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Antifungal Potential of Corolla Extracts from *Butea monosperma* and *Calotropis procera* against Wheat Fungal Diseases Identified from District Bhimber, Azad Kashmir

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ABSTRACT

The present study documented the fungal diseases of wheat crop and their biological management by using corolla extracts of two medicinal plants '*Butea monosperma* and *Calotropis procera*'. The highest infection rate (57.14%) was observed against *Fusarium graminearum* while minimum infection rate (20.8%) was observed against *Blumeria graminis* pathogen. The highest severity rate was recorded at 80% while the minimum severity rate was recorded as 40%. The antifungal responses of corolla extracts have been applied against the most prevalent fungal pathogens of wheat. The highest zone of inhibition was observed in methanolic extract of *Butea monosperma* petals against fungi *Alternaria triticina.* Similarly, the maximum zone of inhibition was observed in methanolic extract of *Calotropis procera* against fungi *Fusarium graminearum*. It was indicated that the treated wheat plants produced better yield than non-treated plants. It was observed that the *Calotropis procera* showed more potential for the management of fungal diseases than *Butea monosperma.* So, it was concluded that the *C. procera* petals are more efficient because they have rich chemical compositions. Therefore, these were considered very effective against selected fungal pathogens of wheat.

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INTRODUCTION

Wheat *(Triticum aestivum* L.) is a grass that is widely harvested for its seeds (Anonymous, 2010). Wheat is grown all over the world and it is only the cereal crop that covers most areas of the world (Isitor *et al*., 1990: Langer and Hill, 1991). Wheat crop is locally used as chuff for livestock and their straw can be used as animal feed after harvesting (Diamond, 1997). Wheat crop is very important in Pakistan due to its multiple and congential uses in different industries. It is locally used as chuff for livestock and their straw can be used as animal feed after harvesting (Diamond, 1997). The waste material of wheat is used in making livestock feed.

Butea monosperma is a medium-sized tree that belongs to the *Fabiaceae* family and the Papilionaceae subfamily. It is a *Butea* species native to the subcontinent's tropical and subtropical regions (Kaur *et al.,* 2018: Rai *et al.,* 2020). Flame of the Forest is a frequent name for it. A range of compounds are obtained from this tree's stem, bark, roots, leaves and flowers. These compounds are being used in the treatment of different kinds of disorders. Flowers of *Butea monosperma* were used to make natural color during the festival of Holi (Rai *et al.,* 2020).

Calotropis procera is a species of flowering plant that belongs to the family Apocynaceae. The plant is harvested from wild areas. It is introduced in Australia as a garden plant or used in the packing of camel saddles which are brought from India in 1900s (Crothers and Newbound, 1998). It is considered an ornamental plant. It is primarily harvested due to its distinctive medicinal properties. The abundance of latex in the green parts of the plant produces and accumulates as a defensive strategy against organisms such as viruses, fungi, insects and large herbivores. It has been found that latex is used by indigenous people to successfully combat some cutaneous fungal infections (Larshini *et al.,* 1997: Begum *et al.,* 2012). *Calotropis procera* is an ideal insecticidal plant. Crude plant extracts are beneficial in terms of efficacy and pest resistance management as the active substances present in them act synergistically (Ajay *et al.,* 2011).

Fungal diseases of wheat crop: A plant sickness happens when an organic entity contaminates a plant and upsets its typical development and propensities. Mostly plant diseases approximately about 85% diseases of plants/crops are caused due to fungi or fungi-like organisms (Isleib, 2012). The wheat crop is exposed to various fungal diseases, which minimize the general production to a large extent (Ahmad *et al*., 2003).

Wheat rust disease: Wheat rust disease is caused due to pathogen *Puccinia graminis* (Cummins and Hiratsuka, 2004; Duplessis *et al*., 2012). The casual specialist of wheat stem (dark) rust is generally dispersed everywhere but it is not uncommon compared to other types of wheat rust (Leonard and Szabo, 2005: Singh *et al*., 2015). The symptoms of contamination usually appear as a cluster of red, black uredinio spores on the leaf surfaces, stems, glumes and awns of injected plants (Kolmer, 2005).

Leaf rust disease: Leaf or brown rust of wheat is caused due to *Puccinia recondita. P. recondita* spread rapidly under the favorable conditions of the environment. (Roefls *et al*., 1992). Identifying symptoms of leaf rust are dusty, reddish-brown fruiting bodies which contain spores that appear on the leaf surfaces, usually causing leaf distortion and defoliation. This disease minimizes the core mass and seed quantities of wheat per head (Kolmer, 2005: Huerta *et al*., 2011).

Stem rust disease: Stem rust also known as cereal rust, black rust or red rust is caused by the pathogen *Puccinia graminis* (Roefls, *et al*., 1992). It mostly occurs in hot and slushy areas. The side effects of this disease include clusters of red, black uredinio spores found on the leaf surfaces, stalks, glumes and canopies on infected part of wheat crop (Kolmer, 2005). In growing season, the spores transformed and become dark colored (black) (Leonard and Szabo, 2005).

Blotch disease: *Bipolaris sorokinaina* is mainly responsible for the spot blotch diseases of wheat (Figueroa *et al*., 2018). The initial symptoms of leaf blotch commonly first appear as yellow spots on the lower sides of leaves, then they increase in size and become grey or brown, ovate-shaped lesions (Chaurasia *et al*., 2000). Spot blotch is totally different from Alternaria blight by the development of dark spot areas, which shows bunches of conidia which produced at late infection stages (Neupane, *et al*., 2010: Viani, *et al*., 2017).

Root rot disease of wheat: The Root Rot disease of wheat is caused by *Bipolaris sorokiniana*, along with other associated fungi which includes *Fusarium pseudograminearum, F. culmorum, Microdochium nivale, Pythium spp.,* and *Rhizoctonia cerealis* (Tunali, *et al*., 2008: Moya-Elizondo, *et al*., 2011 and Saremi, *et al*., 2011). Root rot of wheat plants turns the roots of the affected plant soft and black/brown in color. When touched the plants affected root may also fall and leaves become wilt, small and discolored. Wheat root rot is diagnosed by the appearance of necrotic bruises on the roots (Al-Sadi and Deadman, 2010: Qostal *et al*., 2019).

Smut disease of wheat: Loose smut of wheat is caused by pathogen *Ustilago tritici* which belongs to the family Ustilaginaceae (Sammour, 2015). The major symptoms of loose smut include smutted grain heads which contain black or brown spores, yellowish leaf streaks and stiff, dark green leaves. Affected wheat plants head out early and produce sterile heads with clumped-black spores (Davila, 1989). The symptoms of this infection are not clearly seen until wheat plant reached the development or heading phase. These spores proceed with the help of breeze and rachis stay in that area (Ivanova *et al*., 2004).

Kernel bunt disease: Kernel bunt or partial bunt is induced by *Tilletia indica*, it is one of the types of wheat smut infection (Mitra, 1931). Kernel bunt usually occurs on bread wheat (*T. aestivum*) and durum wheat (*T. turgidum*) (Agarwal *et al*., 1977). Seed-like bunt balls replace sick wheat plant ears, which contain oily, black, and foul-smelling spores. In extreme circumstances, the entire field may reek of decaying fish. Capsules in embryonic kernels got contaminated by *Tilletia indica*

pathogen and it also spread the gains fully or partially into thin flocks with dull shadows of teliospores (Shakoor *et al*., 2014).

Powdery mildew of wheat: Powdery mildew of wheat caused by obligate, bio-trophic ascomyceteous fungus *Blumeria graminis* f. sp. Tritici, *Erysiphe graminis,* both of belongs to family Erysiphaceae (Wiese, 1987; Gacek 1983). Powdery mildew symptoms include fluffy, white powdery fungal spore growths on the surface of the leaf and glumes of the head (Esmail and Draz, 2017). Before mycelia develop, early signs may appear as yellow spots on leaves. The infection of wheat powdery mildew is from lower to the apex of leaves and stem (Draz *et al*., 2019). The spots on the infected parts of wheat are initially white but after some time they change their color to black and become (Daamen, 1989).

***Fusarium* head blight of wheat:** *Fusarium graminearum* causes *Fusarium* head blight (also known as wheat scab or ear blight) (Parry *et al*., 1995), which causes premature senescence of the wheat head (Stack, 2003). *Fusarium* head blight begins to show symptoms soon after flowering. As the pathogen develops and spreads within the head, diseased spikelet's bleach prematurely (Miller, 1994). Infected spikelet's bleach, and the disease spreads upward and downward by infecting spikelet one by one, especially nears the base. Spikelet's may also have little, black fruiting structures (Windels, 2000).

Leaf blight of wheat: *Alternaria triticina* causes leaf blight in wheat (Prasada, 1962). Small, oval, discolored lesions randomly spread on the leaves are the earliest signs of infection (Prabhu and Prasada, 1966). The lesions turn dark brown to grey in color and uneven in shape as they grow larger. Only hosts older than three weeks will become infected, with symptoms developing at 7-8 weeks of age. Lesions begin as oval scars on the lower leaves and spread to more leaves as the plant matures (Perello *et al*., 1992). The lesion may be covered in black powdery conidia. The lower leaves are the first to exhibit signs of illness (Prasada, 1962).

Biological management of wheat diseases: Biological methods are very effective, safe and economically sound methods for the management of diseases (foliar and seed-borne) of wheat caused by fungi (Ehab *et al*., 2016). This technique uses different medicinal plants derived products are used as a spray on crops. Biological control of weed pests is important to lower the impact of diseases (Naraynanasamy, 2013). Currently, local farmers in our country are using crude extracts of different medicinal plants as spray on foliar parts of wheat and maize crops for control of antifungal infections from parts of plants (Askar and Rashad, 2010). In vitro laboratory tests have shown that extracts from a variety of higher plants have antifungal properties. Wild plants appear to be a prospective source of beneficial metabolites (Iason *et al*., 2012).

Chemical treatment of fungal diseases is a current trend for biological management. Fungicides and different chemicals are used at large scale for the management of fungal diseases of crops, but they have lot of hazardous side effects on crops and surrounding environment. The chemical also changes their genetic makeup. So, a good alternative to chemicals is biological management of fungal diseases. Hence, the biological management of fungal diseases through medicinal plant extracts is a more appropriate and effective method that has less environmental side effects (Maj *et al*., 2024).

Objectives of the Study: The current study was focused on the identification of fungal diseases of wheat crop from district Bhimber, Azad Kashmir. The most prevalent fungal diseases of wheat were selected for their bio-management. The corolla of two medicinal plants were used for the effective control of fungal diseases of wheat crop. To explore the comparative results of treated and non-treated samples with corolla extracts of two selected medicinal plants for the assessment of their medicinal potential.

MATERIAL AND METHODS

Sample collection

The floral and fresh samples of *Butea Monosperma* and *Calotropis procera* petals were collected from different local areas of District Bhimber, AJK. The petals of these plants were surface sterilized with 5 % dilutions or concentration of Sodium hypochlorite for 2 to 3 minutes then washed with distilled water thoroughly to remove debris. Then these floral samples were shade dried for 10-15 days until all the water molecules evaporated and they become dried completely for the grinding. After drying of petals, they were grinded by using a mechanical blender into a fine powder. A similar collection method was used by Jabeen *et al*., (2013) with some modifications.

Assessment of disease incidence and severity

For the gathering of wheat cultivar Galaxy-13 samples, a survey was conducted in the field by X diagonal transect method. The plants in the plot were tested for wheat

diseases, and leaf samples were collected by X diagonal transect method as following by Sharma and Duveiller, (2003) from specified locations and brought to the lab for further fungal analysis (Iftikhar *et al*., 2012). Using the technique of Teng and James, (2001), wheat plants were selected for documentation of the occurrence of various forms of foliar fungal infections.

$$Incidence(\%) = \frac{\text{No. of infected Plants}}{\text{Total no. of plants observed}} \text{ X } 100$$

$$Severity(\%) = \frac{\text{No.of infected plants in field}}{\text{Total no.of plants in a field}} \text{ X } 100$$

Isolation of fungi on crop

In the agar plate method collected samples were transferred to sterile PDA media. Before this step, PDA medium was prepared by mixing 39g Agar in 1000ml distilled water. The medium was placed on stirrer for about five minutes to make a uniform mixture. Then this mixture is autoclaved at 121°C temperature in autoclave for sterilization. The PDA media transferred into each petridish (15ml per petridish) under aseptic conditions when temperature of the sterilized media reached 45°C or easily touched media containing flask (Domsch *et al*., 1980). Different types of infectious species which indicate appearance of pests were isolated from wheat crop. Isolated fungi were also purified by sub culturing on sterilized PDA media (Usmani and Ghaffar, 1982).

Field management strategies

Crude extracts of medicinal plants *Butea monosperma* and *Calotropis procera* petals were sprayed with a concentration of 25 ml dilutions of desired plants in 100 ml water on selected wheat plants for reduction of fungal contaminations and better yields. The yield of each sample plot was weighed after threshing, adequate drying, and cleaning (Pathak and Razia, 2013). The quantity of yield of each wheat variety was documented as shown in results.

Preparation of extracts

Petals of *Butea monosperma* and *Calotropis procera* were collected from the District Bhimber. According to the approach given by Kareem *et al*., (2008), the petals of collected plant samples were ground to semi-powder using a grinder. The petal extracts were prepared by adding 25g powder of both plant petals i.e. *Butea monosperma* and *Calotropis procera* and mixed in 250ml of different solvents i.e. petroleum ether, Di ethyl ether, methanol, chloroform, distilled water in conical flasks. The flasks that contain powder of petals were shaken thoroughly so that all the contents mixed well, corked, and saved them at room temperature for 7 days. Removed all the solid materials from extract by simple filtration method by using Whatman No. 1 filter paper. The process was repeated three times, and the obtained extracts were pooled. A stock of solution of each extract was kept in the refrigerator at 4 degrees Celsius until use (Draz, *et al*., 2019).

Agar well diffusion method

The agar well diffusion method (AWD) is extensively used to assess the antifungal activities of plant extracts or fungal extracts. The AWD method was applied for the measurement of zone of inhibition (ZI) during in vitro antifungal activity. Inoculate the agar plate surface with this method by spreading a volume of fungal inoculum over the entire agar surface. Then, using a sterile cork-borer or a tip, make a hole of 6-8mm in diameter and cuff it aseptically. Introduced an antifungal agent or plant extract solution into the well in a volume of 20-100µL at the desired concentration. Then, depending on the test microorganisms, incubate the agar plates under favorable conditions at room temperature (25^{o}C) for 3 days. The antifungal drug diffused into the agar medium, preventing the fungus strain from growing (Silici *et al*., 2006). After 3 days of incubation period, ZI was measured by using simple scale in millimeter and documented in tabulated form as mentioned in results below.

RESULTS

This study covered the medicinal potential of *Butea monosperma* and *Calotropis procera* petals against the fungal pathogens that affect the wheat crop. Fungal infected wheat plants were collected from different regions of District Bhimber Azad Jammu and Kashmir (Gurha Rehman, Gochar, Gurha Liliyan, Gurha Matyal and Dheri Wattan) and these fungal infected wheat plants were examined by using two different plant petals extract (*Butea monosperma* and *Calotropis procera*). Various diseases of wheat crop are described with their causative agents, symptoms and part effected by fungi were shown in Table 1. The discussed diseases were already reported in previous work that described the symptoms of diseases which showed on different parts of wheat plant (leaves, stem, root, kernels, glumes etc).

Table 1. Survey of fungal diseases of wheat crop based on morphological characters collected from district Bhimber, AJK.

Sr. No.	Fungal pathogen	Disease Name	Parts affected of wheat plant	Symptoms
01	*Puccinia graminis*	Wheat stem rust	Both sides of leaf, stem and glumes	Red, black uredinio spores (Kolmer, 2005)
02	*Puccinia recondita*	Wheat leaf rust	Upper surface of leaf and leaf sheath	Dusty- reddish brown fruiting bodies (Kolmer,2005; Hureta *et al*., 2011)
03	*Bipolaris sorokinaina*	Blotch disease	Leaf spikes and roots	Yellow to grey or brown lesions (Figueroa *et al*., 2018)
04	*Fusarium culmorum*	Root rot disease	Roots or crown tissues	Black or brown lesions (Tunali *et al*., 2008; Moya-Elizando, *et al*., 2011)
05	*Ustilago tritici*	Loose smut disease	Glumes and kernel spikes	Yellowish leaf streaks, smutted grain heads (Davila, 1989)
06	*Tilletia indica*	kernel bunt disease	Grains	Dark color and fishy smell on infected kernels (Shakoor *et al*., 2014)
07	*Blumeria graminis*	Powdery mildew disease	Leaves and glumes of head	Fluffy white powdery growth Gacek,1983
08	*Fusarium graminearum*	*Fusarium* Head Blight Disease	Spikelets and kernels	Shriveled, chalky and discolored white-pink kernels (Windels, 2000)
09	*Alternaria triticina*	Leaf Blight of Wheat	Leaves	Small oval, discolored lesions irregularly scattered (Parsada, 1962)

Different contagious species were isolated from regularly contaminated wheat leaves and other natural items (soil and seeds) demonstrating the indications of scourges and were recognized as *Fusarium graminearum*, *Bipolaris sorokhiniana, Alternaria triticina, Ustilago tritici* and *Blumeria graminis.* In Table 2, fungal infected wheat plants were examined and were identified by using microscope. The dominant disease-causing pathogen was *Fusarium graminearum* whose incidence value was recorded as 80% and the minimum incidence of fungal pathogens were recorded by *Alternaria triticina, Blumeria graminis* and *Ustilago tritici* was 40%.

Table 2. Assessment of incidence of fungal species of wheat crop collected from different sites of study area.

Sr. No.	Fungi identified	Incidence of fungi collected from different study sites					Total	%age
		Gurha Rehman	Gochar	Dheri Wattan	Gurha Matyal	Gurha Lilyan	05	100
1	*Bipolaris sorokiniana*	+	-	+	+	-	03	60
2	*Alternaria tritricina*	+	+	-	-	-	02	40
3	*Blumeria graminis tritici*	+	-	-	+	-	02	40
4	*Ustilago segetum tritici*	-	+	-	-	+	02	40
5	*Fusarium graminearum*	+	-	+	+	+	04	80
6	Total isolates from each site	04	02	01	03	02		48
7	Each sample %	80	40	20	60	40		48

The dominant fungal diseases and their severity rate on the wheat crop in the study area are shown in Table 3. To further minimize possible error sources that might be incurred by an investigator, the estimated damage percentage was classified into = classes: 0-3%= (no disease), 4-9% (disease index) 4-5=20%, 5-6=40%, 6-7=60%, 7-8=80%, 8-9=100%. DI from 7-9 has the highest percentage of severity rate. Graphical representation of

severity rate is shown in Figure 1.

Infection rates (IR) of identified fungal species from wheat plants collected from different sites of study area were measured in table 4. It was observed that the highest IR was shown by *Fusarium graminearum* which is 57.14%. While the minimum IR was *Blumeria graminis* which was 20.8%. It was observed that less infected wheat plants showed better yield. This may be due to more resistance against fungal pathogens.

Table 3. Measurement of disease severity rate of isolated fungal pathogens of wheat crop collected from different localities of study area.

Sr. No.	Dominant diseases of wheat and pathogens	Severity rate of dominant diseases in study area					Mean severity rate (%)
		Gurha Rehman	Gochar	Dheri Wattan	Gurha Matyal	Gurha Lilyan	
1	Blotch disease (*Bipolaris sorokiniana*)	09	02	04	04	03	60
2	Leaf blight (Alternaria *triticina*)	06	05	03	03	02	40
3	Powdery mildew (*Blumeria graminis tritici*)	06	03	02	04	02	40
4	Loose smut (*Ustilago segetum tritici*)	03	05	03	02	04	40
5	Root Rot (*Fusarium pseudo-graminearum*)	07	03	08	06	07	80
6	Mean severity rate	80	40	40	60	40	

Key: Severity rate ranges from 1-10 1-3%= no disease, 4-09%= disease index, 4-5=20%, 5-6=40%, 6-7=60%, 7-8=80%, 8-9=100%.

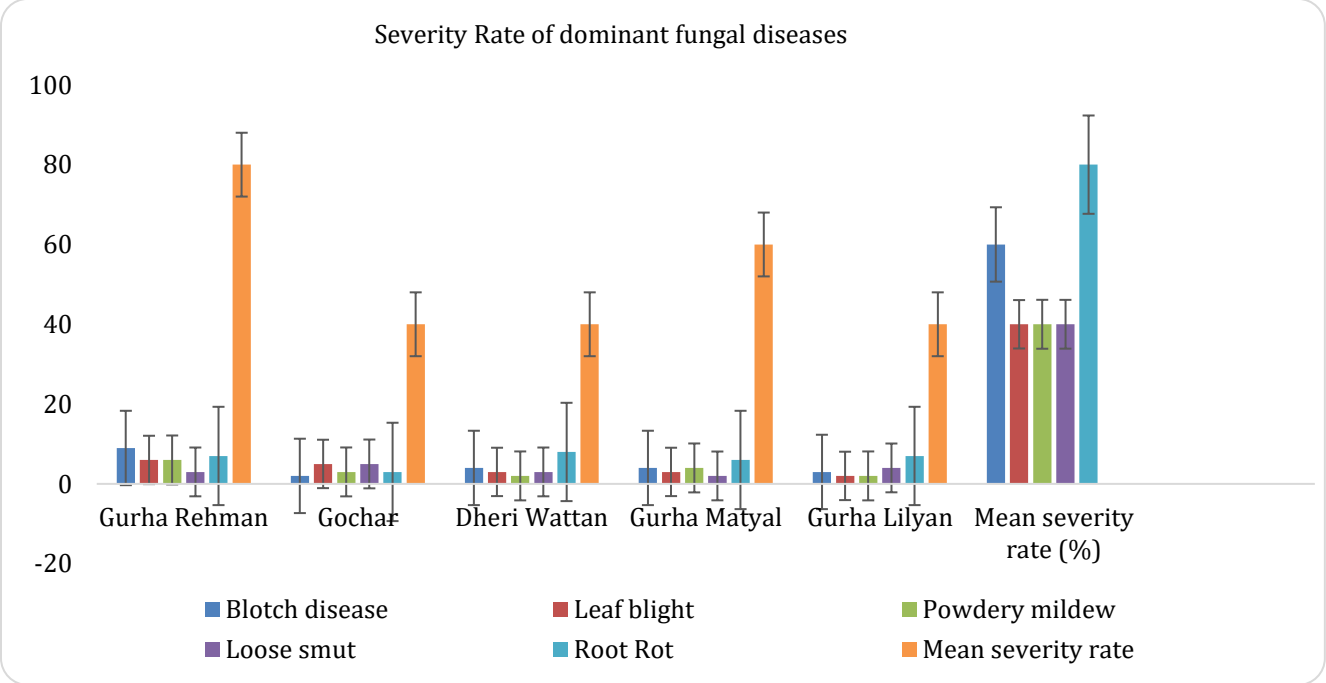

Figure 1. Graphical representation of severity rate of dominant fungal diseases of wheat in different localities of study area.

Table 4. Assessment of infection rates of identified fungal pathogen isolated from wheat crop sample from selected sites of study area.

Sr. No.	Identified fungal species	Infection rate of fungal pathogen from selected site of study area (%)					Total infection rate (%)
		Gurha Rehman	Gochar	Dheri Wattan	Gurha Matyal	Gurha Lilyan	
1	*Bipolaris sorokiniana*	82	0	55.7	48	0	37.14
2	*Alternaria triticina*	88	66	62	0	0	43.2
3	*Blumeria graminis tritici*	60	0	0	44	0	20.8
4	*Ustilago segetum tritici*	74	0	66	0	0	28
5	*Fusarium pseudo-graminearum*	96	0	78	56	55.7	57.1
6	Total infection rate of specific area	80	13.2	52.34	29.6	11.14	37.25

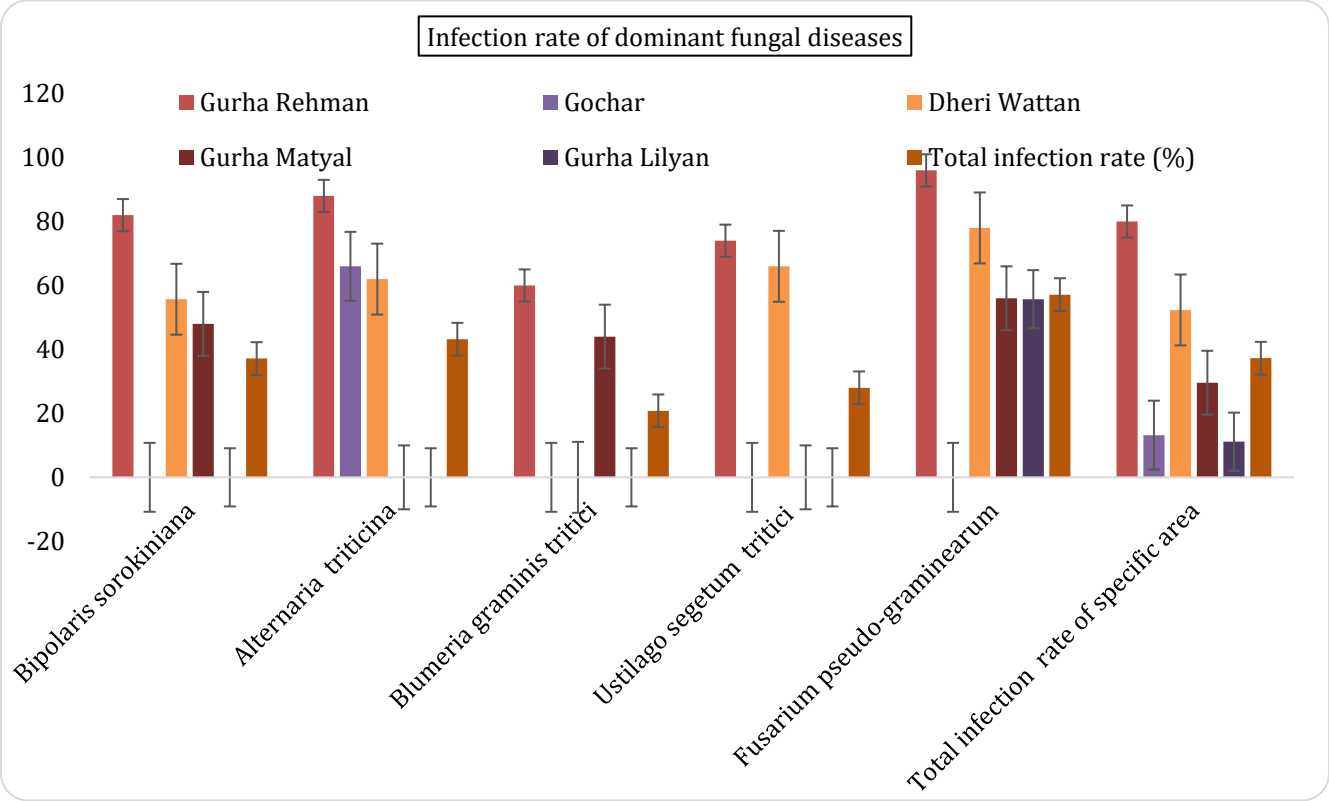

Figure 2. Graphical representation of infection rate of dominant fungal pathogens of Wheat in different localities of study area.

***In-vitro* responses of petals extracts**

Antifungal activity of *Butea monosperma* and *Calotropis procera* in different solvents against wheat plant pathogens was measured in Table 5 and 6. In which the highest zone of inhibition was observed in methanolic extract of *Butea monosperma* against fungi *Alternaria triticina* (33.6mm) and *Calotropis procera* against fungi *U. tritici* (34.67mm). Di ethyl ether extract of *B. monosperma* exhibit growth of fungi *B. sorokhiniana* (31mm) and *C. procera* exhibit *U. tritici* (32mm). On the other hand, chloroform extract of *B. monosperma* exhibit highest growth of fungi *B. sorokhiniana* (31.1mm) and *C. procera* exhibit highest growth of fungi *U. tritici* (29.6mm), Distilled water extract of *B. monosperma* exhibit growth of fungi U. *tritici* by (30.67mm) and *C. procera* exhibit *B. graminis* by (24.25mm) at the end petroleum ether exhibit highest growth of fungi *Alternaria triticina* by (28mm) and *C. procera* exhibit *F. graminearum* by

(31.5mm). Results revealed that all the tried petals solution of two different plants including *Butea monosperma* and *Calotropis procera* caused a huge decline in the direct development of these pathogens. Plant petal extracts like, Cedar extract, Neem, Clove and *Anthi mandhaari* extracts are effective and induce resistance against *Puccinia tritici* that cause leaf rust disease (Shabana, *et al.,* 2017).

Table 5. Measurement of zone of inhibition (mm) by using *Butea monosperma* petals extract.

S. N.	Fungal pathogen	Measurement of zone of inhibition (mm) by using *B. monosperma* petals extracts					
		Methanol	Di ethyl ether	Chloroform	Petroleum Ether	Distilled Water	Control
01	*Bipolaris sorokhiniana*	33.6±2.08	26.33±52	28.33± 1.15	28±4.56	25±1	35.33± 0.58
02	*Alternaria triticina*	28±1	22.3± 1.53	26.33± 3.79	24± 1	21.76± 1.53	29.5 ± 4.54
03	*Blumeria graminis*	32.67± 2.52	29.33± 3.97	22.33± 4.04	21± 5	30.67 ± 2.52	34.0± 7.40
04	*Ustilago tritici*	30.3±3.21	31± 1	31.1± 3.21	27.11± 5.6	27± 3	31.42± 5.51
05	*Fusarium graminearum*	26±5.29	25± 5.6	22± 5	21.25 ± 6.99	21.55± 6.7	31.55± 6.4

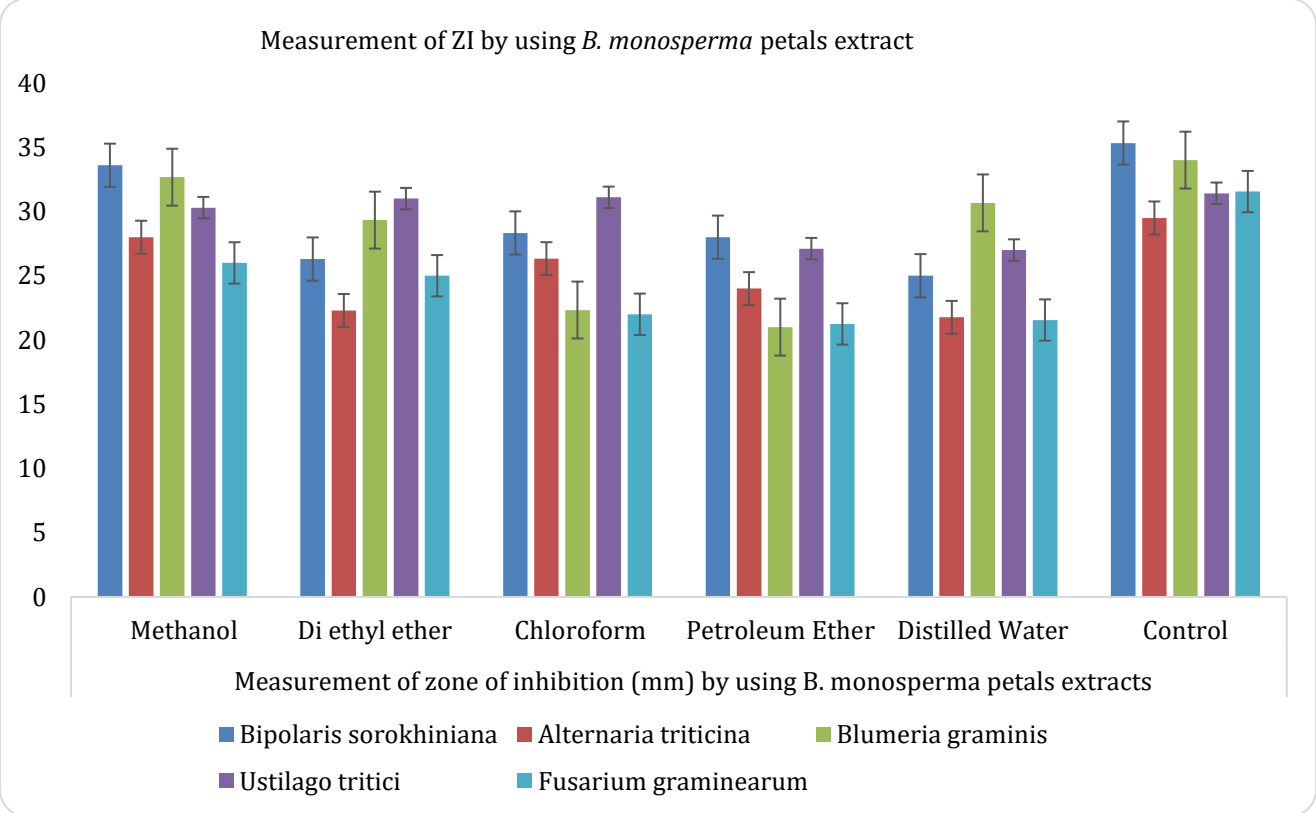

Figure 3. Graphical representation of measurement of zone of inhibition of *C. procera* petals extract.

Table 6. Measurement of zone of inhibition (mm) by using *Calotropis procera* Petals extract.

Sr. No.	Fungal pathogen	Measurement of zone of inhibition (mm) by using *C.procera* petals extracts					
		Methanol	Di ethyl ether	Chloroform	Petroleum Ether	Distilled Water	Control
01	*Bipolaris sorokhiniana*	29.6±2.08	25.33± 2.52	23.33± 1.15	26±4.56	21±1	31.33± 0.58
02	*Alternaria triticina*	36.67±1	22.3± 1.53	28.33± 3.79	21± 1	22.76± 1.53	31.5 ± 4.54
03	*Blumeria graminis*	34.67± 2.52	28.33± 3.97	20.33± 4.04	24± 5	29.67 ± 2.52	35.0± 7.40
04	*Ustilago tritici*	32.3±3.21	32± 1	32.1± 3.21	23.11± 5.6	26± 3	36.42± 5.51
05	*Fusarium graminearum*	34±5.29	23± 5.6	26± 5	24.25 ± 6.99	24.55± 6.7	34.55± 6.4

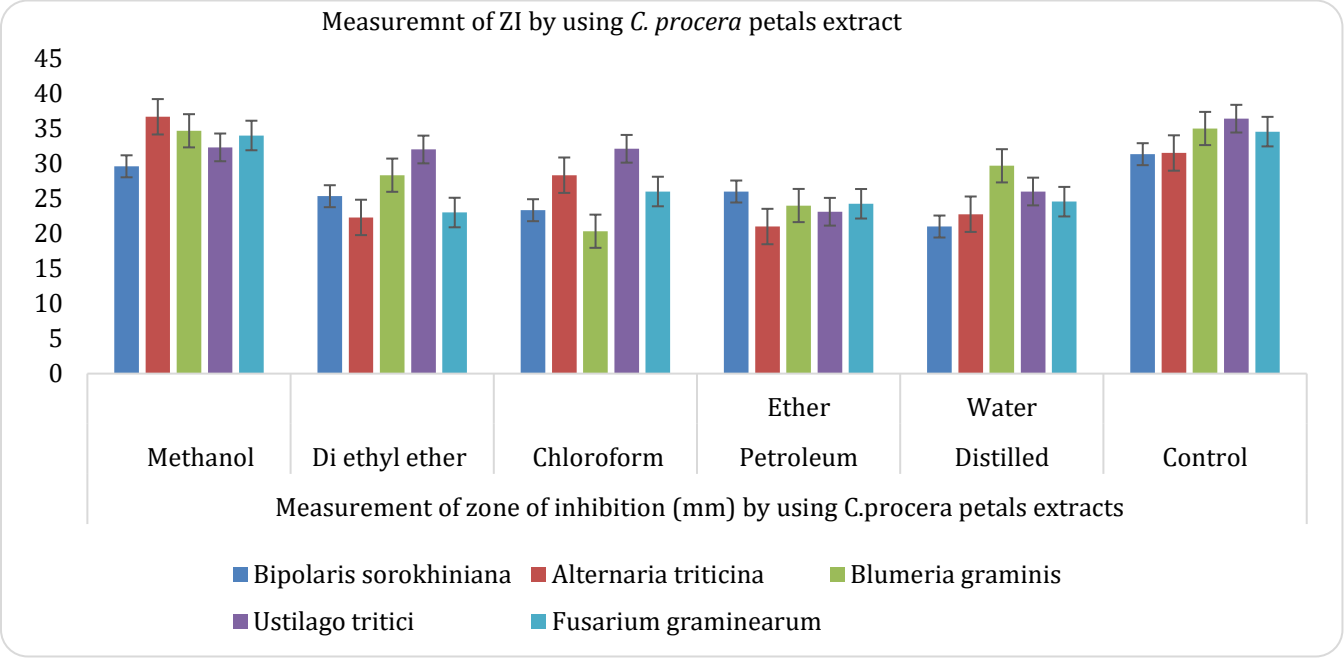

Figure 4. Graphical representation of measurement of Zone of Inhibition of C. *procera* petals extract.

Determination of minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was the lowest concentration of the extract inhibiting the visible growth of any micro-fungi. The MIC of petals extracts of *B. monosperma* and *C. procera* which ranged between 250µL to 1000µL against the isolated fungi (Table 7). The MIC values obtained for two plants petals extracts i.e., *Butea monosperma* and *Calotropis procera* against the fungal strains vary from each other. Both petals' extracts were active against the selected fungal pathogens of wheat. Moreover, *Calotropis procera* had the best antifungal activity against three of the fungi, with minimum inhibitory concentration (MIC) values as low as 0.02mg/ml, 0.05mg/ml and 0.08mg/ml against *Fusarium graminearum, Bipolaris sorokhiniana* and *Ustilago tritici.* Sen and Batra (2012) measure the minimum inhibitory concentration of *Melia azedarach* in different solvents viz. Methanol, Ethanol, Petroleum ether and Water. The results revealed that all the extracts are potent antimicrobials against all the microorganisms studied. Among the different extracts studied methanol and ethanol showed high degree of inhibition followed by petroleum ether and aqueous extract.

Table 7. Measurement of minimum inhibitory concentration (MIC) of two petals extract in different solvents against wheat attacking fungi.

Sr. No	Petals Extract Used	Solvents	Measurement of Minimum Inhibitory Concentration MIC (µg/ml)				
			B. sorokhiniana	*A. triticina*	*B. graminis*	*U. tritici*	*F. graminearum*
01	*Butea monosperma*	Methanol	35	37	39	41	45
		Chloroform	31	35	37	34	37
		Di ethyl ether	29	33	35	31	30
		Petroleum ether	27	31	33	29	35
		Distilled water	24	29	31	25	40
		Control	20	25	27	23	35
02	*Calotropis procera*	Methanol	49	47	45	43	51
		Chloroform	47	45	43	41	49
		Di ethyl ether	43	43	40	39	45
		Petroleum ether	41	39	37	35	41
		Distilled water	37	35	33	30	39
		Control	35	33	31	25	37

Plant extracts treatment under field conditions

The results depicted that the effect of *Butea monosperma* and *Calotropis procera* (flower extract) spray on yield and growth components of wheat plants grown in the study area. Root length was the very first character investigated. Other characteristics were also observed including shoot length, root weight and shoot weight between treated and non-treated samples. Data depicted those plants sprayed with *Butea monosperma* extract showed significant results in increasing the growth components of wheat crop as shown in Table 8. There appeared a significant difference between root length and shoot lengths of treated and non-treated samples among wheat plants trials. Root and shoot lengths also showed significant results between treated and non-treated samples. Kumar *et al*., (2017) also used this method to determine the effect of foliar spraying of plant extracts on the leaf rust infection and wheat yield components evaluated under field conditions. All the used plant extracts improved yield.

Table 8. Effect of *Butea monosperma* petals extract spray on wheat crop.

Sr. No.	WP	Root length (cm)		Shoot length (cm)		Root weight (g)		Shoot weight (g)	
		T	N.T.	T	N.T.	T	N.T.	T	N.T.
1	P1	5.1	4.8	4.1	3.4	2.03	1.09	1.19	1.0
2	P2	4.9	4.5	3.9	3.6	4.13	4.0	5.87	5.4
3	P3	4.5	4.0	4.7	4.6	2.91	2.4	7.01	6.8
4	P4	3.8	3.1	4.4	4.0	2.08	1.9	5.19	4.9
5	P5	4.9	4.4	4.8	4.5	5.51	5.0	6.53	6.2
6	P6	4.2	3.8	4.2	3.6	3.99	3.4	8.01	7.4
7	P7	4.9	4.5	3.7	3.3	2.19	2.0	5.8	5.4
8	P8	4.0	3.5	4.5	4.0	4.82	4.4	6.57	6.2

Key: P1, P2------P8= wheat plant Samples, T= treated, N.T.= non-treated.

In Table 9 data depicted that the plants sprayed with *Calotropis procera* extract showed a more significant result in increasing the growth components of wheat crop than *B. monosperma*.

Table 9. Effect of *Calotropis procera* petals extract spray on yield components of wheat plants collected from study area.

Sr. No.	WP	Root length (cm)		Shoot length (cm)		Root weight (g)		Shoot weight (g)	
		T	N.T.	T	N.T.	T	N.T.	T	N.T.
01	P1	3.01	2.9	2.4	2.01	6.1	5.9	5.1	4.9
02	P2	4.40	3.8	3.71	3.07	4.7	3.8	4.9	4.4
03	P3	3.91	3.41	4.40	4.10	7.4	7.1	7.4	7.1
04	P4	2.04	2.45	5.41	5.04	5.9	5.0	6.3	5.9
05	P5	2.99	2.09	7.43	7.00	8.0	7.5	7.1	6.7
06	P6	3.19	3.00	6.41	5.91	4.5	4.1	4.8	4.4
07	P7	5.82	4.47	8.4	7.80	6.3	5.8	5.9	5.5
08	P8	4.7	4.40	5.8	5.4	5.5	5.1	6.1	5.0

Key: P1, P2------P8= wheat plant Samples, WP wheat plant T= treated, N. T= non-treated.

DISCUSSION

The results confirmed the antifungal activity of *Calotropis procera* and *Butea monosperma* for the better management of most prevalent fungal diseases of wheat variety (Glaxy-13). We used the X diagonal transect method to measure severity and infection rate of fungal diseases of wheat crop. A similar study was also conducted by Gashaw *et al*., (2014) to measure the severity rate of finger millet. They observed that severity of finger millet blast was higher at the station compared to farmer's fields. The severity rate varies from locality to locality due to cultivation of different varieties in different areas and due to different environmental conditions.

The results revealed that methanolic extract of *C. procera* petals were more effective against fungi *U. tritici* with 34.67mm zone of inhibition and the highest zone of inhibition was observed in methanolic extract of *Butea monosperma* against fungi *Alternaria triticina.* Shabana *et al*., (2017) measured the antifungal activity of 18 medicinal plants leaf extracts against 5 seed borne fungi in form of zone of inhibition (mm). Latha *et al.,* (2009) described that leaf extract of *Allium sativum, Aegle marmelos* and *Catharanthus roseus* flowers extracts were also hindered the spore germination and myclelial development of *Aspergillus solani.* These findings strongly supported our results. The comparative impacts of various plants extract solution like *Artemisia tenuifolius* and *Euphorbia guymiana* were powerful against *Fusarium graminearum* (Salhi *et al.,* 2017). They described that aqueous extracts of these plants have strong antifungal properties which make these plants of potential interest for the control of fungi affecting both wheat yield and safety due to natural phytochemicals.

The results also depicted that the effect of *Butea monosperma* and *Calotropis procera* (corolla extracts) spray effected yield and growth components of wheat plants in the study area. The growth and yield of wheat significantly increased after the treatment of these two selected plants corolla extracts. Their floral parts contained secondary metabolites that inhibited the growth fungal pathogens. The spray of these extracts with 25 micro dilutions indicated more ZI as compared to other dilutions. Therefore, we sprayed 25 micro dilutions concentrations on the crop for good results. Kumar *et al*., (2017) also used this method to determine the effect of foliar spraying of plant extracts on the leaf rust infection and better wheat yield components evaluated under field conditions. All the used plant extracts improved yield of crop.

The minimum inhibitory concentration (MIC) obtained for two plants petals extracts i.e. *Butea monosperma* and *Calotropis procera* against the fungal strains varied from each other. Both petals' extracts were active against the selected fungal pathogens of wheat. Moreover, *Calotropis procera* had the best antifungal activity against the three fungi, with minimum inhibitory concentration (MIC) values as low as 0.02mg/ml, 0.05mg/ml and 0.08mg/ml against *Fusarium graminearum, Bipolaris sorokhiniana* and *Ustilago tritici.* Sen and Batra, (2012) measured the minimum inhibitory concentration of *Melia azedarach* in different solvents viz. Methanol, Ethanol, Petroleum ether and Water. The results revealed that all the extracts are potent antimicrobials against all the microorganisms studied. Among the different extracts studied methanol and ethanol showed high degree of inhibition followed by petroleum ether and aqueous extract. Previous literature indicated that these two plants have a broad spectrum of

antimicrobial activities. Therefore, these plants were selected for the management of selected fungal diseases of wheat variety Galaxy-13. The literature of mycology explained the broad range of antimicrobial activities of these two selected plants. So, we can apply their extracts against different types of fungal diseases. Hence, the compound isolation from these plants is a future target to hit the specific fungal disease. They are rich in phytochemicals. Hence, the researcher should isolate the phytochemicals and secondary metabolites for preparation of spray for good agricultural products.

CONCLUSION

Wheat crop treatment with petals extracts may be an effective approach to reduce or eliminate pathogens. Wheat treatment with petals extract is an eco-friendly measure for controlling wheat crop pathogens. The research work on two medicinal plants petals extract as an antifungal activity showed that *Butea monosperma* and *Calotropis procera,* can be utilized for the control of the fungal pathogens of wheat crop and were capable of reducing growth of fungi responsible for alternations in wheat due to the presence of different bioactive compounds. *Calotropis procera* petals have chemical compounds like cyclosadol, multiflorenol, procestrol, terpenes and flavonoids. Qualitative phytochemical analysis of *B. monosperma* confirmed the presence of phytochemicals like saponins, terpenoids, steroids, alkaloids and tannins. These groups of compounds are important for the physiology of plants contributing properties confer resistances against microorganisms like fungi. However, both petals extract produced total inhibition of fungal pathogens of wheat but *Calotropis procera* has more potential in inhibiting fungal pathogens of wheat crop rather than *Butea monosperma*. Further studies are required to determine the effect of these petals extract in vivo and invitro to evaluate their potential as natural treatments for wheat crop fungal diseases.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

Tanveer Hussain and Aleeza Moqaddas conducted lab experiments and wrote this paper. Dr. Muhammad Ishtiaq corrected and re-rewrite the paper. Dr. Fahim Ahmed Khan performed data analyses and assisted with study design. All authors read and contributed to earlier versions and approved the final version.

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REFERENCES

Ahmad, J., M.I. Marwat and H.K. Ahmad. 2003. Effect of herbicides and row spacing on different trades of wheat (*Triticum aestivum).* Pakistan Journal of Weed Sciences Research*,* 9(1-2): 33-40.

Al-Sadi, A.M. and M.L. Deadman. 2010. Influence of seed-borne *Cochliobolus sativus* on crown rot and root rot of barley and wheat. Journal of Phytopathology, 158: 683-690.

Anonymous, 2010. Agricultural Statistics of Pakistan 2008-2009, Ministry of Food and Agriculture, Economic Wing, Government of Pakistan, Islamabad, Pakistan, 25-29.

Askar, A.A. and Y.M. Rashad. 2010. Efficiency of Some Plant Extracts against *Rhizoctonia solani* on Pea. Journal of Plant Protection Research*,* 50: 239-243.

Chaurasia, S., R. Chand and A.K. Joshi. 2000. Relative dominance of *Alternaria triticina* Pras. Et Prab. and *Bipolaris sorokiniana* (Sacc.) Shoemaker in different growth stages of wheat (*T. aestivum* L.). Journal of Plant Disease Protection, 107: 176-181.

Cummins, G.B. and Y. Hiratsuka. 2004. Illustrated Genera of Rust Fungi. St. Paul, MN: APS press.

Daamen, R.A. 1989. Assessment of the profile of powdery mildew and its damage function at low disease intensities in field experiments with winter wheat. Netherland Journal of Plant Pathology, 95(2): 85-105.

Davila, F.G. 1989. Studies on the incompatibility system of *Tilletia indica*. Review Mexician Phytopathalogy, 7: 201-207.

Diamond, J., G. Germs and Steel. 1997. A Short History of Everybody for The Last 13,000 Years. Viking UK Random House, 86.

Domsch, K.H., V. Gams and T.H. Anderson. 1980. Compendium of Soil fungi. Volume 1. Academic press. A subsidiary of Harcourt brace, 96.

Draz, I.S., A.A. Elkhwag and A.A. Elzaawely. 2019.

Application of plant extracts as inducers to challenge leaf rust of wheat. Egypt Journal of Biological Pest Control. 5(3): 1-15.

Duplessis, S., D.L. Joly and P.N. Dodds. 2012. Rust effectors. In: Effectors in Plant Microbe Interections. Wiley Blackwell, 155-193.

Ehab, E., M. Alias. M. Rasha, K. Hassan, A. Elaziem and B. Zaidan. 2016. Antifungal Activities of Ethanolic Extracts of Sunut (*Acacia nilotica* L.) Bitter Apple (*Citrullus colocynthis* L. Schrad) and Mint (*Mentha piperita* L.) against *Fusarium moniliforme* Sheldon. University of Bakht Alruda. Science Journal, 18: 30-38.

Esmail, S.M., I.S. Draz, M.A.E.H. Abou-Zeid and T.A.E.M. Essa. 2017. Powdery mildew susceptibility of spring wheat cultivars as a major constraint on grain yield. Annual of Agricultural Science, 64(1): 39-45.

Figueroa, M., N.M. Upadhyaya, J. Sperschneider, R.F. Park, L.J. Szabo, B. Steffenson and P.N. Dodds. 2018. Changing the game: using integrative genomics to probe virulence mechanisms of the stem rust pathogen *Puccinia graminis*. Frontiers in Plant Science, 19(6): 1523-1536.

Gacek, E. 1983. Analiza patogenicznosci grzybow porazajacych jeczmienia. Biuletyn IHAR, 150:11-15.

Iason, G., M. Dicek and S. Hartley. 2012. The integrative roles of plant secondary metabolites in natural systems: a synthesis in the ecology of plant secondary metabolites from genes to Global Processes, 1-9.

Iftikhar, S., M.L. Collazo-Clavell, V.L. Roger, J.S.T. Sauver, R.D. Brown, S. Cha, and D.J. Rhodes. 2012. Risk of cardiovascular events in patients with polycystic ovary syndrome. The Netherlands journal of medicine., 70(2): 74-80.

Isitor, S.U., M.A.T. Poswal J.A. Shebayan and R.Y. Yakubu. 1990. The accelerated wheat production programme in Nigeria: A case study of Bauchi State, 230-249.

Isleib, J. 2012. Signs and symptoms of plant diseases, Michigan State University Extension.

Ivanova, E.A., V.G. K and T. N. B. 2004. Physiologo-biochemical analysis of pathogenesis of lose smut of wheat at early infectious stages. Seryya Biyalagichnykh Navu, 4: 69-73.

Jabeen, K., W. Nidra and I. Sumera. 2013. Antifungal Potential of *Calotropis procera* against *Macrophomina phaseolina*. Life Science Journal, 10(12): 1-13.

Kareem, S.O., L. Akpan and O.P. Ojo. 2008. Antimicrobial activity of *Calotropis procera* on selected pathogenic organisms. African Journal of Biomedical Research, 11:105-110.

Kolmer, J.A. 2005. Tracking wheat Rust on a continental scale. Current Opinion of Plant Biology, 8: 441-449.

Langer, R.H.M., and G.D. Hill. 1991. Physiological basis of yields: Agricultural Plants, Cambridge University Press., 348.

Leonard, K.J. and L.J. Szabo. 2005. Stem rust of small grains and grasses cause by *Puccinia graminis.* Molecular Plant Pathology, 6: 99-111.

Maj, W., G. Pertile, S. Różalska, K. Skic and M. Frąc. 2024. Comprehensive antifungal investigation of natural plant extracts against *Neosartorya spp*. (*Aspergillus* spp.) of agriculturally significant microbiological contaminants and shaping their metabolic profile. Scientific Reports, 14(1): 8399.

Miller, J.D., and H.L. Trenhlom. 1994. Mycotoxins in Grain, Compounds other than Aflatoxin, Eagan Press, St. Paul. 19-36.

Mitra, M. 1931. A new Bunt on wheat in India. Annual of Applied Biology, 18: 178-179.

Moya-Elizonodo, E.A., L.J. Rew, B.J. Jacobsen, A.C. Hogg and A.T. Dyer. 2011. Distribution and prevalence of fusarium crown rot and common root rot pathogens of wheat in Montana. Plant Diseases, 95: 1099-1108.

Naraynanasamy, P. 2013. Biological management of diseases of crops. Characteristics of Biological control agents. 978-94-007, 6379-1.

Neupane, A., R. Sharma, E. Duveiller and S. Shrestha. 2010. Sources of *Cochliobolus sativus* inoculum causing spot blotch under warm wheat growing conditions in South Asia. Cereal Research Community, 38: 541-549.

Parry, D.W., P. Jenkinson and L. McLeod. 1995. *Fusarium* ear blight (scab) in small grain cereals- a review. Plant Pathology, 44: 207-238.

Pathak, N. and R.J. Zaidi. 2013. Comparatively study of seed dressing fungicides and *Calotropis procera* latex for the control of seed borne mycoflora of wheat. Annals of Biological Research, 1-6.

Perello, A.E., C. Cordo and H.E. Alippi. 1992. Tizon de la hoja del trigo ocasionado por *Alternaria triticina*

Pras. and Prab. En Sud America. In: Proceedings of the VIII Jornads Fitosanitarias Argentinas, 22.

Prasada, R. and A.S. Prabhu. 1962. Leaf blight of wheat caused by a new species of *Alternaria*. Indian Plant Pathology,15: 292-293.

Qostal, S., S. Kribel, M. Chliyen, S. Serghat, A.O.T. Karima and H. Zaarati. 2019. Study of the fungal complex responsible for root rot of wheat and barley in the North-West of Morocco. Plant Archeology, 19: 2143-2157.

Sammour, R. H., M.M. El Shamy, A.E.Z.M., Mustafa and E.S. Yousef. 2015. Inheritance of resistance to loose smut *Ustilago tritici* of wheat, 10(4): 137-146.

Saremi, H., S.M. Okhovvat and S.J. Ashrafi. 2011. Fusarium diseases as the main soil borne fungal pathogen on plants and their control management with soil solarization in Iran. African Journal of Biotechnology, 10(80): 18391-18398.

Shakoor, M. A., M. Ahmad, H.Z. Ghazali, S. Ahmad, M.A. Balouch, R. Anjum and M. Hussain. 2014. Chemotherapy of Karnal bunt of wheat: A Review. International Journal of Advances in Research and Biological Sciences, 1(9): 163-188.

Sharma, R.C. and E. Duvellier. 2003. Selection index for improving Helminthosporium leaf blight resistance, maturity and kernel weight in spring wheat. Crop Sciences, 43: 2031-2036.

Silici, S.I.B.E.L. and A.N. Koc. 2006. Comparative study of *in vitro* methods to analyze the antifungal activity of propolis against yeasts isolated from patients with superficial mycoses. Letters in Applied Microbiology, 43(3): 318-324.

Singh, R.P., D.P. Hodson Y. Jin, E.S. Lagudah M.A. Ayliffe, S. Bhavani, M.N. Rouse, Z.A. Pretouris L.J. Szabo, J.E. Huerta, B.R. Basnet, C. Lan and M.S. Hovmoller. 2015. Emergence and spread of new races of wheat stem rust fungus, continued threat to food security and prospects of genetic control. Phytopathology, 105: 872-884.

Stack R.W. 2003. History of Fusarium head blight with emphasis on North America. APS Press. 1-34.

Teng P.S. and W.C. James. 2001. Disease and yield loss assessment. In Plant Pathologists pocketbook, 25-38.

Tunali, B., J.M. Nicol, D. Hodson, Z. Uckun, O. Buyuk, D. Erdurms. 2008. Root and crown rot fungi associated with spring, facultative, and winter wheat in Turkey. Plant Diseases, 92: 1299-1306.

Usmani, S.M.H. and A. Ghaffar. 1982. Polyethylene Mulching of soil to reduce viability of Sclerotia of *Sclrotium oryzae*. Soil Biology and Biochemistry, 14: 203-206.

Viani, A., P. Sinha, T. Sharma and L.M. Bhar. 2017. A model for forecasting spot blotch disease in wheat. Australas Plant Pathology, 46: 601-609.

Wiese, M.V. 1987. Compendium of wheat diseases. APS Press, St. Paul, MN. Compendium of wheat diseases. 2nd ed. APS Press, St. Paul, MN.

Windels, C.E. 2000. Economic and social impacts of Fusarium head blight: changing farms and rural communities in the Northern Great Plains. Phytopathology, 90: 17-21.