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# PLANT-DERIVED FUNGICIDAL INNOVATIONS: UTILIZING *BUTEA MONOSPERMA* AND *CALOTROPIS PROCERA* EXTRACTS TO SAFEGUARD WHEAT FROM KEY FUNGAL INFESTATIONS

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**Abstract**

*Fungal diseases significantly hinder wheat crop production worldwide, necessitating effective and eco-friendly management strategies. The use of botanical fungicides offers a sustainable alternative to chemical treatments, capitalizing on the natural antifungal properties of medicinal plants. This study aimed to evaluate the antifungal efficacy of crude extracts from the petals of two medicinal plants, Butea monosperma and Calotropis procera, against prevalent fungal pathogens affecting wheat crops. After IRB approval Petals (800g) from B. monosperma and C. procera were harvested from different local areas of district Bhimber AJK and processed to obtain methanolic extracts using cycles of gentle heating boiling and condensation methods. Fungi was isolated and cultured. Infection and severity rates were recorded, along with the zones of inhibition exhibited by each extract. Agar well diffusion technique was used to measure the Zone of Inhibition. SPSS was used to calculate Mean±SD and Graph-Pad Prism 9.0 was used draw the analysis of results. The highest infection rate observed was 57.14% against F. graminearum, while the lowest was 20.8% against B. graminis. Severity of infections ranged from 40% to 80%. Methanolic extract of B. monosperma petals demonstrated a significant zone of inhibition against Alternaria triticina, whereas the extract from 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. Treated wheat plants showed improved yields compared to untreated controls, with C. procera extracts providing better disease management than B. monosperma. The study confirmed the potential of C. procera petal extract as a more effective botanical fungicide against selected fungal pathogens, likely due to its rich chemical composition. This finding supports that identifying the specific bioactive compounds in C. procera petals, optimizing extraction methods, and conducting large-scale field trials to fully establish the practical applications and efficacy of these extracts in agricultural settings.*

**Keywords:** *Antifungal activity, Botanical fungicides, Butea monosperma, Calotropis procera, Fungal diseases, Wheat*

## INTRODUCTION

Wheat (Triticum aestivum L.) 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 (1). Evaluated antifungal efficacy of crude extracts from the petals of two medicinal plants, *Butea monosperma* and *Calotropis procera*, against prevalent fungal pathogens affecting wheat crops has been the among the revolutionary research areas in agricultural Management.

*Butea monosperma* (Lam) 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. 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 (2). *Calotropis procera* is specie of a flowering plant which belongs to the family Apocynaceae. The plant is harvested from wild. It is considered as an ornamental plant. *Calotropis procera* is an ideal insecticidal plant, crude plant extracts are beneficial in the

terms of efficacy and pest resistance management as the active substances present in them act synergistically (3, 4).

Wheat rust disease is caused due to pathogen *Puccinia gramini* and Leaf or brown rust of wheat is caused due to Puccinia recondita. Stem rust also known as cereal rust, black rust or red rust, which is caused by pathogen *Puccinia graminis* (5). *Bipolaris sorokinaian* is mainly responsible for the spot blotch diseases 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* (6, 7). Seed-like bunt balls replace sick wheat plant ears, which contain oily, black, and foul-smelling spores. Powdery mildew of wheat caused by obligate, bio-trophic ascomyceteous fungus *Blumeria graminis f. sp. Tritici*, *Erysiphe graminis*, both of belongs to family Erysiphaceae (8, 9). Fusarium head blight begins to show symptoms soon after flowering. Infected spikelet's bleach, and the disease spreads upward and downward by infecting spikelets one by one, especially nears the base. Spikelets may also have little, black fruiting structures (10).

Biological methods are very effective, save and economically sound method for management of diseases (foliar and seed-borne) of wheat caused by fungi. This technique use different medicinal plants derived products are used as spray on crops. Biological controls of weeds pests are important to low the impact of diseases. 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 (11). In vitro laboratory tests have shown that extracts from a variety of higher plants have antifungal properties. Wild plants, in particular, appear to be a prospective source of beneficial metabolites (12).

This study aimed to evaluate the antifungal efficacy of crude extracts from the petals of two medicinal plants, *Butea monosperma* and *Calotropis procera*, against prevalent fungal pathogens affecting wheat crops.

# MATERIALS AND METHODS

## STUDY APPROVAL

This Study was conducted after Institutional Review Board (IRB/04/2021) approval of supervisory committee Department of Botany, Mirpur University of Science and Technology, Bhimber, Azad Kashmir.

## SAMPLE COLLECTION

The floral and fresh samples of *Butea monosperma* and *Calotropis procera* petals are collected from different local areas of district Bhimber AJK. The petals (800g) of these plants were surface sterilized with 1% 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 and blended into a fine powder.

## ASSESSMENT OF DISEASE INCIDENCE AND SEVERITY

For the gathering of wheat sample plants leaves, a survey was used. The plants in the plot were tested for wheat diseases, and leaf samples were collected by X pattern from specified locations and brought to the lab for further fungal research. Using the technique of wheat plants were chosen for documenting 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 Agar plate method collected samples were transferred to sterile PDA media. Before this step, PDA medium was prepared by mixing of 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 121ºC

temperature in autoclave for sterilization. The PDA media transferred into each petridish (15ml per each petridish) under aseptic conditions when temperature of the sterilized media reached at 45ºC or easily touched media containing flask. Isolated fungi were also purified by sub culturing on sterilized PDA media.

### FIELD MANAGEMENT STRATEGIES & PREPARATION OF EXTRACTS

Crude extracts of medicinal plants *Butea monosperma* and *Calotropis procera* petals were sprayed 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. The petal extracts were prepared. 25g powder of both plant petals i.e. *Butea monosperma* and *Calotropis procera* are mixed in 250ml of different solvents i.e. petroleum ether, Di ethyl ether, methanol, chloroform, distilled water in conical flasks.

### AGAR WELL DIFFUSION METHOD (AWD)

The methodology brief as Inoculation of 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. Then introducing 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. The antifungal drug diffuses into the agar medium, preventing the fungus strain from growing.

### STATISTICAL ANALYSIS

SPSS was used to calculate Mean±SD and Graph-Pad Prism 9.0 was used draw the analysis of results.

## RESULTS

This study covers 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*). Infection rates (IR) was highest shown by Fusarium graminearum which is 57.14%. While Blumeria graminis had 20.8%. It was observed that less infected wheat plants showed better yield. This was may be due to more resistance against fungal pathogens.

### IN VITRO RESPONSES OF PETALS EXTRACTS

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) as shown in Fig. 1. 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.

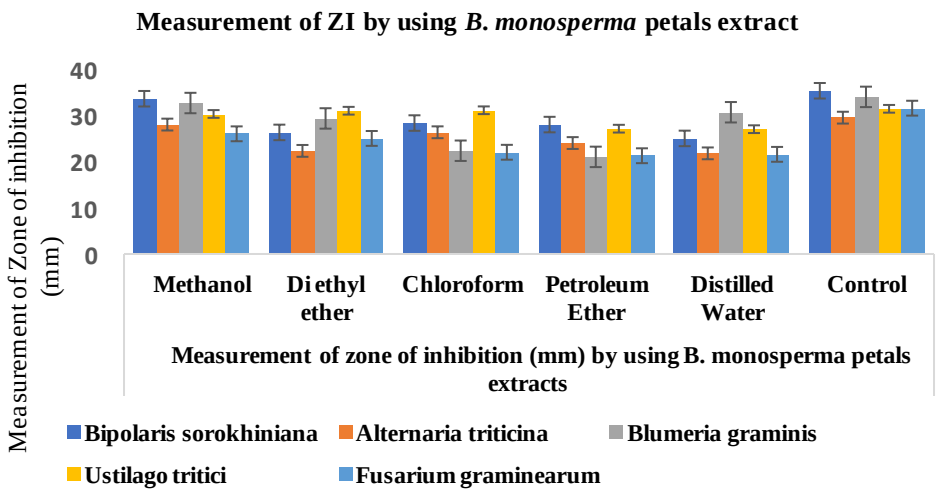


**Fig. 1.** Graphical representation of measurement of Zone of Inhibition of C. procera petals extract

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 as shown in Fig. 2, Clove and *Anthi mandhaari* extracts are effective and induce resistance against *Puccinia tritici* which cause leaf rust disease.

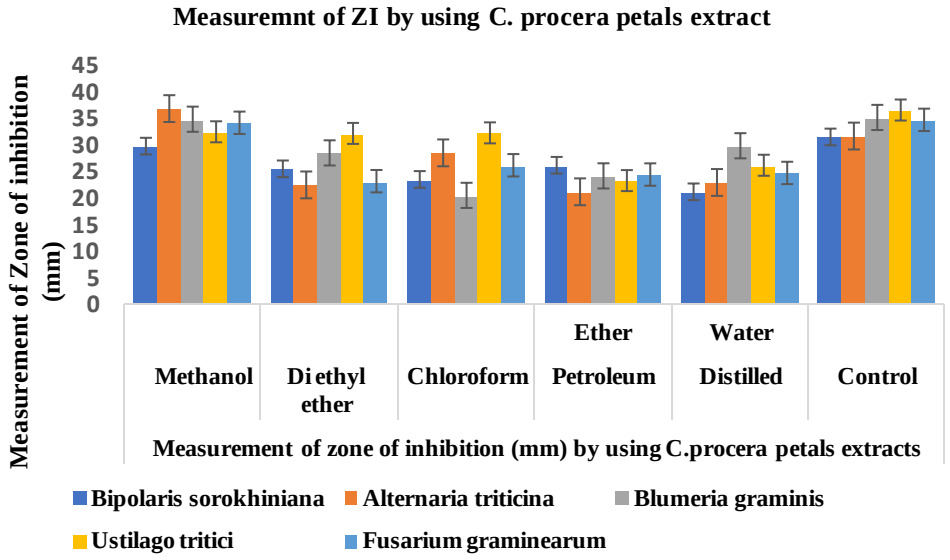


**Fig. 2.** Graphical representation of measurement of Zone of Inhibition of C. procera petals extract.

## 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 extract of *B. monosperma* and *C. procera* which ranged between 250µL to 1000µL against the isolated fungi (table 7). 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. It 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.

## PLANT EXTRACTS TREATMENT UNDER FIELD CONDITIONS

Data depicted that plants sprayed with *Butea monosperma* extract showed significant results in increasing the growth components of wheat crop as shown in Table I. There was 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. All the used plant extracts improved yield.

**Table I.** 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 |

*P1, P2——–P8= wheat plant Samples, WP wheat plant T= treated, N. T= non-treated

**Table II.** 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 |

*P1, P2——-P8= wheat plant Samples, WP wheat plant T= treated, N. T= non-treated

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

## DPPH SCAVENGING ACTIVITY

The DPPH scavenging approach was adopted to determine the antioxidant activity of *A. spinosissima*. Vitamin C served as a standard, showed $IC_{50}$ of 0.08 µM (Table III).

**Table III.** DPPH scavenging activity of *A. spinosissima*

| Sr. No. | Sample Tested | Concentration (mg/ml) | Inhibition (%) | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|
| 1 | G1 | 5 | 90 ± 2.54 | 0.00099 ± 1.15[a] |
| 2 | Vitamin C[b] | 0.5mM | 90 ± 0.11 | 0.08 ± 1.14(µM) |

Results are presented as Mean ± SME, (n=3); a = $p < 0.05$ versus control; [b]Standard antioxidant

# DISCUSSION

One of the groundbreaking research domains in agricultural management has been the assessment of the antifungal potential of crude extracts derived from the petals of two medicinal plants, namely *Butea monosperma* and *Calotropis procera*, against prevalent fungal pathogens impacting wheat crops. A group of researchers measured the antifungal activity of 18 leaf extracts against 5 seed borne fungi as zone of inhibition in mm. It was described that leaf extract of *Allium sativum, Aegle marmelos* and *Catharanthus roseus* flowers extract also hindered the spore germination and myclelial development of *Aspergillus solani* (13). The results confirm the antifungal activity of *Calotropis procera and Butea monosperma* in controlling of dominant fungal diseases of wheat crop. We use X Transect method to measure severity and infection rate of fungal diseases of wheat crop, same method was also used by many researchers to measure the severity rate of finger millet. The severity of finger millet blast was higher at the station compared to farmer's fields (14).

Recent studies have shed light on the efficacy of methanolic extracts derived from *Calotropis procera* petals, demonstrating pronounced effectiveness against the fungal pathogen Ustilago tritici, with a notable zone of inhibition measuring 34.67mm (15). Similarly, investigations have highlighted the potent antifungal properties of methanolic extracts from *Butea monosperma* petals, particularly evident in their ability to inhibit the growth of *Alternaria triticina*. Comparative analyses have underscored the effectiveness of various plant extract solutions, such as *Artemisia tenuifolius* and *Euphorbia guymiana*, against *Fusarium graminearum*. Researchers have emphasized the antifungal potential of aqueous extracts from these plants, suggesting their viability for managing fungal infections that affect wheat yield and safety (16). Moreover,

empirical evidence has demonstrated the positive impact of *Butea monosperma* and *Calotropis procera* flower extract sprays on the yield and growth parameters of wheat plants in the study area.

The results also 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. Another Scientist 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 (17). The MIC values obtained for two plants petals extracts i.e. *Butea monosperma* and *Calotropis procera* against the fungal strains varies 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* (18). It was measured that 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 (19).

## CONCLUSION AND FUTURE PROSPECTS

Wheat crop treatment with petals extracts may be an effective approach to reduce or eliminates 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 show 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. Qualitaive phytochemical analysis of *B. monosperma* confirmed the presence of phytochemicals like saponins, terpenoids, steroids, alkaloids and tannins. These group of componds are are important for the physiology of plants contributing properties confer resistancesagainst microorganisms like fungi, viruses and bacteria etc. However both petals extract produced total inhibitionof 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 in-vitro to evaluate their potential as natural treatments for wheat crop fungal diseases.

### Conflict of Interest:

Authors have no conflict of interest.

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