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Application of aqueous plant extracts and fungicides for management of Fusarium head blight of wheat

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Efficacy of different plant extracts including *Hibiscus rosa*, *Melia azedarach*, and *Cassic fistula* at their concentrations 25%, 50%, and 100% per liter of potato dextrose agar medium and fungicides Alliete and Agro supporter at 1000 ppm, 2000 ppm and 4000 ppm concentrations were studied for the management of Fusarium Head Blight (FHB) of wheat. The experiment was laid out in completely randomized design (CRD) with three replications. Efficacies of the treatments were assessed based on colony diameter of the fungus (*in vitro*). Significant differences were recorded for the interactive effect of plant extracts and its different concentration on colony diameter. *Melia azadarach* seems good with minimum colony diameter (0.9017) with 41.83% inhibition over control at 50% concentration. However, minimum inhibition over control 10.59% with maximum colony diameter 1.3533 cm was recorded for *Cassic fistula* at 100% concentrations. Similarly, the interactive effect of fungicides and their concentrations were also significantly different. Maximum inhibition over control 91.79% with minimum colony diameter (0.3333 cm) was achieved for Agro supporter at 2000 ppm concentration followed by 90.89% inhibition over control and 0.3700cm colony diameter at 1000 ppm concentration of the same fungicide. However, minimum inhibition over control 66.71% with maximum colony diameter (1.3967cm) was recorded for Alliete at 1000 ppm concentrations. These findings highlight the potential of both the phytobiocides and fungicides for the control of Fusarium head blight of wheat.

Keywords: Fusarium head blight, yield reduction, wheat, fungicides, botanicals

INTRODUCTION

Wheat belongs to family *gramineae* (Shewry, 2009), is a staple food for almost all population of the world (James and Mauseth, 2014). It is one of the important agricultural crops of Pakistan, grown on an area of about nine million hectares, which is equal to 40 % of the country's total cultivated land (David, 2017). However, its production is far below than the world annual production. Various biological, cultural and socio-economical constrains are contributing to reduce its yield. Rust, barley yellow dwarf, root rot, and fusarium head blight are important wheat diseases in the region.

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a devastating disease of wheat. The fungus produces a mycotoxin known as deoxynivalenol (DON) which possess a significant threat to human health. Various control strategies have been developed based on importance of the disease. Cultivation of resistant varieties (Barbara et al. 2017), removal of crop residue to reduce inoculum load of the fungus (Johan et al. 2013) and application of balanced fertilizers are in practice (Bernhoft, 2012). Moreover, fungicides can also provide more effective control of FHB but the effects may be variable. Increasing pesticide residues concerns in agricultural products and environment as well as the incidence of resistance in plant pathogens are not in favor of the fungicides use. However, when pedoclimatic conditions favorable to fungal disease development occur, direct control of fungi through the use of fungicides could be an option to consider.

The use of plant extracts for the management of plant diseases has gained importance in recent decades. These are safe alternatives to fungicides and microbial biocontrol agents (Amodioha, 2003; Bowers and Locke, 2004). Furthermore, phytobiocides are non-phytotoxic and less pollutant to environment (Singh, 1994; Simin et al. 2011)).

Studies on the mechanisms of disease control by plant extracts have revealed that the biologically active constituents present in them may have either direct antimicrobial activity (Ansari, 1995; Amodioha, 2000) or induce host plants defense response resulting in reduction of disease development (Schneider and Ullrich, 1994). Several studies have confirmed the role of phytobiocides in controlling FHB. (Alexendra et al. 2013; Rajesh et al**.** 2013; Drakopoulos, et al. 2019**).**

Considering importance of the disease, present study was designed to evaluate the efficacy of plant extracts *(Hibiscus rosa*, *Melia azedarach* and *Cassic fistula)* and fungicides (Alliete and Agro supporter) at its different concentrations against the disease.

MATERIALS AND METHODS

The present study was carried out at fungal pathology laboratory, Crops Disease Research Institute (CDRI)-National Agriculture Research Center (NARC), Islamabad, during 2019 to test the efficacy of plant extracts including Dhareek; *Melia azedarach*, Gul khairo; *Hibiscus rosa* and Amtlas; *Cassic fistula*) and fungicides Alliete (active ingredient: Fosetyl) and Agro supporter (active ingredient: copper oxychloride + Kasugamycin) at its different concentrations against *Fusarium graminearum* for controlling Fusarium Head Blight (FHB) of wheat. Various wheat fields in the area were visited; disease samples were collected, properly labeled in zipper bags, transferred to fungal pathology lab and stored at 4 ºC for further study.

Media Preparation

Potato Dextrose Agar (PDA) medium was prepared using the ingredients given in (Table 1). Potato tubers were washed thoroughly, peeled with sharp knife, cut into pieces and boiled in 1 liter distilled water for 30 minutes. The boiled potatoes were filtered through double layered muslin cloth after cooling, dextrose and agar were added and sterilized in an autoclave at 121ºC for an hour. The hot media was poured into petrilpates @ 20 ml media per plate and left for cooling in a laminar flow unit.

Table 1: Basic ingredients used in the preparation of potato dextrose agar (PDA) medium.

Ingredients	Amount used
Potato	300 gm
Agar	24 gm
Dextrose	45 gm
Distilled water	1.5 L (1500 ml)

Isolation and Identification of *Fusarium graminearum* from disease samples

Disease samples were washed thoroughly under tap water. Pieces of disease samples (Approximately 5mm² sized) were cut and disinfested using 0.1% mercuric chloride solution for 30 sec followed by rinsing in sterilized distilled water. The excised pieces were placed on PDA medium in petri plates and incubated at 25ºC for 3-7 days. Cultures showing morphological resemblance with *Fusarium* spp. were transferred into fresh water agar petri plates. The cultures were further purified by single spore isolation technique. The purified culture was maintained on PDA medium at 5ºC for further study (Fig-1).

Figure1: Pure culture *of Fusarium graminearum.*

Identification of Fusarium species was made based on morphological characteristics and colony morphology (Lorens et al. 2006).

Preparation of medicinal plant leaf extracts

Leaf extract of each plant was prepared by following the procedure of Rajesh et al. (2013). Leaves of each plant were washed with sterilized distilled water separately, crushed with the help of grinder and 200ml sterile distil water was added. The aqueous extract was further stabilized in a conical flask and kept untouched overnight. The extract was stained through double layered muslin cloth and finally through filter paper (Fig-2).

Incorporation of plant extracts into media and adjustment of different concentrations

Treatments of plant extracts including *Hibiscus rosa*, *Melia azedarach*, and *Cassic fistula*, at different concentration 25%, 50%, and 100% were prepared separately by mixing 25% each plant extracts into 75ml distilled water, 50 ml plant extracts into 50ml distilled water and finally 100ml pure plant extract without adding distilled water respectively. Each plant extract was added into 100ml PDA media in different conical flasks and autoclaved at 121ºC for one hour. The media were poured into petri plates @ 20 ml media per plate and left for cooling. Approximately, 5mm sized plugs from the margin of 7-day old culture of *Fusarium graminearum* were put at the center of each petri plate under aseptic conditions in a laminar flow unit.

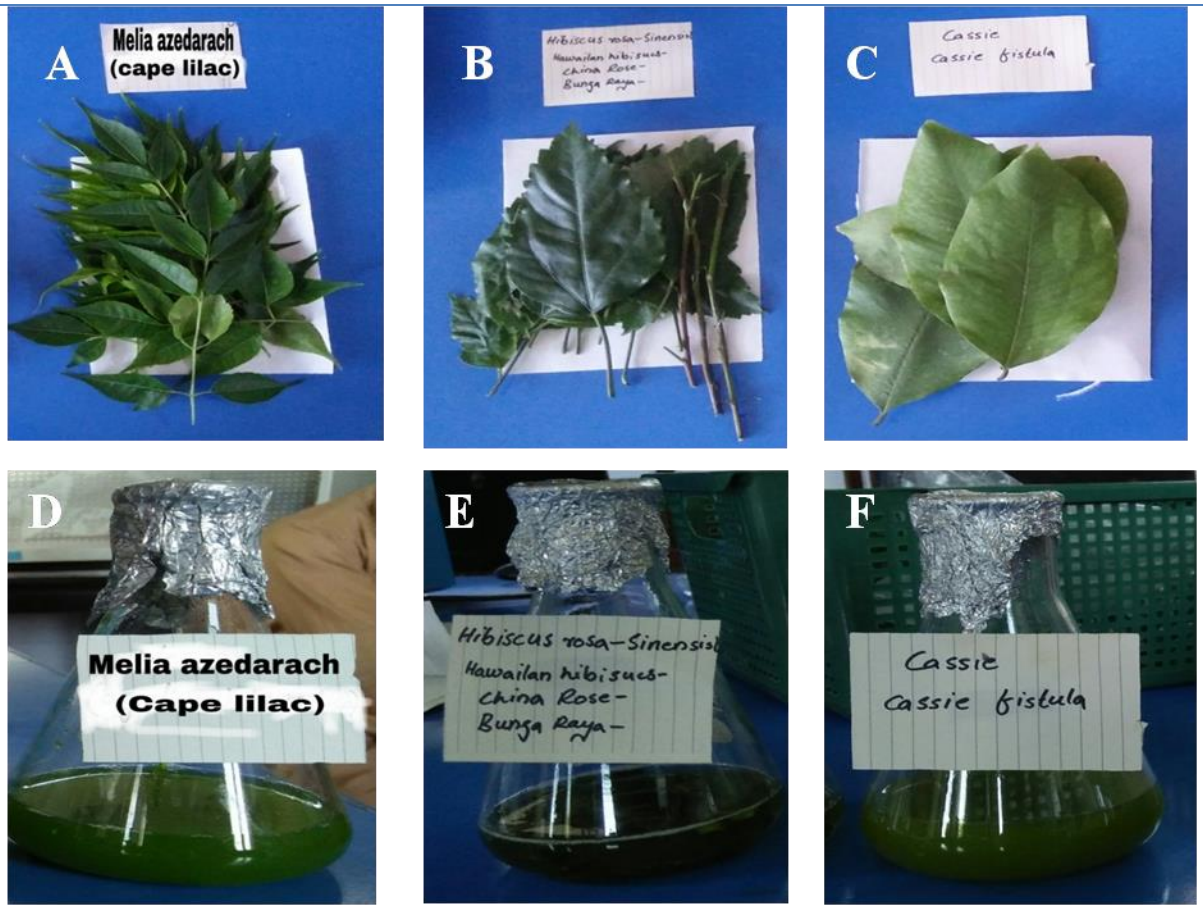

Figure 2: leaves of *Melia azedarach* (A), *Hibiscus rosa* (B) and *Cassic fistula* (C) and their extracts (D, E, F) respectively.

The plates were labeled, sealed with paraflim and incubated at 25ºC for 3-7 days. A control treatment was adjusted using PDA medium without addition of any plant extract. The study was designed in completely randomized design (CRD) with three replications. Data on colony diameter were recorded after 7-days of incubation.

Fungicides Assay (*in vitro*)

Efficacy of fungicides Alliete (active ingredient: Fosetyl) and Agro supporter (active ingredient: copper oxychloride + Kasugamycin) at different concentrations against FHB was determined by using poisoned food technique. The study was designed in completely randomized design (CRD) with three replications.

A weight of 0.5gm, 1gm and 2 gm of both fungicides was measured with the help of electric balance, added to 500 ml media in separate flasks to adjust its concentration at 1000 ppm, 2000ppm and 4000ppm respectively. A control (check) was adjusted using same amount of media without any fungicide. The media amended with fungicides and control check were poured into petriplates @ 20 ml media per plate and left for cooling. Approximately, 5mm sized plugs from the margin of 7-day old culture of *Fusarium graminearum* were put at the center of each petri plate under aseptic conditions in a laminar flow unit. The plates were labeled, sealed with paraflim and incubated at 25ºC for 3-7 days. Data on colony diameter were recorded after 7-days of incubation.

Statistical Analysis

All the data were subjected to analysis of variance (ANOVA) to reveal significant differences using statistix 8.1 software. LSD (Least Significant Different) test was applied to separate the treatment means.

RESULTS

Efficacy of plant extracts and their concentrations in reducing growth of *Fusarium graminearum*

Data regarding mean colony diameter of *Fusarium graminearum* under the influence of different plant extracts at different concentration is presented in Table 2. Statistical analysis of the data showed significant variations for plant extracts ($p = 0.01$) and their concentrations ($p = 0.00$). Minimum colony diameter 1.1156 cm and 28.03% inhibition over control was observed for plant extracts of *Melia azadarach,* followed by *Cassic fistula* and *Hibisuc rosa* with colony diameter 1.2332 cm and 1.2628 cm giving 20.35% and 16.57% inhibition over control respectively.

Regarding concentration minimum colony diameter 1.0579 and maximum inhibition over control 47.95% was achieved while using 25% concentration of plant extracts. This is statistically at par with 50% concentration (1.0650 cm colony diameter and 47.24% inhibition over control) and 100% concentration (1.1550 cm colony diameter and 38.24% inhibition over control) of all plant extracts. However, all the three concentration were statistically different from control.

The interactive effect of plant extracts and its different concentration was also significantly different (P=0.01), Maximum inhibition over control 41.83% with minimum colony diameter (0.9017) was achieved for *Melia azadarach* at 50% concentration followed by 39.36% inhibition over control and 0.9400 colony diameter at 100% concentration of the same plant. However, minimum inhibition over control 10.59% with maximum colony diameter (1.3533) was recorded for *Cassic fistula* at 100% concentrations.

Efficacy of fungicides and their concentrations in reducing growth of *Fusarium graminearum*

Data on mean colony diameter of *Fusarium graminearum* under the effect of Alliete and Agro supporter at its concentrations of 1000ppm, 2000ppm and 4000ppm is presented in Table 3. Non-significant variations were observed for fungicides in terms of its effect on colony diameter however, the effect of their concentrations is significantly different. Maximum inhibition over control (90.35%) with minimum colony diameter (0.3983cm) was observed at 2000 ppm concentration.

The interactive effect of fungicides and their concentrations were also significantly different. Maximum inhibition over control 91.79% with minimum colony diameter (0.3333 cm) was achieved for Agro supporter at 2000 ppm concentration followed by 90.89% inhibition over control and 0.3700cm colony diameter at 1000 ppm concentration of the same fungicide. However, minimum inhibition over control 66.71% with maximum colony diameter (1.3967cm) was recorded for Alliete at 1000 ppm concentrations.

Table 2:Efficacy of plant extracts and their concentrations in controlling *Fusarium graminearum* in terms of inhibiting its colony growth.

Treatments	Concentrations				Means
	C1 (25%)	C2 (50%)	C3 (100%)	Control	
Melia azadarach	1.0703 DEF(30.96%)	0.9017 F (41.83%)	0.9400 EF(39.36%)	1.5503 A	1.1156 B (28.03%)
Hibiscus rosa	1.0993 DE(28.99%)	1.1133 DE(28.09%)	1.1717 CD(24.32%)	1.5483 A	1.2332 A (16.57%)
Cassic fistula	1.0040 DEF(33.67%)	1.1800 CD(22.04%)	1.3533 BC(10.59%)	1.5137 AB	1.2628 A (20.35%)
	1.0579 B (47.95%)	1.0650 B (47.24%)	1.1550 B(38.24%)	1.5374 A	

LSD for plant extracts 0.0969
LSD for concentration 0.1119
LSD for interaction 0.1939

Means within the row or column followed by the same letters are non-significantly different at 5% level of significance. Letters in parenthesis are denoting inhibition over control.

Table 3: Efficacy of fungicides and its concentrations in controlling *Fusarium graminearum* in terms of inhibiting its colony growth

Treatments	Concentrations				Means
	1000ppm	2000ppm	4000ppm	Control	
Agro supporter	0.3700 C (90.89%)	0.3333 C (91.79%)	0.5333 C (86.87%)	4.0633 A	1 1.3250 A (67.39%)
Alliete	1.3967 B (66.71%)	0.4633 C (88.96%)	0.5015 C (88.05%)	4.1967 A	1.5142 A (63.91%)
	0.8833 B (79.82%)	0.3983 C (90.35%)	0.5174 C (87.47%)	4.1300 A	

LSD for fungicides 0.2825
LSD for concentration 0.3996
LSD for interaction 0.5651 Means within the row or column followed by the same letters are non-significantly different at 5% level of significance.Letters in parenthesis are denoting inhibition over control.

DISCUSSION

Fusarium head bligh (FHB) is a destructive diseases of wheat crop, results in significant economic losses due to reduced grain yield (Li et al. 2011). It causes accumulation of a mycotoxin Deoxynivalenol (DON) to an unacceptable level in wheat kernels (Demeke et al. 2005; Paul et al. 2005; Browne, 2007). Levels of DON above 2 ppm may render grain and their by-products unfit for commercialization and consumption (Blandino et al. 2017). This warrants proper control measures for the disease. Literally, no control measure is completely effective for FHB. Fungicides, although good but hazardous to environment and human health. Phytobiocides, being environmentally safe and easily biodegradable (Simin et al. 2011) is a good option for the disease control.

This study indicates that all the aqueous extracts obtained from the leaves of *Melia azedarach*, *Hibiscus rosa* and *Cassic fistula* possess antimicrobial properties. However, marked variability was observed for *Melia azedarach.* It was highly effective in reducing growth of *Fusarium graminearum* at higher concentrations. The antifungal effects of these plant extracts can be due to the presence of various phytochemicals that can act alone or in synergy as found in other studies (Field et al. 2006). *Melia azedarach* is rich in limonoids, which has superb anti-microbial properties (Lee et al. 1991). Similarly, Hibiscus species contain flavonoids, anthocyanins, terpenoids and steroids. *Cassic fistula* contains components like saponin, triterpenoids, glycosides, anthraquinone, steroids and flavonoids that possess excellent antimicrobial property (Draughon, 2004).

The *in vitro* efficacy of fungicides namely, Alliete and Agro supporter at its different concentrations declared that both fungicides restrict mycelial growth of the fungus in comparison to control check, necessitating the use of fungicides in disease control strategies. However, Agro supporter caused maximum inhibition of colonial growth of the fungus at 2000 ppm concentration. Kasugamycin and Copper oxychloride being the active ingredient of Agro supporter inhibit the development of amino acids in ribosome system of the pathogen, thus prevent protein synthesis and cause subsequent death of the pathogen.

All the tested botanicals and fungicides could be candidates for inclusion in a management strategy to reduce overwintering mycelium in the soil and on infected crop residues.

CONCLUSION

It is concluded that *Melia azedarach* (Dhareek) and Agro supporter are capable to inhibit colonial growth of *F. graminearum* at 50% concentration. However, these preliminary results, obtained from *in vitro* experiments, may be supplemented by other more comprehensive studies both in controlled greenhouse conditions and in open field to practically evaluate the use of these treatments in the frame of an integrated pest management system.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

AA and AN provided conceptual frame work and develop the manuscript. RK performed analysis of the data. HK and MYK conducted the experiment. ZA and NK recorded data on all parametres. UI helped in development of the manuscript. All authors read and approved the manuscript.

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