a very effective and quick way to control diseases. Chemicals are fast, effective, and highly efficient in controlling specific pathogens. In this study, four different fungicides, Tilt (propiconazole), Amistar Top (azoxystrobin + difenoconazole), Cabrio Top (pyraclostrobin), and Champion (copper hydroxide), were used against *Rhizoctonia solani* using the poisoned food technique under lab conditions. The greatest inhibitory effect on the mycelial growth of *R. solani* was shown by Tilt at a 150 ppm concentration. Tilt contains propiconazole, which is a demethylation inhibitor, and inhibits sterol biosynthesis affecting fungal cell wall production (Uppala and Zhou, 2018; El-Enany et al., 2019).

In a previous study (Liu et al., 2021), the results showed that the EC50 of propiconazole and pyraclostrobin applied against *R. solani* on rice crops to check sensitivity revealed a resistance frequency of 16.7% for propiconazole and 13.3% for pyraclostrobin. In this study, the antifungal efficacy of various fungicides, including mancozeb, propiconazole, hexaconazole, carbendazim, and copper oxychloride, against *R. solani in vitro* was investigated using the poisoned food technique. When the medium was modified with fungicides propiconazole and carbendazim, the fungus growth was completely inhibited (Kumar et al., 2017).

Commonly, control of *Rhizoctonia* root rot of soybean relies on the application of chemicals, and the drawbacks of the long-term use of these chemicals produce resistance in the pathogen. The negative impact of chemical control on the environment has stimulated researchers to develop alternative strategies to promote sustainable agriculture. To overcome this problem, the use of phytoextracts is effective nowadays (Shuping and Eloff, 2017). In the present study, four plant extracts, Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Eucalyptus (*Eucalyptus* spp), and Turmeric (*Curcuma longa*), were used against *R. solani* using the poisoned food technique under lab conditions. The greatest inhibitory effect on the mycelial growth of *R. solani* was shown by Garlic with a 7% concentration, followed by Ginger, Eucalyptus, and Turmeric, as compared to the control. In a previous study, results by phytoextracts such as Garlic, Ginger, and Eucalyptus spp. showed 100 percent inhibition of the growth of *R. solani*, followed by *A. indica*, *Ocimum sanctum*, *Hibiscus rosa-sinensis*, and *Pongamia pinnata* (Patole and Narute, 2011). Our work is also supported by Dłużniewska (2018), who discussed that the botanical extract of garlic and grapefruit extract were significantly effective against *R. solani*. Results showed that all treatments stopped the mycelial growth, sclerotia germination, and biological activity of the pathogen.

The most effective plant extract of garlic and the most effective fungicide Tilt, along with their combined effect Tilt + Garlic, were used in controlled conditions against root rot of soybean in the greenhouse. Tilt + Garlic showed the minimum disease incidence, followed by Tilt and plant extract Garlic. The inhibition capacity of chemicals and plant extracts increased when used in combination against root rot of soybean caused by *R. solani*. More growth reduction was observed when the pathogen was treated with the most effective plant extract Garlic (7%) and the most effective chemical Tilt (1.5%) in their combination, followed by the individual use of Garlic plant extract and chemical Tilt. The chemical was more effective than the plant extract in greenhouse conditions. The effect of combination treatments of plant extract along with chemicals has been already reported by Kachelo et al. (2022) and Atiq et al. (2018) on different fungal pathogens.

In the past 30 years, research on oilseed crop diseases has grown significantly, particularly in an effort to lessen losses caused by various factors such as crop diseases (Chattopadhyay et al., 2015). Knowledge on agricultural diseases and how to treat them must be updated and improved to combat hunger and malnutrition.

AUTHORS' CONTRIBUTION

NAR and MA conceived the idea; AB conducted the research; NAK analyzed the data; AH and MEK helped in data collection; GAK and HT helped in laboratory experiments; NAR and MA wrote the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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