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Antagonistic Potential of Halophilic Fungi from Lonar Lake Against Soil-Borne Plant Pathogens

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Abstract | **Original Research Article**

***Background**:* Effective plant disease management is still one of the major concerns in Agriculture based countries like India. Meanwhile the use of chemical fungicides to tackle them raises environmental and health concerns. The excessive use of chemical fungicides also contributes to increase in soil salinity posing another threat to crop production. In saline environments the efficiency of the fungicides reduces remarkably due to osmotic interactions. This compels us to find novel and extremophilic biocontrol strategies which can work under the adverse conditions. Halophilic fungi, living in hypersaline conditions can be exploited for such reasons. ***Methodology**:* Lonar Lake, a unique crater ecosystem, in Buldhana district of Maharashtra, India is a home for such halophilic fungi. Water and Sediment samples were collected. Halophilic Fungi were isolated by using serial dilution on selected media. These were supplemented with varying NaCl concentrations. The isolated fungi were identified as *Aspergillus species* and *Penicillin species* via morphological characterization. The antagonistic activity of selected halophilic fungi fungal isolates (HFI) was assessed against soil borne plant pathogen viz. *F oxyporum, R. solani* and *M. phaseolina* by dual culture assay under 5% and 10% salinity. Percentage Growth Inhibition (PGI %) was calculated for the quantification of antagonistic potential. ***Results**:* Total 48 halophilic fungal isolates were obtained from sediment and water samples from Lonar Lake. These isolates exhibited diverse colonial morphology and pigmentation. The dual culture assay displayed the significant inhibitory potential against the tested pathogens. Few isolates demonstrated exceptional PGI% values more than 70% against *F. oxyporum* and *R solani* under 10% NaCl and the highest antagonism up to 82% PGI against *M. phaseolina* at 10% NaCl. ***Conclusion**:* The halophilic fungi from lunar lake are promising biocontrol agents towards the Plant Disease Control insulin habitats their salinity tolerance and the antagonistic potential display their significance in developing novel biocontrol strategies which gives us an eco-friendly alternative against the chemical specifically in saline environments.

Keywords: Halophilic Fungi, Biocontrol, Antagonistic Potential, Lonar Lake, Dual Culture Assay.

1. INTRODUCTION

Considering the global population is growing, and land and water supplies are depleting, it is critical to increase food production while reducing agricultural losses due to factors such as crop diseases. Every year, plant diseases brought on by pathogenic microbes result in significant losses in the production of food crops. Given the possibility that continuing climate change may create circumstances that could encourage the geographic spread of disease, this loss poses a danger to the sustainable supply of high-quality food and raises questions regarding food security [1].

Conventional management strategies rely heavily on broader-level chemical pesticides (fungicides, bactericides, nematicides, and broad-spectrum fumigants), which, despite their efficacy, have shown some serious concerns. Pesticides are harmful chemicals or biological agents intentionally placed into the environment to control or eliminate unwanted pests such as weeds, insects, fungi, and rodents. Pesticides work by tempting, captivating, and ultimately killing or controlling pests [2]. Pesticides, while beneficial, can also be harmful and bio-accumulative. Pesticides can remain in the environment for years due to their long-lasting nature and difficulty in degradation. They remain in the soil and release lots of accumulated salts, making the soil saline, which infiltrates groundwater and surface water, and damages the ecosystem on a vast scale. Chemical qualities can allow them to infiltrate organisms, accumulate in food systems, and affect human health. Occupational usage of pesticides can expose humans to hazardous residues [3].

Soil salinization is one of the major environmental and economic issues around the world. This is caused by rising sea levels or lithological factors resulting from the weathering and dissolution of salt and mineral-containing rocks (primary salinization), or by human activities such as the use of low-quality water for agricultural purposes and fertilizer overuse (secondary salinization). It is estimated that around the world, up to 20% of total cultivated lands and more than 30% of irrigated agricultural fields are saline soils, and this number may increase by up to 10% per year [4]. Salinity is one of the harshest environmental variables restricting crop plant yield since most crop plants are sensitive to salinity induced by excessive salt concentrations in the soil, and the area of land impacted is growing by the day. High saline concentrations result in impairment of plant growth, disruption of crucial physiological processes, and contribute to the proliferation of plant diseases by creating favorable conditions, especially those which are caused by soil-borne pathogens. The growth of plants in the saline habitat can result in salinity/salt stress.

Plants' physiological responses to salt stress include osmotic, ionic, and oxidative stressors. Halophytes are suited to high salt concentrations, whereas lycophytes are more sensitive to salinity stress. Salinity stress has a wide range of effects on plants, including osmotic stress, ionic toxicity, and oxidative damage, which influence nutrient intake, photosynthesis, and reproduction [5]. The synthetic pesticides are not only harmful due to their toxicity, but also due to the complex interactions between the salt stress and virulence of soil-borne pathogens reduce the effects of traditional chemical pesticides [6], highlighting an urgent need for a sustainable, environment-friendly alternative for disease management.

Many organisms have substantial restrictions due to high osmotic pressure, ion toxicity, poor soil physical conditions, and/or soil floods; hence, salt-affected habitats are specialized ecotones. Organisms have evolved to live in harsh environments, resulting in several endemism's. Halophilic bacteria, sometimes known as "salt-loving" microorganisms, thrive in conditions with high salt concentrations that kill most other germs. Halotolerant and halophilic microorganisms can thrive in hypersaline conditions; however, only halophiles need at least 0.2 molar (M) of salt for growth. Halotolerant microorganisms can only withstand salt concentrations of less than 0.2 M [7]. These halophilic microorganisms can be leveraged for the biocontrol of soil-borne pathogens.

Among these biocontrol agents, halophilic fungi not only show high tolerance against high concentrations but also show promise in the management of diseases caused by soil-borne plant pathogens. Halophilic fungi survive at high salinities by employing a variety of adaptation mechanisms at various levels, including cellular (e.g., increased cell wall thickness and specialized transporter systems), genetic (e.g., transport-related genes and high acidic amino acid content), enzymatic, and metabolic pathways (e.g., oxidoreductase, superoxide dismutase, and the high osmolarity glycerol signalling pathway). Halophilic microorganism membrane structure is critical to their ability to adapt to saltwater conditions. It shields the cell from the damaging effects of excessive salt concentrations and maintains osmotic balance in the cell through adequate membrane fluidity [8].

The increased salt of the environment initially affects the cell wall and cell membrane of fungal cells. Fungi exhibit structural changes in the cell wall and cell membrane, as well as the presence of pigments and/or hydrophobins in response to increased salt [9]. The cell wall thickening and changes in lipid content are examples of structural changes caused by increasing salt in the media [10].

The generation of secondary metabolites in halophilic fungi can be regulated by salt concentration. Some salt-tolerant fungi in sun salterns produce antifungal and antibacterial chemicals, implying that they may influence microbial composition in saline environments [11]. One of the most serious risks to public health is the growing resistance of multiple pathogenic microbes to antibiotics. Their overuse over the years has weakened or destroyed their effectiveness.

Fungi are regarded as effective makers of secondary metabolites having antibacterial capabilities. In recent years, halophilic and halotolerant fungi have gained attention for their potential as a source of novel antimicrobial compounds with antibacterial and antifungal characteristics [12]. Some research has revealed that halophilic fungi produce bioactive molecules with antiviral activity [13]. Secondary metabolites released by halophilic and halotolerant marine fungi include steroids, peptides, terpenes, alkaloids, and polyketides, all of which have antiviral, antibacterial, antifungal, anticancer, and cytotoxic properties [14].

Halophilic fungi have also been investigated for their capacity to create antifungal chemicals. Because of their difficult ecological habitat, they have evolved powerful antifungal metabolites as part of their defence systems. These chemicals have potential applications in agriculture, food preservation, and pharmaceuticals [8]. It has been demonstrated that several halophilic fungi of these genera are capable of synthesizing a variety of polyketides, including antibiotics such as penicillins, extrolites, and penicillic acids [15].

Enzymes encompassing phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO) play critical roles in these defense systems. PAL is an important enzyme in the phenylpropanoid pathway, facilitating the conversion of L-phenylalanine to trans-

cinnamic acid, the initial step in the biosynthesis of phenolics, flavonoids, and lignin. PPO, on the other hand, is involved in the oxidation of phenolic compounds to quinones, which are harmful to pathogens and help to reinforce the cell wall [16].

Halophilic fungi, organisms adapted to grow in salt-saturated environments, have specific physiological and biochemical characteristics that allow them to survive and remain active under very high osmotic conditions. These responses include intricate stress response pathways, such as the High Osmolarity Glycerol (HOG) pathway that controls intracellular osmolyte accumulation to counterbalance external osmotic pressure. In addition, these fungi frequently have membrane lipid changes to change the fluidity and integrity of their membranes in concert with high salt tolerance. In addition, ROS-modulating systems and increased thickened cell wall deposition have also contributed to their resistance. A unique characteristic of halophilic fungi is the production of various stress-induced secondary metabolites. These include polyketides, alkaloids, extrolites, and pigments, many of which have strong antimicrobial or antifungal activity [17]. This diversity in metabolism is a natural weapon against phytopathogens. The ability of halophilic fungi to grow in saline environments, where biocontrol agents such as many Trichoderma spp. Usually, failure makes them excellent prospects for the development of resistant biocontrol agents for salt-affected agricultural lands [18]. Their specialized adaptations make them particularly well-suited for ecological niches in which other beneficial microbes cannot survive.

Notwithstanding the outstanding extremophilic variations in Lonar Lake, halophilic fungi from this exotic crater ecosystem are neither adequately studied for their strain-specific biocontrol efficacy vis-à-vis soil-borne phytopathogens under salinity stress. Halophilic fungi from Lonar Lake display a strong antagonistic potential towards important soil-borne plant pathogens, which is the consequence of their unique salinity-induced metabolic adaptation. The objective of the study was to recover and purify a variety of halophilic fungi from different ecological regions of Lonar Lake.

2. MATERIALS AND METHODS

2.1. Situate Analysis:

Lonar Lake is the world's only Hypersaline-alkaline Natural Soda Lake created by high velocity crater impact. Water body of this lake's ambient environment is associated with extreme physicochemical conditions, usually a pH of 9–11 and high salinity levels frequently above 10% (w/v) NaCl, in addition to high alkalinity The unique hydro-geochemistry coupled with its isolated characteristics have led to a specialized microbial community adapted to these extreme parameters, which makes the Lonar lake a promising source for extremophilic microorganisms [19].

2.2. Sample Collection:

For the isolation of halophilic fungi, a total of 16 environmental samples were collected from eight distinct sites around Lonar Lake. These included eight water samples and eight sediment samples. The sampling sites were carefully selected to represent diverse ecological zones, including open water, submerged areas, and potential microbial hotspots. Sediment samples were collected using a sterile core sampler by accessing the bottom layers of the lakebed. Approximately 5–10 cm depth of sediment was retrieved and immediately transferred into sterile containers. All samples were annotated with their location. The lake's sediment and water have a high mineral content, which has been shown to select for microbes with extreme-tolerant survival mechanisms, conditioning them for halophilic growth. Water samples were obtained by submerging sterile 50 mL Falcon tubes in the lake to a depth of 10-15 cm. The canisters were tightly closed and had site-specific tags. All collected specimens were transported promptly to the laboratory in insulated containers, stored at 4 °C, and processed [20,21].

2.3. Isolation of Halophilic Fungi

For the isolation of halophilic fungi, sediment and water samples were handled under aseptic conditions. 1 g of each sediment sample was suspended in 9 mL sterile saline (0.85% NaCl) independently, and the sediments were well homogenized manually by vortexing to ensure uniform distribution of microbial content. Successive dilutions from 10^{-1} to 10^{-6} were prepared by adding 1 mL from each previous dilution into 9 mL fresh sterile saline solution. Similarly, 1 mL of the water sample was directly combined with 9 mL of sterile saline solution to start the dilution of the water sample. The serial dilutions for sessile samples were done using the same method as sediment samples [17]. 100 µL of each dilution was plated in Modified Sabouraud Dextrose Agar (SDA) containing 5% NaCl to favour the growth of halophilic fungi. The spreading was made with the aid of a sterile L-shaped spreader. The inoculated plates were then incubated between 25 and 30 °C for 7-14 days inside the laboratory. After the period of incubation, fungal colonies with halophilic growth were noticed. Pure isolates were obtained by sub-culturing different colonies onto fresh halophilic SDA plates. From there, a sum of eleven pure (HFI) Halophilic Fungal Isolates were successfully isolated from the samples [22].

2.4. Antagonistic Activity under Salt-stress Conditions

The antagonistic potential of the halophilic fungi against soil-borne plant pathogens was evaluated by the dual culture method on Modified Potato Dextrose Agar (PDA) medium containing 5% NaCl. The selective medium was poured into Petri dishes under aseptic conditions. The pathogen *M. phaseolina, F. oxysporum and R solani* [22], were each aseptically inoculated at the edges of the plate. The plates were then placed onto the

agar surface of a PDA culture that had been inoculated with the pathogen, and after the pathogen was colonized for 2 days, they were inoculated at an equal distance (about 2–3 cm) from the pathogen point of origin with halophilic fungal isolates. The structure was such that direct contact of the test pathogen and halophilic fungi could be viewed. At constant temperature (25–30 °C), all plates were incubated for 5–7 days to promote growth and interaction of the fungi [24]. After the incubation period, the plates were checked for the presence of antagonistic activity. The inhibition zone of pathogen growth was determined as the distance between the pathogen and the halophilic fungal colony, and the ratio of the control plates, which were the pathogen grown without any antagonist, was measured. A separate zone between the halophilic fungus and the pathogen was taken as evidence of antifungal activity. A third bioassay was conducted to determine if there was potential for antibiosis, to further assess the mechanism of antagonism. Plates were examined for inhibition zones around the colonies of the halophilic fungus, indicating production of antifungal metabolites or lytic enzymes. These inhibition zones were compared with the control plates to affirm the suppressive effect [25]. From these colonies, the radial growth of both pathogens in dual culture (T) and in control © was measured. For each extract, the percentage growth inhibition (PGI%) was calculated as follows: [26].

$$PGI\ \% = \frac{(C - T)}{C} \times 100$$

2.5. Mechanistic Studies

Preliminary mechanistic studies of the antagonistic action, including extracellular lytic enzyme production. Activity assays for chitinase, β-1,3-glucanase, and protease work were performed. Chitinase and β-1,3-glucanase for isolates were inoculated on selective agar media with colloidal chitin (6.25 g) or laminarin (8 g) as the sole carbon source, respectively. After incubation and staining, the detection of a clear halo around the fungal colony suggested that enzymes were secreted [27]. Protease activity was measured on skim milk agar plates, where the clear zone surrounding the colonies represented hydrolyzed protein. To quantify enzyme production, the diameter of these zones was measured. This enzymatic potential is often linked to mycoparasitism of biocontrol fungi, aiding pathogens' cell wall degradation [28].

3. RESULTS

3.1. Sample Collection

Samples of water, soil, and sediment were collected from various sites of Lonar Lake. Some were collected near the shoreline, and others from the mid-lake. A total of sixteen samples, eight sediments and eight water samples were collected. All samples were labelled immediately and stored at 4°C during fungus isolation work. The sediments, which were all different colours from brown to darker, show that the organic matter and mineral content were not uniform. The differences also indicated that the fungal diversity may differ across locations.

3.2. Isolation and Purification

Extraction from selective media containing 5–20% NaCl yielded 48 separate fungal morphotypes. This variation was observed in terms of growth rate, pigmentation (white to yellow, orange, green and black), and colony texture (velvety, cottony, and leathery) among these isolates. These isolates were successively subcultured, leading to the purification of axenic cultures. 30 fungal colonies were chosen and purified. From these isolates, 11 exhibited considerable growth even at higher saline concentrations. Fig.1 and Fig.2. provides the summary of these isolates, their provenance and their pigmentation which was observed.

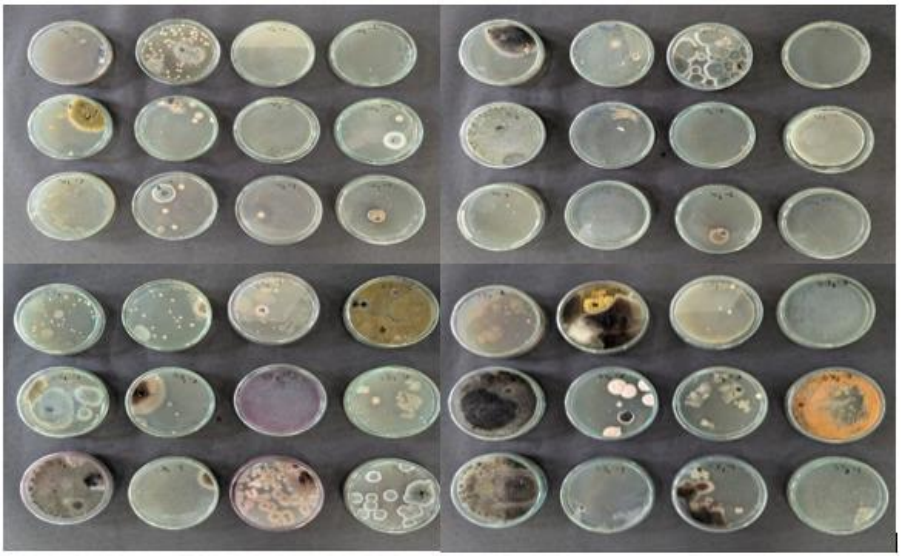

Fig.1. Isolated Fungal Colonies from Lonar Lake Samples

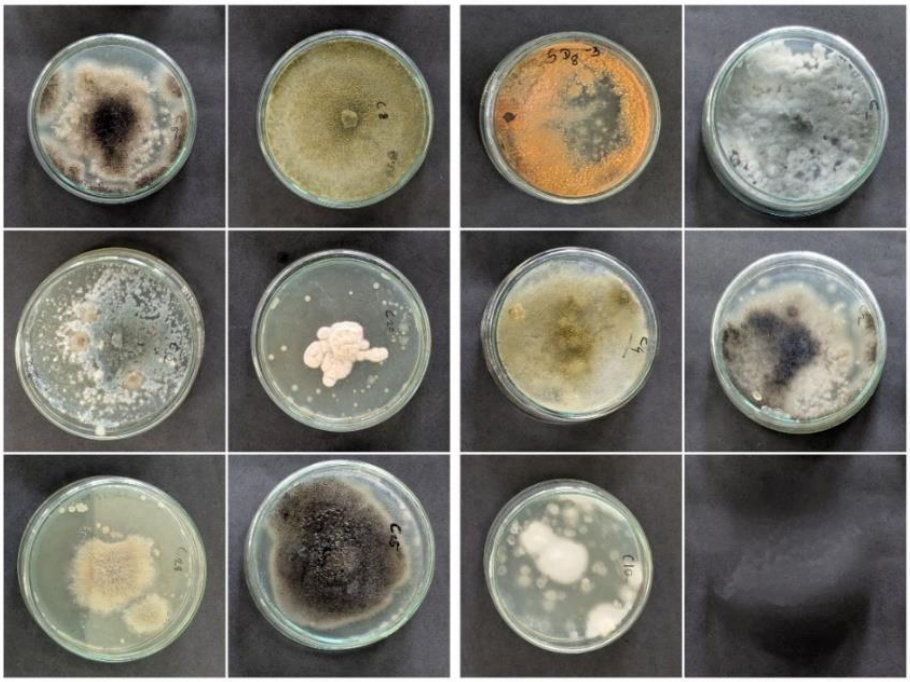

Fig. 2. 11 Selected Fungal isolates

3.3 Macroscopic Characterization of Fungal Colonies

The macroscopic characterization of purified fungal isolates included area of the colony, rate of growth, nature (cottony, powdery, leathery), elevation, margin, and pigmentation from the obverse and reverse sides of PDA and SDA plates supplemented with 10% NaCl. Slide preparation from cultures in log-phase growth allowed for micrometric analyses. Fungal mycelia were mounted in Lactophenol Cotton Blue stain in small portions. Using a microscope, the microscopic features of the species, including hyphal septation, conidiophore presence and morphology, conidia size, shape, and arrangement, and other reproductive structures, were observed [17]

3.4. Morphological and Microscopic Characterization:

Observations at the macroscopic level show a high level of variability in colony morphology. Example: isolate HFI-S-03 grew rapidly as a spreading dark green colony with a white margin, similar to *Aspergillus spp*. In contrast, isolate HFI-W-01 produced a small but vigorous colony with a velvety and orange-yellow aspect. A more detailed insight from microscopic analysis (Lactophenol Cotton Blue). Septate hyphae, characteristic of many Ascomycota isolates, were observed in this study. The conidiophores and conidial structures of several isolates were reminiscent of those in the genera *Aspergillus* and *Penicillium*. As an example, HFI-S-03 showed the typical Aspergillus morphology with globose vesicles, biseriate phialides, and dark green conidia in chains (Fig. 3). The sizes and shapes of conidia varied between isolates but were determined by micrometry to assist with preliminary characterization.

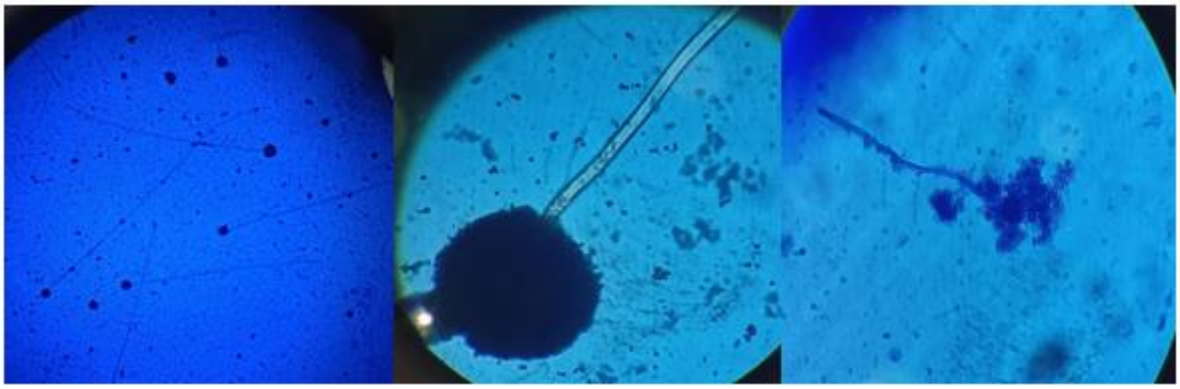

Figure 3: Microscopic view of isolated halophilic fungi mentions the fungi species viz. *Aspergillus* spp. and *Penicillin* spp.

3.5. Antagonistic Activity

The isolated fungi were then screened using the dual culture method and tested for their antagonism toward soil-borne plant pathogens, namely *Fusarium oxysporum, Rhizoctonia solani,* and *Macrophomina phaseolina*. These experiments were carried out on SDA plates supplemented with 5–10% salt to simulate saline soil conditions. The pathogens were inhibited, and a transparent colour zone was developed among all the isolates against the pathogens. The halophilic fungal isolates from all three dual cultures revealed extensive antagonistic activity against all three soil-borne plant pathogens. The PGI% differed based on the fungal isolate, the target pathogen, and the salinity level.

Percentage Growth Inhibition (PGI%) of selected Potentially Strong Antagonistic Isolates against Three Pathogens at 5% and 10% NaCl (Data summarized in Table 1).

Table 1: PGI% of Potentially strong Antagonistic isolates at 5% and 10% NaCl

ISOLATE	PATHOGEN	PGI% in 5% NaCl	PGI% in 10% NaCl
HFI-S-03	*F. oxysporum*	68.5 ± 2.1	75.2 ± 1.8
HFI-S-03	*M. phaseolina*	61.2 ± 3.5	69.8 ± 2.4
HFI-S-03	*R. solani*	72.8 ± 1.9	82.1 ± 2.5
HFI-W-01	*F. oxysporum*	55.3 ± 2.8	63.7 ± 2.2
HFI-W-01	*M. phaseolina*	50.1 ± 3.1	58.9 ± 2.6
HFI-W-01	*R. solani*	60.5 ± 2.5	70.3 ± 1.9

FI-S-03 had significantly stronger inhibitory effects against all pathogens (where the isolate HFI-S-03 was always most effective), with 82.1% PGI against *Macrophomina phaseolina* at 10% NaCl. For many isolates, we observed a tendency for antagonistic activity to be either retained or, in multiple cases, greatly increased under elevated salinity, relative to non-saline conditions. This observation is particularly salient because it indicates potential for adaptive plasticity in biocontrol settings in saline environments that promote growth. Coiling of antagonistic hyphae around pathogen hyphae, indicative of mycoparasitism, was recorded frequently, as well as the visible inhibition zones consistent with antibiosis.

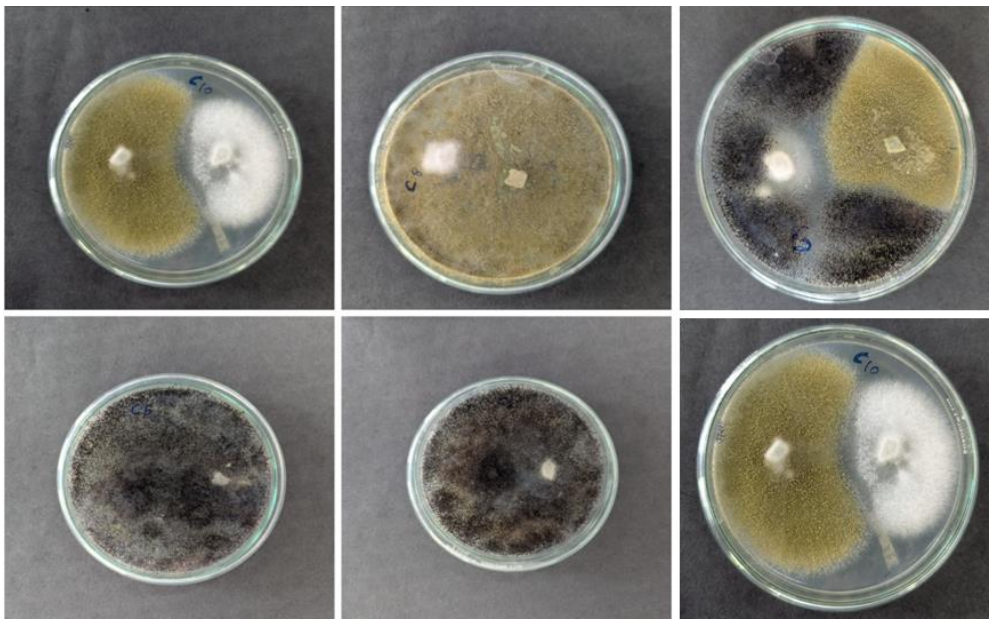

Figure 4: Antagonistic effect of Isolated Fungal Colonies from Lonar Lake Samples

3.6. Mechanistic Insights

While the antagonistic activity was detected in solid medium, enzyme assays allowed us to gain preliminary insight into potential underlying mechanisms. Most extracellular chitinase, β-1,3-glucanase, and protease activities were exhibited by various halophilic isolates, especially the isolates that showed marked inhibition of fungal growth in the dual culture. Such clear zones of hydrolysis produced by HFI-S-03 on chitin agar (diameter of 18 mm), laminarin agar (diameter of 22 mm), and skim milk agar (diameter of 15 mm) demonstrated the efficient secretion of these cell-wall degrading enzymes. These enzymes are known to be able to degrade cell wall structural components of fungi, indicating that mycoparasitism may play a role in pathogen suppression. The display of such enzymatic properties in particular salinity conditions highlights the direct ability of these halophiles to disrupt the target pathogen structure and compromise its viability.

4. DISCUSSION

The results of the experimental studies presented here indicate that halophilic fungi from Lonar Lake have considerable potential to be biocontrol agents in saline environments may help overcome a crucial limiting factor for controlling salt-induced soil-borne diseases in salt-affected agroecosystems [29]. The isolation of 24 halophilic or halotolerant fungal isolates, predominantly classified to genera like Aspergillus, Penicillium, and Cladosporium, amply demonstrates that hypersaline habitats serve as sinks for metabolically versatile fungal populations adapted for osmotic stress, strengthening an earlier description reported from other saline and hypersaline ecosystems [17]. The fact that several isolates grew best between 5% and 10% NaCl and still showed appreciable growth at as high as 20% NaCl also complements their potential for commercial use under salt-stressed soils, where proven bio-control agents or chemical fungicides often fail to perform or work inefficiently [18].

One of the main findings of this work was that the antagonistic activity against *Fusarium oxysporum, Rhizoctonia solani, and Macrophomina phaseolina* was retained or even significantly increased under saline conditions; instead, none of the salt stress effects caused a repression of the antagonistic activity. HFI-S-03, for instance, consistently produced high Percentage Growth Inhibition (PGI) values >70% against *F. oxysporum and R. solani* (10% NaCl), while also reaching about 82% against *M. phaseolina* (10% NaCl), which together indicated a strong antagonistic performance, when compared to the other bioagents, under conditions reflective of salinized agricultural soils. This salinity-related enhancement of antagonism corroborates the concept that, under abiotic stress, adaptations to extreme environments will indeed confer enhanced biocontrol efficacy, an observation that likely holds for other extremophilic organisms [30] and contrasts with many of the traditional biocontrol fungi whose efficacy tends to diminish with increasing salinity [18].

The mechanistic tests add another understanding to the mode of action of these antagonists of halophilic fungi. The high antagonistic isolates, such as HFI-S-03, showed strong extracellular activities of chitinase, β-1,3-glucanase, and protease, suggesting that their enzymatic degradation of pathogen cell walls plays a vital role for this isolate [18], lending support to established models of fungal mycoparasitism. The common observation of mycelial coiling around pathogen hyphae, as well as clear inhibition zones in dual culture assays, implies a combination of direct mycoparasitism and antibiosis with cell wall–degrading enzymes and antifungal metabolites working synergistically to inhibit the growth of the pathogens [17]. These mechanisms are particularly useful under saline stress, as plants experiencing osmotic and ion toxicity are physiologically compromised and thus need potent external antagonists capable of efficiently alleviating pathogen pressure [31].

In addition to their direct antagonistic activity, the ecological origin of these isolates brings about other agronomic benefits. Lonar Lake features high salinity and pH, as well as unique hydrogeochemical conditions; thus, fungi surviving in such an environment are expected to be tolerant not only to salt but also other co-occurring stresses such as alkalinity and fluctuating nutrient availability [32]. Thus, halophilic isolates (e.g., HFI-S-03 and HFI-W-01) may have more promising potential as bioformulations that could maintain their effectiveness under variable and often difficult conditions of salt-stressed fields than biocontrol agents from non-extreme habitats [33]. This is in line with general trends towards the use of extremophiles for sustainable agriculture, with stress-adapted microorganisms being increasingly considered as interesting tools to enhance plant resistance to stresses induced by climate change [31].

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Despite these findings, several limitations of the current study should be noted in the interpretation of these data. Next, all antagonistic and enzymatic assays took place in vitro with limited extrapolation to rhizosphere interactions, soil physicochemical heterogeneity, and plant physiological processes present in the environments found in the field. Second, the study addressed three important soil-borne pathogens and did not assess any potential non-target impacts on beneficial soil microorganisms or symbiotic partners, such as arbuscular mycorrhizal fungi, which are important for nutrient acquisition and stress resistance in many crops [30]. Despite the proof of extracellular enzyme production, the secondary metabolites responsible for antibiosis and the genetic/physiological framework controlling halotolerance and antagonism were not described, thus confining mechanistic insights at a molecular level [33].

Insight: Future research should prioritize multistep validation of these halophilic fungi as

biocontrol agents. It is also essential to conduct greenhouse and field trials along salinity gradients in order to understand if those high in vitro PGIs are translated into consistent reductions of disease incidence and severity, as well as on plant growth and yield under real agronomic conditions [18].

Parallel multi-approach methods as monitoring the antifungal compound production through metabolomics, and identifying genes and pathways that are associated with halotolerance and antagonism alike by means of (trans) genomics, would contribute to a more profound mechanistic basis for rational selection as well as improvement of promising isolates [33]. Overall, this study introduces Lonar Lake halophiles as a potential form of strain sources for the production and application of next-generation salinity-tolerant bio-fungicides that can act to control plant disease sustainably in the growing salt-affected agricultural territories across the globe [29].

5. CONCLUSIONS

This study, for the first time, reports a high level of diversity of halophilic fungi from hypersaline Lonar Lake, and demonstrates their significant salt tolerance. Majority of these isolates exhibited significant antagonistic activity against important soil-borne plant pathogens [*Fusarium oxysporum, Rhizoctonia solani and Macrophomina phaseolina*]. Importantly, the biocontrol efficiency of some isolates was not only retained but also improved in a saline environment, giving them an edge over traditional biocontrol agents in salt-affected farmlands. Initial mechanistic studies have indicated that this observed antagonism is in part due to the induction of extracellular lytic enzymes such as chitinase, β-1,3-glucanase and proteases. Taken together, these results place halophilic fungi isolated from Lonar Lake as potential candidates for exploitation in the development of novel and eco-friendly bio-fungicides suitable for saline agricultural soils, which is an urgent requirement given the global demand on food product. There is a need for more research to fully characterize their secondary metabolites, and for extensive field validation to translate these laboratory findings into effective agronomic tools.

Author contributions:

"Conceptualization, R.B. and S.W.; methodology, R.B.; A.M. and V.C.; formal analysis, A.M.; investigation, R.B.; resources, A.T.; data curation, V.C.; writing—original draft preparation, A.T. and S.W; writing—review and editing A.T. and S.W. All authors have read and agreed to the published version of the manuscript."

Conflict of interest: The authors declare no conflict of interest.

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