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Antifungal activity and role of *Terminalia* extracts in imparting resistance in barley against spot blotch by modulating metabolic defense mechanisms

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Running title: Ecofriendly management of spot blotch of barley

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ABSTRACT

The objective of the current investigation was to examine the effects of botanically-induced resistance against the *Bipolaris sorokiniana* causing spot blotch disease in susceptible barley cultivar (PL 426). Different concentrations of botanical extracts prepared from leaf, fruit and bark of *Terminalia bellerica* and *Terminalia chebula* were tested against *B. sorokiniana* using poisoned food approach. The ethanolic and aqueous fruit extracts resulted in more than 70% mycelial inhibition of *B. sorokiniana*. The foliar spray of promising doses of botanical extracts was done 2 days prior to inoculation under *in-vivo* conditions. The physiological and biochemical variations were recorded after treatments from 1 to 7 days after treatment (DAT). Foliar spraying of botanical extracts resulted in an increase in total phenol content in inoculated barley plants from 1 DAT to 7 DAT. The malondialdehyde content was significantly lower in plants treated with botanicals as compared to inoculated and uninoculated controls. The activity of peroxidase and phenylalanine ammonia lyase was significantly higher in all plants sprayed with botanical extracts as compared to controls. Furthermore, botanicals reduced the percentage of disease severity in treated plants while increasing the percentage of yield increase. Plants treated with *T. chebula* fruit extracts had the highest yield increase, followed by *T. bellerica* fruit extracts. From the present study, it can be concluded that the fruit extracts of *T. chebula* and *T. bellerica* are a promising approach for the eco-friendly management of spot blotch.

Keywords: Barley, spot blotch, *Bipolaris sorokiniana*, extracts, resistance.

INTRODUCTION

Barley (*Hordeum vulgare* L.) a member of the Poaceae, is one of the most versatile cereal crops, ranking fourth after wheat, maize and rice (Giraldo *et al*. 2019). It is a major dietary component of human beings and is also known as the poor man's crop as it requires low input

and has better adaptability to salinity, drought, alkalinity and marginal lands (Kaur *et al.*, 2021). It is grown during the rabi season in almost all parts of the world with arid or semi-arid climates (Golla, 2021). In recent years, fungal infections in cereals have been reported in various regions of the world and are considered to be one of the primary factors affecting yield and grain quality (Smagacz and Martyniuk, 2001). Plant diseases are abnormal conditions that primarily affect crop plants' primary and secondary metabolisms by disrupting their biochemical and physiological processes (Chhabra *et al.,* 2019). The most devastating fungal disease against barley is spot blotch caused by *Bipolaris sorokiniana* (Sacc.) Shoemaker, which produces irregular, oval, light to dark brown blotches on the leaf blade and sheath. These blotches later spread to cover the entire leaf surface (Kaur *et al.,* 2021). *B. sorokiniana* which is widespread throughout the world and is particularly aggressive in conditions of high relative humidity, high temperature and unbalanced soil fertility, annually causes significant economic losses in cereal harvests (Kumar *et al.,* 2002). Estimated yield losses as a result of spot blotch have been observed to range from 15.5% and may reach up to 100% under high disease incidence conditions (Kumar *et al.,* 2020).

In today's agricultural system, the need to produce high quality and quantity of grains per unit area is causing rapid changes in agricultural setup to meet the needs of an enormously growing population (Kaur *et al.,* 2023). The management of plant diseases heavily relies on fungicides. However, the excessive and inappropriate use of fungicides harms the ecosystem and has unintended impacts on plants and humans. Consequently, a sustainable approach to disease management is required, and biological control is one such secure and efficient approach that leads to the stimulation of induced resistance against the pathogen (Chhabra *et al.,* 2023c). In the present study, the *Terminalia* species were selected to determine their effectiveness against spot-blotch infection, as these are the most widely used medicines for traditional purposes worldwide (Cock, 2015). *Terminalia* is the second-largest genus in the Combretaceae family and consists of over 200 species. The plants in this genus have the highest level of genetic variety in South-East Asia, which is the original habitat for most of the world's tropical regions. (Das *et al.,* 2020). *Terminalia chebula* Retz. and *Terminalia bellirica* (Gaertn.) Roxb are highly adaptable botanical species, offering a distinct reservoir of many chemicals characterized by a wide range of chemical structures. Various Terminalia species possess nutraceutical properties that offer numerous health advantages, including the potential to treat certain disorders (Cock, 2015). For instance, the fruits of T. *bellirica* (Gaertn.) Roxb. and *T. chebula* Retz. typically combine to create Triphala, a renowned polyherbal mixture used in Ayurvedic medicines. This plant contains many phytochemical bioactive rich compounds but the antifungal activities of

Terminalia species' fruit may be attributed to their elevated tannin content (Das *et al.,* 2020). Furthermore, the utilization of decoctions and macerations derived from the leaves and stem bark shown notable antibacterial efficacy (Abraham *et al.,* 2014). A total of sixty chemicals were detected in the leaf ethyl acetate extract, exhibiting significant antifungal properties. The compounds di-, tri-, and tetra-gallotannins, chebulinic acid and ellagitannins have been demonstrated to have antifungal effects (Salih *et al.,* 2022).

Plants produce reactive oxygen species as defense mechanism against different biotic stressors to initiate subsequent defence reactions (Asada, 2006). The induced defence mechanism consists of a variety of enzymatic and non-enzymatic components which form the antioxidant system of the host to act against the oxidative stress in crop plants which help the plant defend itself and increase its yield and productivity (Akter *et al.,* 2015). Moreover, secondary metabolism in plants which, includes the synthesis of numerous active metabolites, also protect the plants against different pathogens (Chhabra *et al.,* 2023b). Plant extracts reduce disease incidence by triggering host defense responses against the invading pathogen (Chhabra *et al.,* 2023a). Plant extracts have the tendency to modulate physiological and biochemical mechanisms in order to make plants withstand stressful conditions. Several studies conducted by various researchers have demonstrated the protective nature of botanical extracts and their exogenous application, which make the plant resistant to invading pathogens (Chhabra *et al.,* 2023a,b; Draz *et al.,* 2019; El-Malik and Abbas,2017). These extracts have the potential to alter the primary and secondary metabolisms, thereby improving the plant's photosynthetic capacity (Butt *et al.,* 2019).

The current study's main goal was to conduct *in-vitro* and *in-vivo* studies of *Terminalia* extracts against barley spot blotch disease. The present investigation was also conducted to assess the variations in antioxidant enzyme activities and non-enzymatic metabolites in treated barley plants as compared to control plants. This study can provide scientific basis to develop an eco-friendly management practices to eliminate the use of hazardous synthetic fungicides.

MATERIALS AND METHODS

Plant material collection and drying

Healthy and mature leaves, bark, and fruits (3 kg each) of *Terminalia chebula* (Harar) and *Terminalia bellerica* (Gaertn.) Roxb. (Bahera) were collected from trees ranging in age from 11 to 13 years growing at the Research farm, Department of Forestry and Natural Resources, Punjab Agricultural University. The mature bark, fruits and leaves of *T. chebula* were collected in the months of October-December while the same plant parts of *T. bellerica* (Bahera) were

collected in May. To make fine powder from each plant material, the material was crushed in an electric grinder after being dried in a hot-air oven at 60°C for a week.

Botanical Extracts preparation

The aqueous and 50% ethanolic extracts of *Terminalia chebula* (Harar) and *Terminalia bellerica* (Bahera) were prepared by mixing dry powdered tissues (100mg/100ml) from each specified part in a 1:1 w/v solution of distilled water and fractionated 50% ethanolic solvent for 48 hours. The extract was filtered through muslin cloth, centrifuged at 4000 rpm for 30 minutes, and later strained with Whatman No. 1 filter paper. The solution served as the crude extract (100 percent concentration) (Hossain *et al.,* 2011). Different concentrations from the crude extract were prepared to test their antifungal effect against the target pathogen.

Fungal inoculum and *In-vitro* studies

Bipolaris sorokiniana the causal organism, was procured from the Department of Plant Pathology Punjab Agricultural University which was used in this study. The identity of the pathogen was confirmed by observing its morphology and conidial characteristics under a light microscope. The pathogenicity of the culture was proven prior to experimentation using a highly susceptible cultivar and the fungi produced typical spot blotch symptoms after 3-4 days of inoculation.

The antifungal efficacy of botanicals was tested at concentrations of 10%, 20%, 30%, 40% and 50% on double strength PDA medium (Channakeshava and Pankaja, 2018). Botanical extracts were sterilised using 0.22-mm filters to evaluate their antifungal effect. To achieve the desired concentration, the necessary amount of each plant extract was added to double strength potato dextrose agar medium. The medium incorporated with botanicals served as treatments and the medium without botanical extracts was kept as a control. Three replications were performed for each concentration, and inoculated plates were then incubated at 25±2°C for 6 days until mycelium growth covered the entire plate in the control plate. The percent inhibition of mycelial growth was calculated using the formula given by Vincent (1947).

Crop establishment, Inoculation and disease assessment

The seeds of the selected susceptible barley cultivar PL 426 procured from the Department of Plant Pathology were sown in the month of October using the standard package of practices followed by Punjab Agricultural University. The plot size was kept to 2×2 meters, and the total number of plots was 30, divided into three rows of ten plots each. The experiment was carried out in a randomised block design with three replications.

To multiply the pure culture of *B. sorokiniana*, tiny mycelial discs were transferred onto PDA-coated petri plates and incubated at 24±2°C for a week. Spore suspension was developed for the disease's artificial inoculations. Conidia were collected from the fungus cultured in Petri dishes and utilized to prepare the conidial suspension. The fungal mycelium was stirred in sterile distilled water and then filtered through muslin fabric. The spore concentration of *B. sorokiniana* was adjusted to 10^6 spores/mL of sterile water using a haemocytometer. The botanical extracts that were found promising under laboratory conditions were evaluated against spot blotch disease of barley under field conditions. A foliar spray of selected botanical extracts of leaf, bark and fruit @ 50% concentration was given prior to the inoculation with pathogens in order for the host plants (at the booting stage) to develop resistance to the pathogen.

After one day of spraying botanical extracts, pathogen inoculation was performed. Plants were inoculated as the conidial suspension of *B. sorokiniana* (5×10^3 conidia mL/L) was applied in the form of a fine mist to the leaves of each plant using an atomizer at noon. Polysorbate 20 (1% w/v) was added to the sterile water to promote conidial adhesion. C1 was kept as an inoculated control that received no botanical treatment. C2 was kept as an untreated control (plants without inoculation and sprayed with sterile water). The plots were tagged with labels with information about the date of spray and inoculation. The disease symptom appeared in the form of minute spots on the leaves of the plant after the 4^{th} day of inoculation. The physiological and biochemical variations were recorded after treatments from 1-7 days after treatment (Chhabra *et al.,* 2023a).

Physiological and biochemical estimations

The barley leaf samples collected from 1 DAT to 7 DAT were used to record the following metabolic variations in host plants. The chlorophyll concentration was determined spectrophotometrically according to the methodology of Hisox and Israeltam (1979). The phenol content was determined spectrophotometrically using standard methodology (Bray and Thorpe 1954). Malondialdehyde content was estimated using the standard procedure of Cheeseman (2006). The activity of peroxidase was determined spectrophotometrically according to the method of Shannon *et al.*, (1966). PAL activity was estimated using the standard methodology of Burrell and Rees (1974). To obtain an average value for the sample, each treatment was replicated three times.

Disease and yield attributes

The Terminal Disease Severity Index (DSI) was recorded and measured using a 0–9 double-digit scale as given by Saari and Presscott, (1975). The first digit of the scale denotes the percent blighted area on the flag leaf, whereas; the second digit represents the percent blighted area on the flag-1 (F-1) leaf. After the plants were harvested, they were dried, threshed, and recorded as the plot's yield. This yield was then converted to the unit of (q/ac) to calculate the total grain yield. The Ibrahim *et al.,* (2003) formula was used to determine the percent yield gain.

Statistical analysis

The field experiment used a randomised block design, and the lab experiment used a completely randomised design. The biochemical parameters were replicated thrice with identical results; the values presented in this manuscript represent the average of those results. The statistical analysis of the two-year pooled data was performed using Tukey's post hoc test through SPSS statistical software**.** The standard deviation of the means was calculated using Microsoft Excel (2016). At the probability level of ($p \leq 0.05$), the differences were considered statistically significant (Easterling, 2015).

RESULTS AND DISCUSSION

In-vitro screening of extracts presenting antifungal activity

The effects of fruit, leaf and bark extracts (aqueous and 50% ethanolic) from *T. chebula* and *T. bellerica* were evaluated at 10%, 20%, 30%, 40% and 50% concentrations against *B. sorokiniana* fungal growth under *in-vitro* conditions. The colony diameter of the mycelium at the given concentrations of tested botanicals incorporated in PDA medium was noted until the growth of mycelium in the control covered the entire plate. Data regarding the antifungal efficacy of *Terminala* extracts against *B. sorokiniana* growth is given in Table 1. At various doses, the tested botanicals effectively inhibited the growth of pathogens and thus showed significant antifungal efficacy (Figures 1 and 2). As the concentration of botanicals either in aqueous or ethanolic solvents increased, the mycelial inhibition (%) also increased.

On the basis of mean values, it was observed that among *T. chebula*, the fruit ethanol extract possessed significantly higher inhibitory potential (57.2%) against mycelial growth of fungal pathogens, followed by the fruit aqueous extract (54.5%). When compared to its bark extracts, a significant antifungal potential of 50.0% and 51.2% was recorded. Among the aqueous and 50% ethanolic extracts of *T. chebula*, it was observed that the 50% ethanolic extract from all parts had significant inhibitory potential in contrast to its aqueous counterparts. The maximum

reduction in the fungal colony of *B. sorokiniana* was recorded for fruit treatments, followed by leaf treatments. In comparison to their other counterparts, the treatments with bark extracts proved to be less effective against the pathogen. Among aqueous and 50% ethanolic extracts of *T. bellerica*, it was observed that the 50% ethanolic extract from all parts had a significant inhibitory effect on fungal growth in contrast to its aqueous extracts.

Among all treatments, Harar fruit ethanolic extract, Harar bark aqueous extract, Bahera leaf aqueous extract and Bahera fruit ethanolic extract reported more than 50% inhibition of fungal colonies at 30%, 40% and 50% concentrations of botanicals, respectively. On comparing both medicinal plants, it was found that treatments with *T. chebula* possess more antifungal potential as compared to *T. bellerica* extracts. In contrast to aqueous extracts in all botanical treatments, ethanolic and aqueous extracts at the highest concentration (50%) from the selected *Terminalia* species inhibited fungal growth in PDA media. Tegegne *et al.* (2008) suggested *in vitro* tests of botanical extracts as an important step in selecting plants with antifungal potential against various plant pathogens. Naz *et al.* (2014) reported that methanolic and aqueous extracts of *Jacaranda mimosifolia* followed by *Thevetia peruviana* at different concentration caused significant inhibition of *B. sorokiniana* growth.

Altered Total chlorophyll content

The data recorded for total chlorophyll content is presented in Table 2. All the plants sprayed with botanical extracts resulted in an increase in total chlorophyll content from the 1 DAT to the 7 DAT. Among treatments of *T. chebula*, in T2 (fruit ethanolic extract), the total chlorophyll content in the leaves of barley was significantly higher (4.13 mg g^{-1} FW) than in plants with treatment T1 (fruit aqueous extract) of 3.32 mg g^{-1} FW. The total chlorophyll contents of plots sprayed with bark aqueous (T3) and bark ethanolic extract (T4) were statistically at par to each other. Plants sprayed with *T. bellerica* fruit extracts (aqueous and ethanolic) had significantly higher chlorophyll content than (T8) plots sprayed with leaf aqueous extract (1.98 mg g^{-1} FW) and (T7) leaf ethanolic treatment (1.61 mg g^{-1} FW). The total chlorophyll content of the plants sprayed with the (T6) fruit ethanolic extract (3.14 mg g^{-1} FW) and the (T5) fruit aqueous extract (2.87 mg g^{-1} FW) was statistically at par. Among control treatments inoculated (without spray), C1 showed significantly lower total chlorophyll content in contrast to C2 uninoculated (water spray). Inoculated control (without spray) plots recorded a decrease in total chlorophyll content from the 2 DAT. On the basis of the mean data, it is clear that the plants sprayed with botanical extracts in all treatments, irrespective of the solvent, possessed significantly higher total chlorophyll content as compared to the control plants. Botanical-induced resistance refers to

increase in the synthesis of some compounds in plants that can inhibit the growth of pathogens as a result of high chlorophyll content as compared to untreated plants. The pathogen's toxic metabolites may be the reason for the decrease in chlorophyll content in inoculated plants (Senthil *et al.,* 2010) and they may prevent the production of chlorophyll rather than damage already-existing pigments (Mandal *et al.,* 2009). Similar results were recorded in the present study, where the inoculated control recorded the lowest chlorophyll content. Dallagnol *et al.* (2011) observed that the pathogen-produced compounds significantly decreased photosynthetic pigments, severely suppressing leaf photosynthesis in the process. This alters the physiology of the leaf and results in necrosis and cell death. Godlewska *et al.* (2021) reported that foliar applications of the plant extracts have led to an increase in the parameters like total yield, biomass, photosynthetic pigments, antioxidants and nutrient elements of radish.

Modulation in Phenol Metabolism

It is evident from the Table 3 that the uninoculated plants (C2) exhibited no significant variation in total phenol content and maintained a constant value throughout the investigation. Among treatments of *T. chebula,* treatment fruit ethanolic (T2) extract recorded an increase in phenol content of (25.0 mg g^{-1} DW) followed by fruit aqueous spray T1 (24.7 mg g^{-1} DW). Similarly, the plants treated with bark ethanolic extract also showed significantly higher phenolic content than their aqueous extract. Fruit extract treatments had significantly higher total phenols than bark extract treatments. Among treatments of *T. bellerica,* the fruit ethanolic extract (T6) recorded significantly high total phenols in contrast to the fruit aqueous spray (T5). But plots treated with leaf aqueous extract spray showed significantly lower phenol content (12.7 mg g^{-1} DW) in contrast to T8 (16.2 mg g^{-1} DW). Uninoculated plots (C2) had significantly higher total phenols than inoculated plots (C1). When compared to healthy and inoculated plots, all botanically sprayed plants accumulated significantly more total phenols. Spraying of botanical extracts resulted in an increase in total phenol content in inoculated barley plants until the last day of observation. The potential inhibitory effect of the plant extracts as potent bio-fungicides was in agreement with the findings of Karavaev *et al.* (2002), who showed that the activity of the aqueous extracts from the leaves of *Padus avium*, *Populus tremula*, *Chelidonium majus* significantly inhibited the *Puccinia triticina* infection and induced the systemic resistance in plants, which was attributed to the high level of total phenols in the treated leaves of wheat.

Lipid peroxidation marker status: malondialdehyde

The results of malondialdehyde content (MDA) obtained from Table 4 demonstrated that the spray of botanical extracts on inoculated plants significantly lowers the MDA content from the 1 DAT till the last day of the observation recorded, whereas the inoculated control (C1) recorded significantly higher MDA content in contrast to plots sprayed with botanicals and uninoculated control (C2). Among the treatments of *Terminalia chebula,* the plots treated with bark extract hold significantly higher MDA content as compared to treatments with fruit (aqueous and ethanolic) extracts. The plants sprayed with fruit ethanolic extract (T2) and fruit aqueous extract (T1) were statistically equal to each other. Similarly, the plots sprayed with bark ethanolic extract (T4) and bark aqueous extract (T3) were statistically at par. Similarly, among the treatments of *Terminalia bellerica,* the leaf extracts recorded significantly higher MDA content in contrast to plots sprayed with fruit extracts. The plots treated with fruit ethanolic extract (T6) and fruit aqueous extract (T5) were statistically at par to each other. Inoculated plants without botanical spray (C1) had significantly higher MDA content than the uninoculated control (C2). In comparison to the inoculated and uninoculated control, all plots sprayed with botanical extracts had significantly lower MDA content. The lowering of cellular reactive oxygen species, which is essential for maintaining cell membrane integrity, is caused by the increased activities of scavenging antioxidant enzymes (Singh *et al.*, 2016). Similarly, Farag *et al.* (2011) found that willow aqueous extracts reduced the disease incidence of *Fusarium* wilt in tomato seedlings after 3 and 7 days of infection by increasing the activities of antioxidant defence enzymes and decreasing the level of malondialdehyde.

Activity of Peroxidase (POX)

The data on peroxidase activity in relation to plants treated with different botanicals are presented in Table 5. Among plants sprayed with *T. chebula*, the enzyme activity of fruit ethanolic extract (T2) and fruit aqueous extract (T1) were statistically at par to each other. Similarly, the activity in plants treated with bark ethanolic extract (T4) and bark aqueous extract (T3) was statistically at par. Among treatments of *T. bellerica* species, the enzyme activity of fruit ethanolic extract (T6) and fruit aqueous extract (T5) was statistically at par to each other. Similarly, the activity in plants treated with bark ethanolic extract (T8) and bark aqueous extract (T7) was statistically at par. Among both species, plants sprayed with fruit ethanolic extract recorded significantly higher peroxidase activity as compared to other botanical treatments. Among control treatments, uninoculated control (C2) resulted in significant high enzyme activity in contrast to inoculated control (C1). From the second day after pathogen (C1)

inoculation, the plants' enzymatic activity decreased dramatically. Peroxidase aids lignification by catalysing the final polymerization step of lignin synthesis, increasing tissue lignification and limiting fungi penetration (Barilli *et al.*, 2010). According to Geetha and Shetty report (2002), inducers upregulate the activity of peroxidase with correlated initiation of systemic resistance in host to confer fungal resistance. Similarly *Reynoutria sachalinensis* extracts provided protection against powdery mildew in cucumber and tobacco plants through modulation of antioxidant defense mechanisms (Sundar *et al.*, 2001).

Activity of Phenyl alanine ammonia lyase (PAL)

PAL is one of the foremost broadly examined proteins in plant secondary metabolism whose expression has also been proposed to play a significant role as a physiological marker for plant resistance assessment. The results of phenyl alanine ammonia lyase (PAL) activity are presented in Table 6**.** The increase in enzyme activity was significant from the first day after treatment to the seventh day in inoculated host plants sprayed with botanicals. Among the treatments of *T. chebula,* the plants treated with the fruit ethanolic extract (T2) showed significantly high PAL activity (6.05 µg min^{-1} $mg^{-1}protein^{-1}$) followed by fruit aqueous extract (T1). The activity of plants sprayed with bark ethanolic extract (T4) and bark aqueous extract (T3) was statistically at par to each other. The spray of fruit extract recorded significantly higher PAL activity in comparison to plants sprayed with leaf extract. Among the treatments of *T. bellerica*, the PAL activity in plots sprayed with fruit ethanolic extract (T6) and fruit aqueous extract (T5) was statistically at par to each other. Similarly, the activity of plants treated with leaf ethanolic extract (T8) and leaf aqueous extract (T7) was statistically at par. Among both species, *T. chebula* had significantly higher PAL activity. Based on the mean data, it is clear that all of the plants treated with botanicals had significantly higher PAL activity than the inoculated and uninoculated control. According to Chakraboty *et al.,* (2007), spraying an aqueous extract of *Cathranthus roseus* to tea plants resulted in a significant increase in the expression of PR (pathogenesis-related) proteins as well as a quick build-up of phenolics in host plant both of which decreased the frequency of foliar blight. Subsequent rise in PAL activity and phenolics leads to upregulation in endogenous salicylic acid biosynthesis, an essential plant systemic resistance signal (Chhabra *et al.*, 2022)

Terminal Disease severity

Table 7 displayed data on the effect of botanical extracts on disease severity and disease control percentage. From the table, it can be noted that the highest terminal disease severity was

recorded in control plants, which received only pathogen inoculation (69.6%). All the botanically treated plants led to lower terminal disease severity as compared to the inoculated control plants. Among the plants sprayed with extracts of *T. chebula* species, the significant lesser terminal disease severity percent was observed with treatment of *T. chebula*, especially treatment T2 which led to greater yield and percent disease control. Among *T. bellerica*, the treatment with a leaf ethanolic extract recorded a significantly lower disease severity of 39% with a percent disease control of 58.4%. Among all botanically treated plots, the leaf aqueous extract of *T. bellerica* resulted in significantly high terminal disease severity and lower disease control. Amongst the plants treated with aqueous and ethanolic extracts of fruits from both species, the ethanolic treatments of both *Terminalia* species showed a significantly higher percentage of disease control in contrast to the aqueous treatments. Due to the high similarity between the phytochemistry of both tree species, it is presumed that the mechanism of action of their antifungal properties is strongly similar (Zhang *et al.*, 2019). However, further studies are required to better understand the molecular and cellular mechanisms behind the antifungal roles of botanical extracts.

Total Yield

It is evident from the data (Table 7) that the yield component recorded was significantly higher in plants sprayed with different botanical extracts when compared with controls, thus demonstrating their protective nature by inducing resistance against pathogens. Among treatments, the maximum percent increase in yield and total yield were recorded in plots treated with (T2) fruit ethanolic extract of *T. chebula* (i.e., 42.4% and 13.9q/ac, respectively), followed by (T1) plants sprayed with fruit aqueous extract. The treatment of fruit ethanolic and aqueous extract from *T. bellerica* recorded a significant increase in total yield in contrast to leaf aqueous and ethanolic extract. The plants treated with *T. chebula* and *T. bellerica* resulted in a significant increase in yield compared to control C1 (water sprayed with pathogen spore suspension). Wheat leaf rust severity was reduced considerably by foliar spraying of pomegranate, eucalyptus, cactus, garlic and neem plant extracts (El-Malik and Abbas, 2017). Foliar applications of these extracts not only impart resistance in infected plants but also increased yield and grain quality. The results obtained are in accordance with Kumar *et al.*, (2017), who found that the foliar application of *Lantana camara* extract to potato plants as an inducer before the inoculation with *Alternaria solani* led to a reduction in the disease severity. Wheat leaf rust severity was reduced considerably by foliar spraying of different plant extracts (El-Malik and Abbas, 2017). Draz *et al.* (2019) demonstrated that treating *Puccinia triticina*-infected wheat

plants with the investigated plant extracts (*Melia azedarach*, *Acalypha wilkesiana*, *Lawsonia inermis*, *Punica granatum* and *Lantana camara)* significantly improved yield components when compared to the untreated control.

In conclusion, the current study's findings clearly demonstrated that these extracts were efficient against barley spot blotch pathogen. The mechanism by which botanical extracts work differ from those of traditional pesticides in that, they restrict its growth both directly and indirectly by eliciting defense mechanisms from plants. In the current context of sustainable agriculture and rising consumer demand for organic food, plant resistance inducers are seen as a potential and environmentally acceptable alternative to conventional fungicides. It is therefore highly advised to incorporate them into eco-friendly disease management strategy.

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Table 1. Inhibitory effect of botanical extracts at different concentration against *B. sorokiniana* under *in-vitro* conditions.

Tree species	Plant part	Solvent	Mycelial inhibition (%)					Mean
Concentration tested (%)			10 %	20 %	30 %	40 %	50 %	
Terminalia chebula (Harar)	Fruit	Aqueous	33.2±1.3	39.8±1.5	44.0±1.2	70.7±1.6	84.7±0.7	54.5^{b}
	Fruit	50% Ethanolic	32.9±1.2	39.4±0.6	52.0±1.5	74.9±2.4	86.8±0.5	57.2^{a}
	Leaf	Aqueous	26.3±2.9	27.4±1.0	28.4±1.5	30.2±2.4	41.0±1.5	29.7^{i}
	Leaf	50% Ethanolic	30.0±1.5	43.1±1.6	49.5±0.7	54.1±1.2	56.6±0.9	46.6^{g}
	Bark	Aqueous	18.4±1.8	33.1±0.9	60.6±1.5	62.8±1.1	75.2±0.6	50.1^{de}
	Bark	50% Ethanolic	30.0±1.6	37.0±0.7	41.0±2.7	69.1±0.8	78.7±0.9	51.2^{cd}
Mean			28.5	35.9	45.9	60.6	70.2	
Terminalia bellerica (Bahera)	Fruit	Aqueous	21.2±1.2	30.4±0.9	49.5±1.5	77.0±0.6	85.7±1.2	52.8^{bc}
	Fruit	50% Ethanolic	41.7±1.7	44.7±1.2	50.8±1.2	58.9±0.5	71.0±2.2	53.4^{bc}
	Leaf	Aqueous	26.7±1.8	19.4±0.6	54.3±1.6	66.5±0.6	69.1±1.0	47.2^{fg}
	Leaf	50% Ethanolic	29.6±1.2	35.9±1.8	41.5±1.4	57.4±1.3	79.5±0.8	48.8^{ef}
	Bark	Aqueous	7.84±0.7	12.2±0.7	32.9±0.7	34.3±1.3	38.5±1.5	25.1^{j}
	Bark	50% Ethanolic	28.4±1.4	32.3±0.7	40.4±1.8	40.6±0.8	63.8±3.0	41.1^{h}
Mean			25.9	29.1	44.9	55.8	67.9	
Overall Mean			27.2^{d}	32.6^{d}	45.4^{c}	58.2^{b}	69.0^{a}	46.5
Propiconazole at 0.1% concentration exhibited 100% mycelial growth inhibition								
Mean±SD, each value with different letter is significantly different ($p \leq 0.05$) as per Tukey's post hoc test.								

Table 2. Effect of foliar spray of botanical extracts on total chlorophyll content of barley.

Total chlorophyll content (mg g^{-1} FW)										
Tree species	Spray	Treatment	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT	MEAN
T. chebula	Fruit (aqueous)	T1	2.77±1.6	2.86±0.5	2.94±0.2	3.42±0.3	3.56±0.7	3.74±0.9	3.97±0.4	3.32^{b}
	Fruit (50% ethanolic)	T2	3.12±0.1	3.59±0.9	3.86±0.3	4.15±0.6	4.63±0.6	4.76±0.9	4.85±0.3	4.13^{a}
	Bark (aqueous)	T3	1.60±0.1	1.66±0.8	1.79±0.3	1.94±0.3	2.03±0.5	2.12±0.2	2.19±0.2	1.90^{cd}
	Bark (50% ethanolic)	T4	1.84±0.2	1.93±0.2	1.98±0.6	2.27±0.6	2.36±0.9	2.48±0.7	2.54±0.5	2.2^{c}
Mean			2.33	2.51	2.64	2.94	3.14	3.28	3.39	
T. bellerica	Fruit (aqueous)	T5	2.31±0.4	2.42±0.6	2.63±0.3	3.04±0.2	3.15±0.4	3.29±0.8	3.48±0.6	2.87^{b}
	Fruit (50% ethanolic)	T6	2.68±0.2	2.74±0.1	2.89±0.2	3.27±0.5	3.34±0.4	3.46±0.4	3.58±0.1	3.14^{b}
	Leaf (aqueous)	T7	1.27±0.5	1.36±0.1	1.49±0.3	1.56±0.5	1.79±0.6	1.86±0.3	1.93±0.3	1.61^{de}
	Leaf (50% ethanolic)	T8	1.42±0.1	1.59±0.6	1.74±0.1	2.09±0.6	2.16±0.3	2.29±0.7	2.57±0.4	1.98^{cd}
Mean			1.92	2.02	2.19	2.49	2.61	2.72	2.89	
Inoculated (without spray)		C1	0.56±0.4	0.85±0.2	0.75±0.2	0.54±0.1	0.58±0.1	0.42±0.7	0.36±0.6	0.58^{f}
Uninoculated (water spray)		C2	1.04±0.4	1.09±0.6	1.13±0.4	1.25±0.4	1.31±0.4	1.26±0.6	1.12±0.8	1.17^{e}
Overall Mean			1.86^{c}	2.01^{bc}	2.12^{bc}	2.35^{b}	2.49^{ab}	2.57^{a}	2.66^{a}	
Mean±SD, each value with different letter is significantly different ($p \leq 0.05$) as per Tukey's post hoc test; DAT refers to days after treatment										

Table 3. Effect of foliar spray of botanical extracts on total phenol content of barley.

Total Phenol content (mg g^{-1} DW)										
Tree species	Spray	Treatment	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT	MEAN
T. chebula	Fruit (aqueous)	T1	19.6±0.7	23.4±0.5	25.7±0.6	26.1±0.9	26.5±0.4	26.8±0.9	26.6±0.7	24.7^{a}
	Fruit (50% ethanolic)	T2	22.3±0.4	23.2±0.7	23.4±0.2	25.6±0.9	27.8±0.7	27.9±0.7	26.7±0.9	25.0^{a}
	Bark (aqueous)	T3	13.3±0.7	14.1±0.6	14.5±0.2	15.2±0.8	16.1±0.7	16.7±0.7	17.0±0.9	15.0^{d}
	Bark (50% ethanolic)	T4	15.1±0.5	15.3±0.5	16.1±0.5	17.3±0.6	17.9±0.8	18.4±0.4	19.2±0.5	16.8^{c}
Mean			17.6	19.0	20.0	21.0	22.1	22.5	20.4	
T. bellerica	Fruit (aqueous)	T5	15.9±0.6	16.2±0.8	16.6±0.7	17.2±1.1	18.4±0.7	19.0±0.5	18.2±0.8	17.1^{c}
	Fruit (50% ethanolic)	T6	18.5±0.7	19.3±0.5	19.5±0.5	20.1±1.1	20.7±0.7	21.1±0.5	21.8±0.8	19.9^{b}
	Leaf (aqueous)	T7	11.3±0.8	11.6±0.7	12.1±0.6	13.4±0.7	13.9±0.5	14.2±0.5	14.3±0.9	12.7^{e}
	Leaf (50% ethanolic)	T8	14.1±1.0	14.6±0.6	16.5±0.9	16.9±0.5	17.1±0.2	17.2±0.7	18.6±1.0	16.2^{c}
Mean			14.9	15.4	16.2	17.0	17.5	17.9	16.2	
Inoculated (without spray)		C1	6.3±0.4	8.2±0.6	7.1±0.5	6.4±0.6	4.7±0.4	3.6±0.4	2.9±0.5	5.60^{g}
Uninoculated (water spray)		C2	9.8±0.6	9.1±0.4	8.9±0.6	9.3±0.3	8.5±0.2	8.6±0.2	9.2±0.3	8.9^{f}
Overall Mean			14.6^{cd}	15.5^{cd}	16.0^{bcd}	16.8^{bc}	17.1^{b}	17.4^{ab}	18.2^{a}	17.8
Mean±SD, each value with different letter is significantly different ($p \leq 0.05$) as per Tukey's post hoc test; DAT refers to days after treatment.										

Table 4. Effect of foliar spray of botanical extracts on malondialdehyde content of barley.

Malondialdehyde content (nM g^{-1} FW)										
Tree species	Spray	Treatment	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT	MEAN
T. chebula	Fruit (aqueous)	T1	1.95±0.5	1.86±0.8	1.72±0.4	1.63±0.4	1.51±0.3	1.50±0.8	1.33±0.5	1.64^{c}
	Fruit (50% ethanolic)	T2	1.84±1.2	1.76±0.5	1.53±0.3	1.47±1.1	1.41±0.6	1.26±0.3	1.19±0.6	1.49^{c}
	Bark (aqueous)	T3	2.59±1.3	2.51±0.2	2.39±0.3	2.34±0.6	2.22±0.2	2.15±0.6	2.08±0.4	2.32^{b}
	Bark (50% ethanolic)	T4	2.43±0.5	2.36±0.1	2.31±0.4	2.25±0.1	2.14±0.6	2.02±0.2	1.97±1.0	2.21^{b}
Mean			2.20	2.12	1.98	1.92	1.82	1.73	1.64	
T. bellerica	Fruit (aqueous)	T5	2.07±0.8	2.01±0.2	1.89±0.4	1.84±0.2	1.76±1.1	1.71±0.5	1.56±0.2	1.84^{c}
	Fruit (50% ethanolic)	T6	1.98±1.0	1.91±0.7	1.82±0.9	1.69±0.4	1.58±0.3	1.43±0.7	1.42±0.8	1.69^{c}
	Leaf (aqueous)	T7	2.84±0.1	2.77±0.9	2.69±0.2	2.49±0.9	2.41±0.5	2.28±0.8	2.21±0.1	2.53^{b}
	Leaf (50% ethanolic)	T8	2.72±0.3	2.63±0.2	2.60±0.7	2.42±0.5	2.39±0.4	2.21±0.7	2.17±0.5	2.44^{b}
Mean			2.40	2.33	2.25	2.11	2.03	1.91	1.84	
Inoculated (without spray)		C1	3.12±1.4	3.26±0.3	3.44±0.8	3.73±0.5	3.85±0.3	4.09±0.6	4.18±0.6	3.67^{a}
Uninoculated (water spray)		C2	1.08±0.3	1.01±0.4	1.11±0.5	1.05±0.7	0.97±0.5	1.02±0.5	1.01±0.5	1.04^{d}
Overall Mean			2.26^{a}	2.21^{ab}	2.15^{abc}	2.09^{bc}	2.03^{c}	1.96^{d}	1.91^{d}	
Mean±SD, each value with different letter is significantly different ($p \leq 0.05$) as per Tukey's post hoc test; DAT refers to days after treatment										

562 **Table 5**. Effect of foliar spray of promising botanical extracts on peroxidase activity of barley.

Peroxidase (µmole min^{-1} mg^{-1} $protein^{-1}$)										
Tree species	Spray	Treatment	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT	MEAN
T. chebula	Fruit (aqueous)	T1	1.41±0.3	1.52±0.5	1.59±0.3	1.64±0.4	1.71±0.2	1.75±0.1	1.76±0.2	1.62^{ab}
	Fruit (50% ethanolic)	T2	1.53±0.3	1.59±0.6	1.65±0.3	1.67±0.9	1.72±0.2	1.79±0.1	1.82±0.2	1.68^{a}
	Bark (aqueous)	T3	1.24±0.5	1.29±0.3	1.31±0.4	1.42±1.0	1.45±0.2	1.51±0.1	1.56±0.2	1.39^{abc}
	Bark (50% ethanolic)	T4	1.27±0.7	1.32±0.3	1.37±0.3	1.43±0.3	1.49±0.2	1.55±0.1	1.59±0.1	1.43^{abc}
Mean			1.36	1.47	1.48	1.54	1.59	1.65	1.68	
T. bellerica	Fruit (aqueous)	T5	1.30±0.6	1.36±0.3	1.41±0.2	1.43±0.4	1.48±0.2	1.50±0.2	1.60±0.1	1.44^{abc}
	Fruit (50% ethanolic)	T6	1.39±0.6	1.44±0.3	1.57±0.2	1.58±0.3	1.66±0.3	1.71±0.2	1.74±0.4	1.58^{ab}
	Leaf (aqueous)	T7	1.17±0.7	1.19±0.5	1.21±0.2	1.26±0.3	1.31±0.5	1.33±0.1	1.42±0.3	1.27^{cd}
	Leaf (50% ethanolic)	T8	1.21±0.7	1.24±0.3	1.31±0.2	1.36±0.4	1.44±0.2	1.50±0.1	1.53±0.3	1.36^{bc}
Mean			1.27	1.31	1.37	1.41	1.50	1.51	1.57	
Inoculated (without spray)		C1	0.89±0.1	1.27±0.4	0.52±0.1	0.41±0.3	0.23±0.1	0.08±0.4	0.03±0.2	0.50^{e}
Uninoculated (water spray)		C2	1.11±0.1	1.03±0.3	1.09±0.3	1.05±0.3	1.10±0.1	1.01±0.2	1.05±0.8	1.06^{d}
Overall Mean			1.25^{d}	1.32^{cd}	1.30^{bcd}	1.33^{bcd}	1.36^{bcd}	1.37^{bcd}	1.41^{a}	
Mean±SD, each value with different letter is significantly different ($p \leq 0.05$) as per Tukey's post hoc test; DAT refers to days after treatment.										

563

564 **Table 6.** Effect of foliar spray of botanical extracts on phenylalanine ammonia lyase activity
565 of barley leaves.

Phenylalanine Ammonia Lyase (µg min^{-1} $mg^{-1}$$protein^{-1}$)										
Tree species	Spray	Treatment	1 DAT	2 DAT	3 DAT	4 DAT	5 DAT	6 DAT	7 DAT	MEAN
T. chebula	Fruit (aqueous)	T1	4.53±0.2	4.58±0.01	5.02±0.01	5.12±0.01	5.13±0.01	5.18±0.01	5.25±0.01	4.97^{b}
	Fruit (50% ethanolic)	T2	5.00±0.02	5.07±0.03	6.18±±0.03	6.24±0.01	6.33±0.01	6.63±0.03	6.87±0.01	6.05^{a}
	Bark (aqueous)	T3	3.86±0.02	3.94±0.05	4.01±0.04	4.04±0.02	4.11±0.10	4.16±0.01	4.20±0.01	4.05^{c}
	Bark (50% ethanolic)	T4	3.95±0.01	4.01±0.15	4.04±0.01	4.13±0.02	4.18±0.02	4.22±0.01	4.35±0.02	4.13^{c}
Mean			4.33	4.4	4.81	4.89	4.94	5.04	5.17	
T. bellerica	Fruit (aqueous)	T5	4.36±0.02	4.41±0.03	4.54±0.02	4.64±0.03	4.87±0.01	4.97±0.01	4.69±0.25	4.69^{b}
	Fruit (50% ethanolic)	T6	4.57±0.01	4.58±0.02	4.88±0.01	4.99±0.01	5.09±0.04	5.14±0.01	5.24±0.02	4.93^{b}
	Leaf (aqueous)	T7	3.67±0.02	3.71±0.02	3.77±0.03	3.87±0.01	3.92±0.02	3.98±0.01	4.42±0.02	3.90^{c}
	Leaf (50% ethanolic)	T8	3.53±0.02	3.59±0.04	3.97±0.03	4.02±0.01	4.07±0.01	4.13±0.01	4.21±0.02	3.93^{c}
Mean			4.03	4.07	4.29	4.38	4.49	4.54	4.64	
Inoculated (without spray)		C1	1.96±0.12	2.54±0.14	2.06±0.35	1.68±0.54	1.32±0.36	1.19±0.49	1.08±0.46	1.69^{e}
Uninoculated (water spray)		C2	2.09±0.06	2.15±0.09	2.10±0.38	2.07±0.41	2.08±0.52	2.11±0.47	2.06±0.47	2.10^{d}
Overall Mean			3.76^{d}	3.86^{d}	4.05^{cd}	4.08^{bcd}	4.11^{bc}	4.17^{b}	4.23^{a}	4.04
Mean±SD, each value with different letter is significantly different ($p \leq 0.05$) as per Tukey's post hoc test; DAT refers to days after treatment										

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570 **Table 7.** Effect of spray of selected botanical extracts on disease and yield parameters in barley.

Spray	Treatment	Dose	Terminal Disease severity (%)	Per cent disease control	Yield (q/ac)	Per cent increase in yield
T. chebula fruit extract (aqueous)	T1	@ 50%	34.3^{g}	63.6^{c}	12.8^{bc}	36.2^{bc}
T. chebula fruit extract (ethanolic)	T2	@ 50%	29.9^{h}	71.1^{b}	13.9^{ab}	42.4^{ab}
T. chebula bark extract (aqueous)	T3	@ 50%	45.2^{d}	44.6^{g}	11.4^{bcd}	20.7^{def}
T. chebula bark extract (ethanolic)	T4	@ 50%	43.6^{e}	47.8^{f}	11.7^{bcd}	25.0^{de}
T. bellerica fruit extract (aqueous)	T5	@ 50%	41.5^{f}	51.7^{e}	11.9^{bcd}	26.6^{de}
T. bellerica fruit extract (ethanolic)	T6	@ 50%	39.0^{g}	58.4^{d}	12.2^{bc}	29.8^{cd}
T. bellerica leaf extract (aqueous)	T7	@ 50%	50.6^{b}	31.8^{i}	10.2^{cd}	14.9^{f}
T. bellerica leaf extract (ethanolic)	T8	@ 50%	48.9^{c}	34.7^{h}	10.8^{bcd}	18.6^{ef}
Control (water sprayed with pathogen spore suspension)	C1	-	69.6^{a}	-	8.41^{d}	-
Uninoculated (only water)	C2	-	6.69^{i}	90.1^{a}	15.3^{a}	62.2^{a}
Each value with different letter is significantly different ($p \leq 0.05$) as per Tukey's post hoc test						

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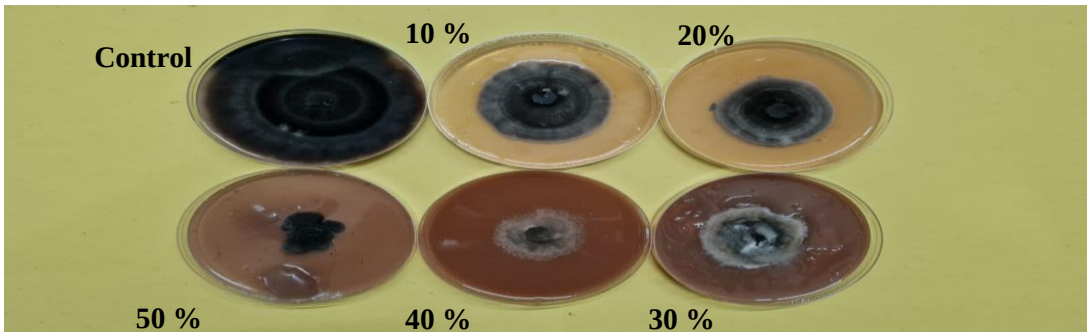
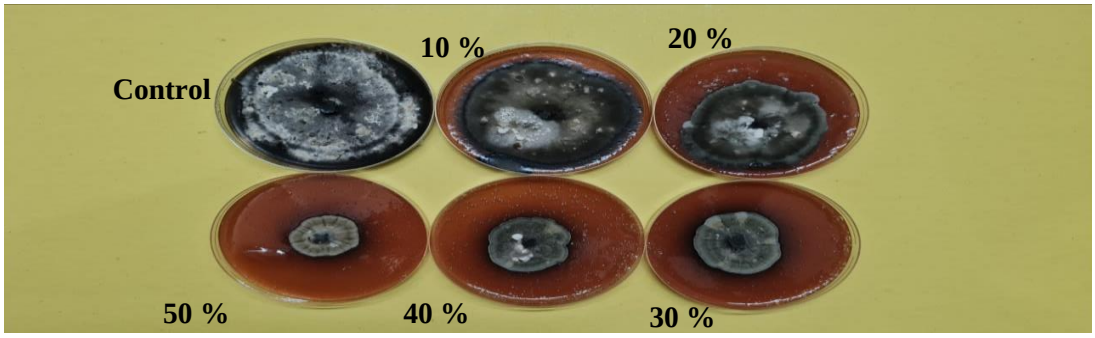
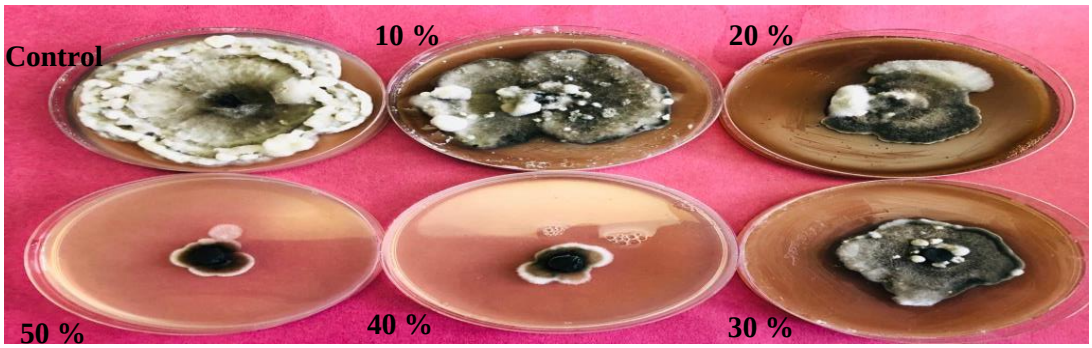






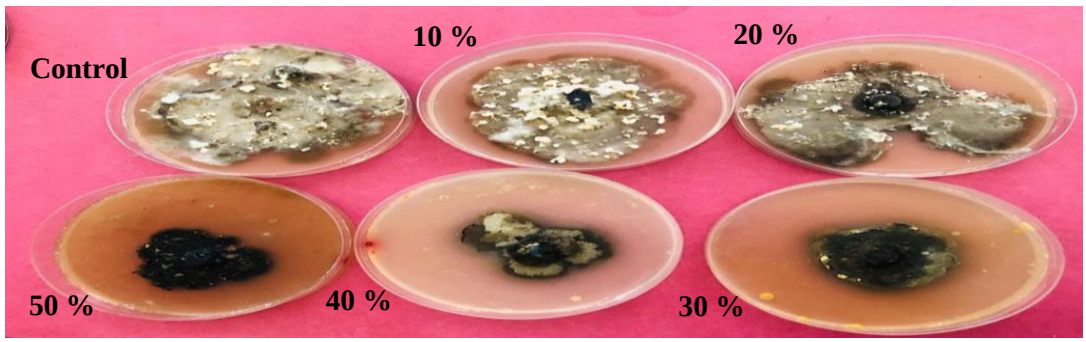


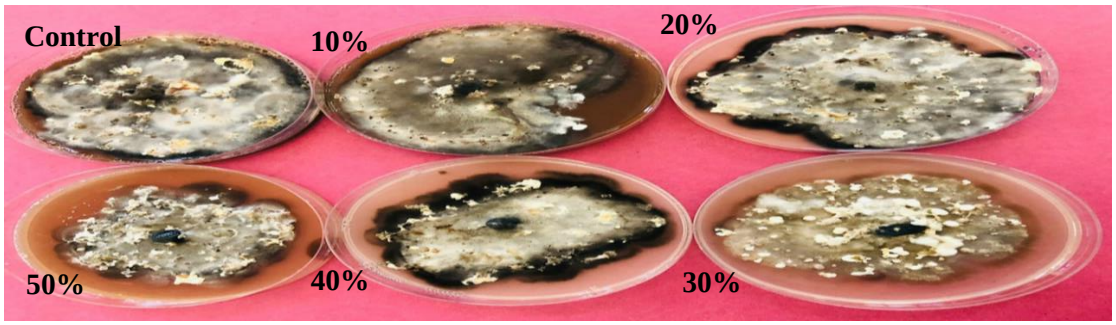
Figure 1. Antifungal activities of fruit extracts of *Terminalia* species against *B. sorokiniana.*

(A) Fruit ethanolic extract of *T.chebula*

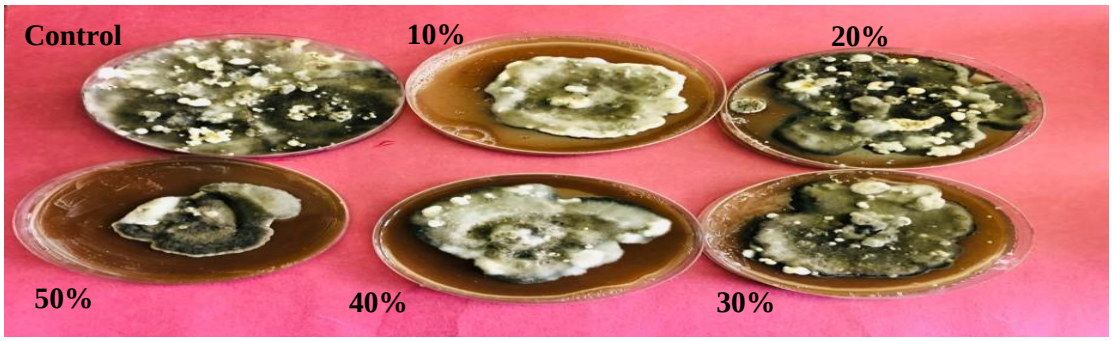
(B) Fruit ethanolic extract of *T. bellerica*

(C) Fruit aqueous extract of *T. chebula*

(D) Fruit aqueous extract of *T. bellerica*



(A)



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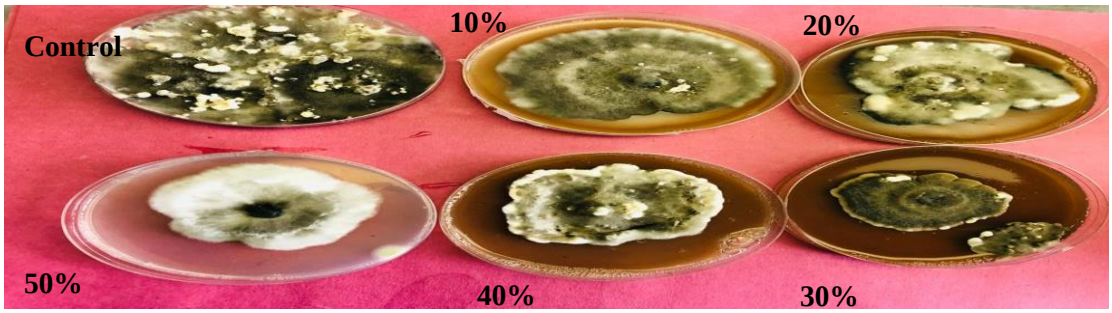

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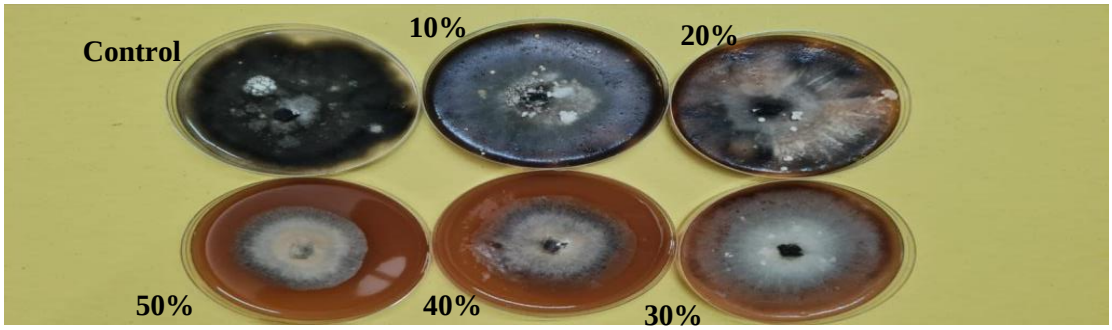

(D)

Figure 2. Antifungal activities of different extracts of *Terminalia* species against *B. sorokiniana*.
(A) Bark aqueous extract of *T. bellerica*
(B) Bark ethanolic extract of *T. bellerica*
(C) Leaf ethanolic extract of *T. chebula*
(D) Leaf aqueous extract of *T. chebula*