

ORIGINAL RESEARCH ARTICLE

Identification of aggressive microfungal pathogens of wheat crop from Bhimber Azad Kashmir and effective biomanagement of a most dominant pathogen *Alternaria solani*

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ABSTRACT

Fungal diseases affect both the productivity and sustainability of wheat crop. The aim of present study was to document the fungal diseases of wheat crop and biological management of a most dominant fungal pathogen *Alternaria solani* by using medicinal plant extracts. Fungal infected wheat plants were collected from different sites of District Bhimber, Azad Jammu and Kashmir (Sukasan, Kool, Gurha Liliyan, Gurha Matyal, Dheri Wattan, and Pothi) and these fungal infected wheat plants were examined under field and greenhouse conditions by using four different plant extracts to check the severity rate of fungal diseases. Three contagious species were isolated from regularly contaminated wheat leaves and other natural items demonstrating the indications of scourges and most dominant species recognized as *A. solani*. The antifungal action of four plant's leaf solutions including *Nerium oleander* (Oleander), *Allium sativum* (Garlic), *Ocimum basilicum* (Sweet Basil) and *Eucalyptus chamadulonsis* (Eucalyptus) have been attempted to control *Alternaria solani* in an artificial medium and in natural conditions. In an artificial medium, decoction of *Allium sativum* bulb at 5% fixation caused the most noteworthy lessening in mycelial improvement of *A. solani* (40.2%), while *Ocimum basilicum* at 1% and 5% fixation and *Nerium oleander* at 5% fixation the most decreases obstruct the mycelial advancement of the microorganism. In nursery analyzes, the most serious diminishing in contamination seriousness was brought about by the bulb extract of *Allium sativum* on 5% fixation as well as *Nerium oleander* on 1% and 5% fixations. The best decline in infection seriousness was accomplished with *Allium sativum* on 5% fixation, but the slightest decrease was observed after treatment of wheat plants with *Nerium oleander* on 1% and 5% application (22.3% and 30.2%, respectively). *Allium sativum* at 5% application expanded natural product yield by 35.2% and 40.2% as opposed to contaminated control. It was concluded that all selected medicinal plants reduced the selected disease and furthermore upgraded grain yield as opposed to contaminated control in field crop diseases especially *Alternaria solani* attack on wheat.

Keywords: Wheat Crop; Fungal Pathogen; *Alternaria Solani*; Biological Management; Antifungal Activity

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1. Introduction

Wheat is a prevalent assembled crop which is utilized as sustenance of human beings and forage for livestock. The flourishing of wheat crop depends relatively upon the adaptability and exceptional yield return potential in addition the part of gluten protein that presents visco-flexible belongings allows the paste of wheat grains to be utilized in bread, pasta, noodles, and other in other items^[1]. It is the main grain producing crop that yields about 20% of the world's food needs. Around two third of the total population of world use wheat as staple food^[2]. *Triticum aestivum* L. is located between the huge grain producing crops

and acknowledges an essential limit in sanitation of proliferating hungry millions in Bangladesh. In forthcoming time span that prepares for 2020, it is believed that the interest for wheat for human utilization in rural nations will create by 1.6% every year^[3]. In this line, increase in wheat yields is a basic step in maintaining global food security. Latest research on environmental change expects a clear increase in precipitation and temperature.

Additionally, wheat crop contributes the fundamental amino acids, minerals, nutrients, advantageous phytochemicals, and dietary fiber segments in the human diet, and is especially wealthy in entire grain items. Nonetheless, wheat items are likewise known to be mindful or proposed to be answerable for various unfavorable responses in human beings, including intolerance (especially celiac illness) and hypersensitivities. Wheat is considered as a significant piece of the “enormous three” grain crops, with in excess of 600 million tons being gathered yearly. For instance, in 2007, the entire outside creation stood around 607 million tons come close to 652 million tons of rice and 785 million tons of corn. However, wheat has no competitor in its evolutionary range^[4]. Among the wheat producing nations, Pakistan remains at tenth spot in term of region (8.5 million hectares) and 59th as far as yield, for example, 21.0 million tons. Current cultivation of wheat in Pakistan is around 95 million tons it assessed that by 2020, the interest will be around 137 million tons. Pakistan began from creation of around 3,814 kg in 1961 and came to up to the degree of 25,214 kg in 2011^[5].

Wheat crop is exposed to various diseases, which lessens its general production to large extent, since wheat plants in all phases of development and

in all common habitats are dependent upon different mechanical, physiologic and natural burdens that meddle with their typical development and advancement. Biotic dangers, bugs, infections, fungal species, nematodes, microbes and weeds play essential roles to deteriorate wheat production^[6]. Wheat, like other different cereal crops is subjected to the varieties of fungal pathogenic attacks. In serious attack, it might result even more than 75% decrease in grain yield^[7]. The fungal pathogens include the maximum reduction and more prominent attack on wheat^[8]. These fungal diseases can be passed starting with one plant then onto the next through wind and different sources^[9]. The causative agent of wheat stem (black) rust, generally dispersed, however, is more unusual than the other types of wheat rusts and *Alternaria* spp. attack^[10,11]. These organisms may cause diverse foliar blight infections like Septoria, spot blotch, tan spot, net blotch, *Fusarium* head blight and *Alternaria* attack^[8,12,13].

Alternaria solani (Ellis and Martin) Jones and Grout, is considered the most damaging causal agent of fungal diseases^[14]. As a result of *A. solani* attack, a major decrease in crop quantity and quality have been documented. The early symptoms of *A. solani* are tiny dark brown spots on the older leaves lower side. The tissue around the primary lesions will turn bright yellow and the entire leaves can become necrosis and chlorotic if the lesions are multiple. The spots are enlarged and they form concentric rings that give them the eye of a bull. Diseases grow in favorable weather conditions, accidents can become numerous and plants defoliate, destroying the quantity and quality^[15]. Then gradually each part of plant can infect by *Alternaria solani* and reduced plant growth^[14]. Therefore, it is

necessary to treat this disease for control. Thus, the use of chemicals for disease control needs to be reduced. Therefore, to test bioagents against *A. solani* is save and eco-friendly management technique to control the disease spread.

2. Objectives

The current research was focused on isolation and identification of dominant diseases of wheat crop growing in District Bhimber, Azad Kashmir, Pakistan. The second objective is to find severity rate of each isolated fungal species from the study area. The third aims of the research project is biological management of a most dominant fungal disease of wheat by use of selected medicinal plant extracts.

3. Methods

In the current research, fungal infected samples of wheat (*Triticum aestivum*) cultivars were obtained from various wheat fields of District Bhimber, Azad Kashmir. After that fungal species were separated from infected parts of wheat by use of microscope. Then these species were identified which indicated various diseases on wheat crop. The infectious species have been isolated from normally infected leaves and wheat grains.

Pathogenicity test was conducted for measurement of efficacy of the most dominant fungal pathogen of wheat crop. The antibody was prepared by testing each of the individuals on PDA refining medium at 27 °C for 15 days. At that point, 10 mL of clean filtered water was added to each plate and the sets were deliberately broken with a sterile needle. The conidic suspension of each boundary was shifted to 5×10^6 spores/mL and was used to inoculate 20 wheat plants with a nebulizer. After inoculation, the plants were covered with

polyethylene packs for 48 bags to maintain high humidity conditions. After 48 h, the bags were removed and the plants were kept in nursery settings. The pots were specially organized during the planting season. Fourteen days after vaccination, the severity of the infection was recorded. Examination was repeated twice^[16]. Decline in inoculum creation followed by a concealment of its capacity to contaminate which make an accumulative impact more than a few infection cycles in patho frameworks where inoculum delivered inside the field has significant contribution to the advancement of epidemics.

4. Preparation of extracts

Extracts from the leaves of 4 medicinal plant species, including *Nerium oleander*, *Allium sativum*, *Ocimum basilicum*, and *Eucalyptus chamadulonsis*, were prepared from different parts of District Bhimber AJK. 10 g of fresh leaf material was collected from each plant species, washed with water, crushed with mortar and gently crushed by adding pure water at the rate of 10 mL/g plant tissue, and the homogenates were centrifuged at 10,000× for 15 min at 4 °C and the supernatant reaction lines were collected. The plant extract was diluted to 1% and 5% dilutions^[17].

4.1 In vitro testing of 4 medicinal plants extracts

To measure the antifungal activity of selected medicinal plants against *Alternaria solani* using the vitro agar well diffusion method. In this method, added dilutions in wells created one PDA media containing petri dishes. The dilutions of 5 mL of leaf extracts with ratio of 1% and 5% of each plant independently freely were inoculated with mycelial circles taken from the floating edges of 7 days old

orders of *A. solani*. In the control a test, refined water was used instead of plant extracts. The embedded media was placed at 27 °C. 4 plates for each treatment were used as replicas. The width or zone of inhibition (ZI) of the attractive settlement was assessed using a one-meter line along the diagonal or two pairs of diagonals on each side of the petri dish. Each treatment was divided into three portions with four plates for each replication.

4.2 Response of medicinal plant extracts against selected fungal disease under greenhouse trials

Plant solutions were treated in 1% and 5% fixations as a leaf spray on 7 weeks old wheat plants, and periodically up to 60 days. Wheat plants were vaccinated with a 20 mL of *A. solani* suspension. After vaccination, the plants were stored in an air chamber with a daily temperature of 28 °C and an average humidity of 85%. The infection was recorded 15 days after the vaccination. The investigation was repeated twice.

4.3 Testing of plant extracts for fungal diseases of wheat under field conditions

Field settings focused on the experiment followed during the growing season. There were 2 rows and 5 field plants for each section, arranged in a completely irregular square layout. 3 plots were used as a duplicate of each treatment identical for unrelated treatments. Improvement of the infection was recorded 15 days after inoculation. Field rounds were repeated twice. At cutting time, the ideal harvest yield was set for each treatment with untreated control. 10 plants per treatment were collected to examine the absolute yield of each treatment.

5. Statistical analysis

The entire investigations were repeated two times. Assessments demonstrated no basic connection between the two tests for any of the medications. In this vein, the consequences of duplicate tests were assembled for the last assessment. Variation in examinations was finished by utilizing MSTAT-C programming variation 2.10. The LSD was utilized to test incredible differentiations between medications $\leq 0.5^{[18]}$.

6. Results and discussion

Fungal infected wheat plants were collected from different regions of District Bhimber, Azad Jammu and Kashmir, and these fungal infected wheat plants were examined under lab and greenhouse conditions by using 4 different plant extracts to check the severity rate of dominant diseases. 7 fungal species were isolated from regularly contaminated wheat leaves and other natural items demonstrating the indications of diseases and the most dominant recognized pathogen named as *Alternaria solani*. **Table 1** showed the dominant fungal diseases with their severity rate present on wheat crop in the study area. Among these diseases of wheat crop, the highest severity rate of disease rate is shown by black mold (*Alternaria solani*) which is 90%, and lowest severity rate is shown by the common root rot, foot rot and crown rot disease which is 50%, respectively.

For the management of *A. solani* infection, 4 types of medicinal plants were distinguished and assessed for antifungal activity against *A. solani*. Results revealed that all the tried leaf solution of different plants including *Nerium oleander*, *Allium sativum*, *Ocimum basilicum* and *Eucalyptus chamadulonsis* indicated a good decline in the di-

rect development of *A. solani* on wheat crop. Comparative impacts of various plants leaf extract solution powerful against *Alternaria* spp. were accounted for by a few investigators^[16,19]. Vijayan^[20] described that leaf solution of *A. sativum*, and *Catharanthus roseus* flower solution hindered spore germination and mycelial development of *A. solani*. The inhibitory influence of tried concentrates might be due to their uninterrupted harmful

impact on microbes. Studies on the processing of infections concealment using plant items proposed that the dynamic properties found in plant concentrates may act straight forward proceeding upon microorganisms^[21], otherwise cause foundational obstruction in plants, which reduces the advancement of the infection^[22].

Table 1. Analysis/measurement of severity of some dominant fungal diseases present on wheat crop in the study area

S. No.	Identified dominant diseases on wheat crop		Severity rate
1	Leaf Rust (Brown Rust)	<i>Puccinia triticina</i>	60%
2	Stem Rust (Black Rust)	<i>Puccinia graminis tritici</i>	80%
3	Stripe Rust (Yellow Rust)	<i>Puccinia striiformis</i>	75%
4	Loose Smut	<i>Ustilago tritici</i>	72%
5	Black Molds (Sooty Molds)	<i>Alternaria solani</i>	90%
6	Powdery Mildew	<i>Sclerophthora macrospora</i>	55%
7	Common Root Rot, Foot Rot, and Crown Rot	<i>Rhizocotonia solani</i>	50%

6.1 Pathogenicity tests results

The results exhibit that all the tried isolates of *A. solani* were equipped for tainting wheat plants causing basic blight signs with changing degrees of infection seriousness. Recorded data revealed that segregates 1 and 3 were profoundly transmittable and triggered noteworthy seriousness of the infection.

6.2 Effect of plant extracts on radial growth of *A. solani*

4 types of plants were distinguished and assessed for antifungal activity against *A. solani*. Leaf concentrates of tried plants with 1% and 5% application were strong in hindering the extensive progression of *A. solani*, as opposed to control. In vitro effects of four plant extracts on the linear

growth of *A. solani*. 5 mm of leaf solutions from every one of the plant tests were blended by using 45 mL PDA medium. Each treatment was repeated several times with 4 plates for each replicate. At 5% fixation, leaf solutions of *D. stramonium*, *A. indica* and *A. sativum* caused the most noticeable expansion in the mycelial growth of *A. solani* (44.4%, 43.3% and 40.2%, respectively). *E. chamadulon-sis*, *D. stramonium* 1% fixation, *O. basilicum* with 1% and 5% application and *N. oleander* with 1% application caused minimal hindrance in improving the mycelial micro flora. These finding were supported by Curtis *et al.*^[23]. The effect of plant extracts on fungal diseases in wheat under artificial infection in greenhouse and field trials on different concentrations of four plants including *N. olean-*

der, *A. sativum*, *E. chamadulonsis* and *O. basasilicum* fundamentally reduced the fungal infections

as mentioned in **Table 2**.

Table 2. The response of selected medicinal plant treatment against *A. solani* at 1% and 5% fixation

Treatments	Concentration (%)	Linear growth (cm)	Reduction %
<i>Nerium oleander</i>	1	5.9	22.3
	5	6.3	30.2
<i>Allium sativum</i>	1	6.5	35.2
	5	5.5	40.2
<i>Eucalyptus chamadulonsis</i>	1	7.5	29.8
	5	6.9	30.0
<i>Ocimum basilicum</i>	1	7.0	25.2
	5	6.8	21.3

Medications by using plant solutions reduce the fungal diseases under field circumstances. The best decline in the seriousness of diseases was accomplished through *A. sativum* with 5% application and slightest diminishing was acquired by treating wheat plants with *N. oleander* on 1% and 5% application (22.3% and 30.2%, respectively). Various medications were observably successful.

Table 3 illustrated that effectiveness of using plant separates is redirected in grain income. Kernels showered by *A. sativum* on 5% fixation expanded harvest to 39.5%, as opposed to the untreated control. Alternately, leaf solutions of *O. basilicum*, *E. chamadulonsis* as well as *N. oleander* modestly expanded the harvest in between 52.1%, 45.6% and 30.5% rather than the contaminated control. Greenhouse and field tests demonstrated that foliar showers comprising plant extracts on wheat crop carried about critical lessening in the premature rust contamination. These consequences were like past efforts preceding the job of plant so-

lutions controlling contagious infections. A few investigators like Curtis *et al.*^[23], Krebs *et al.*^[24] and Latha *et al.*^[16] validated those essences of twenty non-plant species triggered the decrease in different contagious infections as well as inhibited mycelial development of *A. solani*. Treatments using plant solutions upgraded harvest of wheat plants contrasted with control of infection.

7. Conclusion

The investigation demonstrated that dominant fungal species was *Alternaria solani* that indicated 90% severity rate. As four medicinal plants application, for example *N. oleander*, *O. basilicum*, *A. sativum* and *E. chamadulonsis* can be utilized for the bio control of the contagious infections of *A. solani* on wheat crop. This control strategy can accordingly add to reduction the risks of poisonous fungicides, particularly on wheat crop which are good alternatives. Therefore, it was concluded that medicinal plants extracts indicated very effective biomanagement of *Alternaria solani* disease spread on wheat crop significantly.

Table 3. Response of four medicinal plant leaf extracts on the dominant diseases of wheat crops under greenhouse trials and yield of wheat under field conditions

Treatment	Concentration (%)	Greenhouse conditions		Field conditions			
		Disease severity (%)	Reduction (%)	Disease severity (%)	Reduction (%)	Yield (t/h)	Increase
<i>N. oleander</i>	1	34.2	31.9	30.2	37.2	2.9	28.0
	5	28.3	40.0	32.1	40.7	3.0	30.5
<i>A. sativum</i>	1	25.7	45.0	34.5	44.5	3.1	33.7
	5	27.4	47.2	36.1	47.5	3.2	39.5
<i>E. chamadulosis</i>	1	29.7	30.5	40.0	50.0	3.3	42.1
	5	25.4	50.1	42.4	52.2	3.4	45.6
<i>O. basilicum</i>	1	30.2	60.6	45.2	54.1	3.5	49.0
	5	32.3	69.7	47.1	57.3	3.7	52.1

Conflict of interest

The authors declare no conflict of interest.

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