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RESEARCH PAPER

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Would it be possible to use nonpathogenic fungi to improve the turnover of crop residues?

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Abstract

This study was aimed to assess the suitability of four fungal species for operating in the residues of three crops in Golestan province, Iran. For this, four experiments were conducted to analyze their ability to grow on five culture media (Experiment I) and on the residues (Experiment II) and their growth responses to different pHs (Experiment III) and temperature levels (Experiment IV). Then, the possibility of establishing these fungi in the cultivated lands of studied crops was examined. Fungal growth was high on soybean and cotton residues and low on those of rice, and all the fungi produced a significant reduction in the carbon to nitrogen ratios in relation to noninoculated residues. The amount of nitrogen in fungal-treated cotton residues increased about four times compared with the control and in other studied residues increased twice as much as the control. The lowest C:N values for cotton and rice residues were found for *Pleurotus ostreatus* while *Aspergillus niger* was the most efficient for those of soybean. The results also showed that these fungi will not show the best performance in respect to temperature and pH, but will not be ineffective. The results could be the basis for further studies on the use of these fungi to improve nutrient cycling, focusing on multicriteria zoning on climatic and soil-related components.

KEYWORDS

Aspergillus niger, crop residues, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Trichoderma viride*

1 | INTRODUCTION

Every year, huge amounts of chemical fertilizers are consumed on agricultural lands resulting in increased production costs for the farmers and environmental issues, like the pollution of both surface and groundwater and contributing to agricultural CO_2 emissions [1], and rely on nonrenewable mineral resources in many cases (e.g., P, K) [2]. The recovery and reuse of nutrients in crop residues can be considered a promising avenue to reduce the dependence of high-yielding cropping systems on chemical fertilizers [3]. However, the use of crop residues usually poses several management issues, which ultimately explains why large amounts of residues are removed from agricultural systems in various forms (e.g., burning). For instance, the accumulation of these

Abbreviations: CMA, corn meal agar; CZA, Czapeck agar; MA, malt agar; PDA, potato dextrose agar; YEA, yeast extract agar.

residues in the soil can prevent easy tillage operations and thus lead to more energy consumption [4].

Soil microorganisms can provide good ecosystem services, of which the recycling of nutrients is one of the most important [5]. The incorporation of exogenous living organisms improving nutrient cycling from crop residues in agroecosystems can be envisaged as a practical application of this study topic. Among all microbial communities (including fungi, actinomycetes, and bacteria), fungi are the most important degraders of lignocellulosic materials. Understanding the environmental conditions and type of crop residues that favor the activity of decomposing fungi and fungal growth-mediated decomposition processes are important as it may help in determining the likely appropriateness or incompatibility of their application in specific agricultural areas. Different environmental factors such as temperature, humidity, light, and other factors could affect microorganisms and their functions as decomposers of crop residues [6]. For example, the growth of *Aspergillus nidulans* over 7 days proceeds at a fast pace on a culture medium of Czapec agar at a temperature of 25°C, while that of *Aspergillus glaucus* is slow. Most species of Aspergillus grow 1–9 cm in such conditions. Aspergillus fungi can tolerate high temperatures and it grows well at 40°C; although suitable temperature for its growth ranges from 20°C to 50°C [7,8].

The acidity of both the soil environment (rhizosphere) and plant residues are also two influential factors in the process of decomposition of plant residues. The pH of residues left by wooden species is usually in the range from 4 to 6, while that of herbaceous plants is typically around 7, becoming gradually acidic as the microorganisms grow and decomposition proceeds [9]. As a consequence, the incorporation and decomposition of plant residues result in changes in the pH of the rhizosphere, with the direction and the magnitude of such changes depending largely on the concentration of organic anions in the residues and initial soil pH [10]. The growth of fungi is known to be sensitive to pH but optimal ranges can be species- or even strain-specific. For instance, different strains of the Trichoderma show different responses to the acidity of their growth environment [11,12]. The best-growing environment for *Aspergillus niger* is potato dextrose agar (PDA), while lingo-cellulose-agar and Czapeck agar [CPA] + yeast extract are in the next rank [13]. Media containing D-galactose promotes vegetative rather than reproductive growth of *A. niger* [14]. These contrasting behaviors among fungal strains respond to the different starch and sugar contents of such culture media.

Having a range of responses for different strains of nonpathogenic fungi to environmental factors and their ability to grow on the residues of various crops can help us in identifying agricultural areas where these fungi could settle well. As a first approximation, this can be done by matching the fungi environmental requirements with long-term averages of climatic factors and soil characteristics. The durability of these factors in the soil, especially concerning soil temperature, can also be studied. This study focused on four fungal strains (*Pleurotus ostreatus*, *Phanerochaete chrysosporium*, *A. niger*, and *Trichoderma viride*) that have been reportedly identified as capable of breaking down crop residues [15–17], forest wastes [15], and composts [18]. The goals of the present study were to (1) determine the best culture medium for four fungal strains, (2) investigate the response of the four fungal strains to the acidity of the growing medium, (3) estimate the cardinal temperatures of the strains studied, (4) explore the suitable cotton, soybean, and rice cultivated areas in the province of Golestan, Iran, for the establishment of the four studied strains considering environmental and land features.

2 | MATERIALS AND METHODS

2.1 | The study area for investigating the possibility of using nonpathogenic fungi to improve the turnover of crop residues

Golestan province has an area of 20,437 km^2, which is located in northern Iran (36° 44′ and 38° 05′ N and the longitudes of 53° 51′ and 56° 14′ E. Based on the De-Martonne climate classification system the Golestan province contains five different climates including the Mediterranean, arid-desert, semiarid, humid, and semi-humid. In this region, total annual precipitation varies from 250 to 750 mm and with increasing from north to south regions without respect to the altitude. The climatic conditions of this region have made it possible to cultivate various crops. Wheat, barley, canola, beans, sugar beet, soybean, rice, corn, sesame, sunflower, cotton, potato, onion, alfalfa, and clover are the most important crops grown in the province as autumn and summer crops.

2.2 | Collection of fungi

All the fungi strains selected for this study (i.e., *P. ostreatus*, *P. chrysosporium*, *A. niger*, and *T. viride*) were collected from the Shast Kola forests and crop-grown fields around Gorgan, northern Iran. After they were isolated and identified, the necessary tests to ensure that these fungi were not pathogenic were performed (data not published).

2.3 | Experiment I: Performance of fungal species under different culture media

This preliminary experiment aimed to identify appropriate culture media for subsequent experiments. Five culture media (including PDA, malt agar [MA], yeast extract agar [YEA], corn meal agar [CMA], and CZA) were selected for the study. The inoculation of each strain was performed by removing hypha fragments of each fungus and transferring them to the target culture media. After 7 days at 25 ± 1°C, growth responses were evaluated by measuring the radius of the fungal colonies with a ruler. Three replicates per treatment (strain × culture medium) were used.

2.4 | Experiment II: Performance of fungal species in relation to crop residues

Dried residues of cotton, rice, and soybean were chopped into 50 mm segments. 10 g of chopped pieces of each crop were transferred to test tubes (5 × 25 cm) and its moisture adjusted to 80%–85% using distilled water and then autoclaved. For inoculation, a piece of fungal colonies of 10 mm diameter was added to the tubes containing the residues. Tubes inoculated with fungal samples were incubated in a growth chamber at a temperature of 25 ± 1°C and the mycelial growth was measured after 20 days with the ruler on the substrate. Three replicates per treatment (strain × type of residue) were used.

As an indication of the decomposing activity of the fungi, the carbon to nitrogen ratio (C:N) was evaluated after 30 days with low values of C:N indicating high decomposition. The carbon to nitrogen ratio of the residues after 30 days of inoculation with the studied fungi was studied as an indicator for the ability of these fungi to decompose crop residues. In this regard, the decomposition process on crop residues causes its contents to decompose and the ratio of nitrogen to organic carbon increases (C:N decreases) with the consumption of materials in the substrate. Nitrogen was measured by the Kjeldahl method in three steps (digestion, distillation, and titration) [19–21]. In the digestion stage, 0.2 g of the plant residue samples treated with the fungi were transferred to the special tube of the device after 30 days along with the control samples and 6 ml of 98% sulfuric acid and one catalyst tablet was added. The samples were then exposed to 250°C for 45 min and then kept at 350°C for 240 min. The extract of the digested straw was used for the distillation step. In the distillation stage, after adding 65 ml of 32% sodium bicarbonate and 35 ml of distilled water, distillation was performed for 5 min. The distillation product was added to 45 ml of 2% boric acid and finally titrated with 0.2 N hydrochloric acid and methyl reagent (pH = 4.8). Nitrogen was measured with a Kjeldahl apparatus (Gerhardt model). The amount of final hydrochloric acid used according to Equation (1) was used to determine the available nitrogen:

$$\%N = \frac{0.2(A - B) \times 14}{(1000 \times C)} \times 100. \quad (1)$$

In this equation, N is the amount of available nitrogen (%); A, the amount of acid consumed per milliliter; B shows the amount of acid consumed in the control sample in milliliters; and C the weight of the sample in grams.

The amount of organic carbon was also measured by the classic dichromate oxidation method of [22]. To do this, from the 30 days-treated samples of crop residues with fungi and the control samples, 1 g was transferred to a 250 ml Erlenmeyer flask with 10 ml of a solution of potassium dichromate and 10 ml of 98% sulfuric acid was added. After 30 min, 100 ml of distilled water were added to it and after cooling the Erlenmeyer contents, four to five drops of Orthophenafetrolein were added and the sample was titrated with ferrous sulfate until the color of the solution turned reddish-brown. Then the percentage of organic carbon remaining at the end of the decomposition process and in the control sample was calculated using the following equation:

$$\%OC = \frac{(M \times 0.39)(Vb - Va)}{S} \times 100, \quad (2)$$

where OC, is the percentage of organic carbon; M, the molarity of ferrous sulfate; S, the weight of the primary sample in grams; Vb indicates the volume of ferrous sulfate consumed in the control sample in milliliters and Va indicates the volume of ferrous sulfate consumed in titration in milliliters.

2.5 | Experiment III: pH responses of fungal species

In this experiment, the growth response under different pH levels was assessed for the four fungi species. PDA culture medium was considered in all cases, adjusting their pH to five different levels (i.e., 4, 5, 6, 7, and 8) using 50% lactic acid and 1 M NaOH. Hypha pieces were inoculated in the prepared culture media from 7-days old cultures. Petri dishes containing cultured samples were incubated in a growth chamber at the temperature of 25 ± 1°C. Then, the radius of each fungal colony grown on different nutrient media was measured with a ruler after 10 days [12,23]. Three replicates per treatment (strain × pH) were used.

As an additional trial in this experiment, the pH of residues left by the three species under study was determined. To do so, the residues were milled and passed through a 35-mesh sieve. Then, 1 g of each residue was soaked in distilled water (10 ml) for 6 h and stirred for 2 and 4 h. Finally, the mixture was filtered by the Whatman filter paper (number 4) in vacuum conditions. The pH extract was measured by a pH meter, Sartorius PB-11 [9].

2.6 | Experiment IV: Temperature responses of fungal species

This experiment aims at estimating the cardinal temperatures for the growth of the studied fungi. A PDA culture media was used in all cases, as the four strains performed well under such media in Experiment I. Hyphae isolated from 7-day fungal colonies were allowed to grow in a growth chamber at different constant temperatures ranging from 5°C to 40°C (with 5°C intervals). After 10 days, the cloning radius of each fungus was measured with a ruler. Three replicates per treatment (strain × temperature) were used. Finally, growth (radius of the colony) data for each strain were fitted to temperature using a triangular function as [24]

$$f(T) = \left\{ \begin{array}{ll} \frac{T - T_b}{T_o - T_b} & \text{if} \quad T_b < T \leq T_o \\ \frac{T_c - T}{T_c - T_o} & \text{if} \quad T_o < T < T_c \\ 0 & \text{if} \quad T \leq T_b \text{ or } T \geq T_c \end{array} \right\}, \tag{3}$$

where T, T_b, T_o, and T_c are, respectively the current, base, optimum and ceiling temperatures. Nonlinear (Nlin) and iterative optimization procedures were used to estimate equation parameters yielding the least square difference.

2.7 | Statistical analysis

The effects of the imposed treatments in each experiment on the growth of the four studied fungi were analyzed assuming a completely randomized experimental design with a factorial arrangement using the SAS program [25]. Fitting of the temperature and pH response functions was done using SigmaPlot version 12.5, from Systat Software, Inc. (www.systatsoftware.com).

2.8 | Theoretical assessment on the suitability of the fungal species for crop-specific agricultural areas of Golestan, northern Iran

2.8.1 | Spatial characterization of cotton, soybean, and rice cultivated areas

Traditional spectral image analysis methods for detecting land use information can be misleading because of the possibility of spectral interference. New object-oriented methods have been created to increase classification accuracy. In the Object-based imagery analysis method, the pixels of the images are divided into objects and sub-objects, and then small land-cover patches are integrated into the ownership parcels. In contrast to edge-oriented methods that document variations in the image, in the region-oriented methods, the pixels are grouped into given objects based on some homogeneity criteria [26].

In this study, using satellite imagery and remote sensing techniques, soybean, cotton, and rice cultivation areas within Golestan province, Iran, were identified during the 2017–2018 growing season. Satellite images of Sentinel 2 and object-based image analysis were used for land detection. All necessary atmospheric and geometric corrections were made on the images to enhance the possibility of detecting the lands. To detect the soybean-, cotton- and rice-grown fields with the cooperation of experts from the Jihad-e-Agriculture Organization in the 13 townships of Golestan province, 400 field coordinates were recorded for each crop. Some of the information reported in other academic studies was also used to complete the database of ground control points [27–30]. Finally, 1674 points were used throughout the province for detection (Figure 1a). After the detection of the land using the object-based land imagery method, the accuracy of the output images to identify the crops was determined using κ and overall accuracy coefficients [31].

2.9 | Spatial characterization of pH and temperatures in cotton, soybean, and rice cultivated areas

Spatial variability in soil pH within the cotton, soybean, and rice cultivated areas of Golestan province was characterized by 2618 soil samples that were taken in a project by Golestan Agricultural and Natural Resources Research and Education Center. For this, a regular grid-based sampling procedure from 15 cm depth was done. In this project, all chemical and physical characteristics of the soils were analyzed. These samples provided the information to generate a GIS pH map of the study area

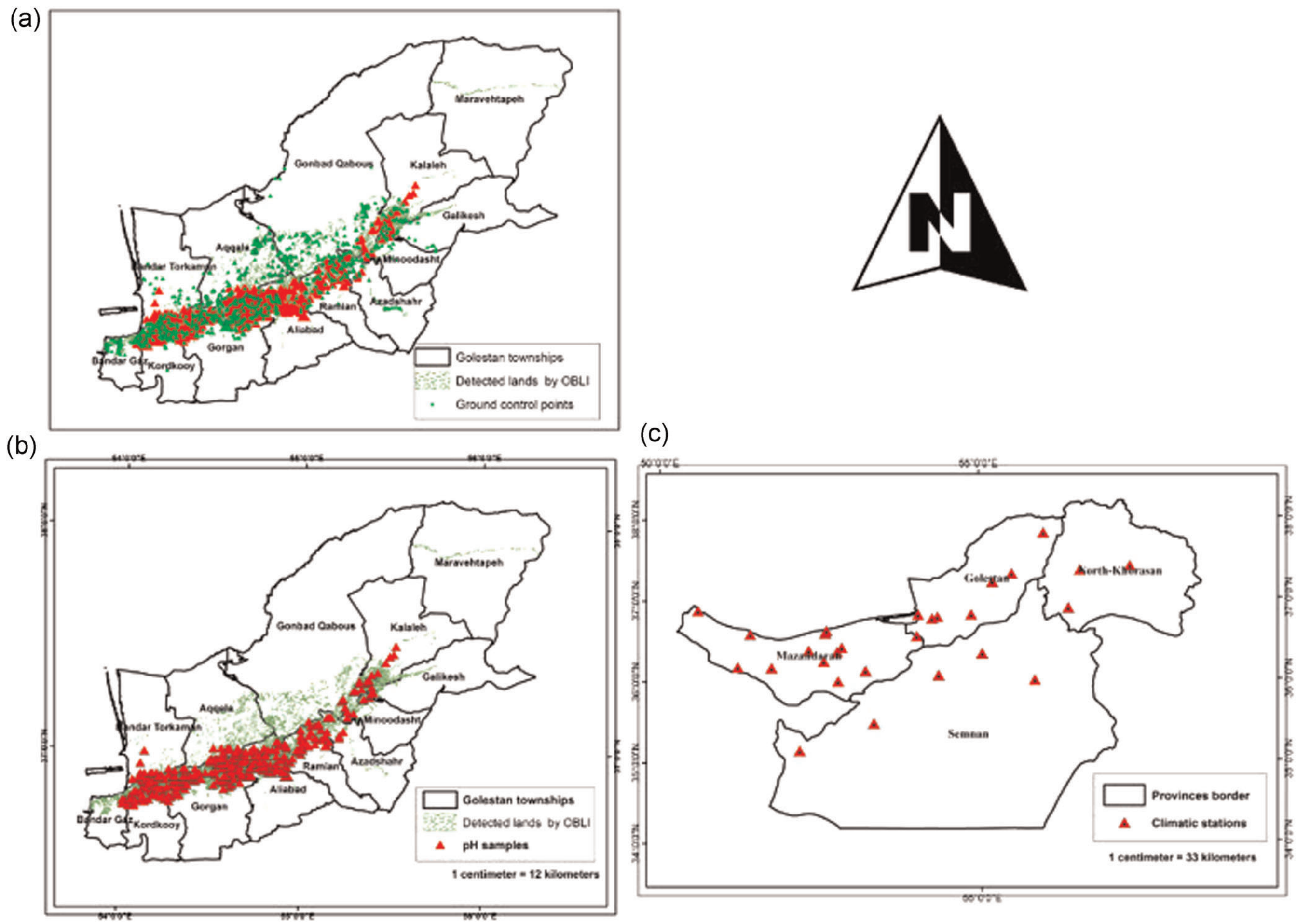

FIGURE 1 (a) Ground control points for detecting cotton, soybean, and rice cultivated lands using the OBLI method (1647 points), (b) soil samples used to measure pH in Golestan province, Iran (2618 points), and (c) climatic stations were used to provide temperature layers. The borders of the townships of Golestan province and the lands under cultivation of the three studied crops are also shown. Green polygons represent entire lands that were detected according to the OBLI method for cotton, soybean, and rice

using the inverse distance weighted method (as a classical interpolation model) (Figure 1b).

Concerning temperature, monthly records from 15 to 30 years long time series were collected from 35 meteorological stations within Golestan and four adjacent provinces (Figure 1c). The average mean monthly temperatures (for the available statistical period) were extracted for all stations and temperature variables. In the next step, to provide different minimum, maximum, and average monthly temperature raster layers, different interpolation methods were tested. To this end, many stations have been excluded from the interpolation process as independent test points to evaluate its accuracy. To interpolate the temperature variables, different classification methods and geostatistical interpolation procedure-models were used (data not shown). After preparing the raster layers, the corresponding numerical values of the stations that were left out as independent data were extracted using the "extract values by points" function in GIS media. The output accuracy of each method was investigated using statistical indices as root mean square error (RMSE), coefficient of determination (r^2), and the deviation of the adjusted regression line to the actual data against the interpolated one. These layers (maximum, average, and minimum temperatures) were prepared separately for each of the 12 months of the year and the cultivated lands of each of the three crops (a total of 108 layers). Then a regular grid of points (200×200) was formed on the layers and the corresponding values of three temperature variables for three crops and 12 months of the year were extracted from the generated rasters using "extract values to points" function. In the next step, using these data, box plots were drawn to characterize the spatial variability of maximum, minimum, and average temperatures for

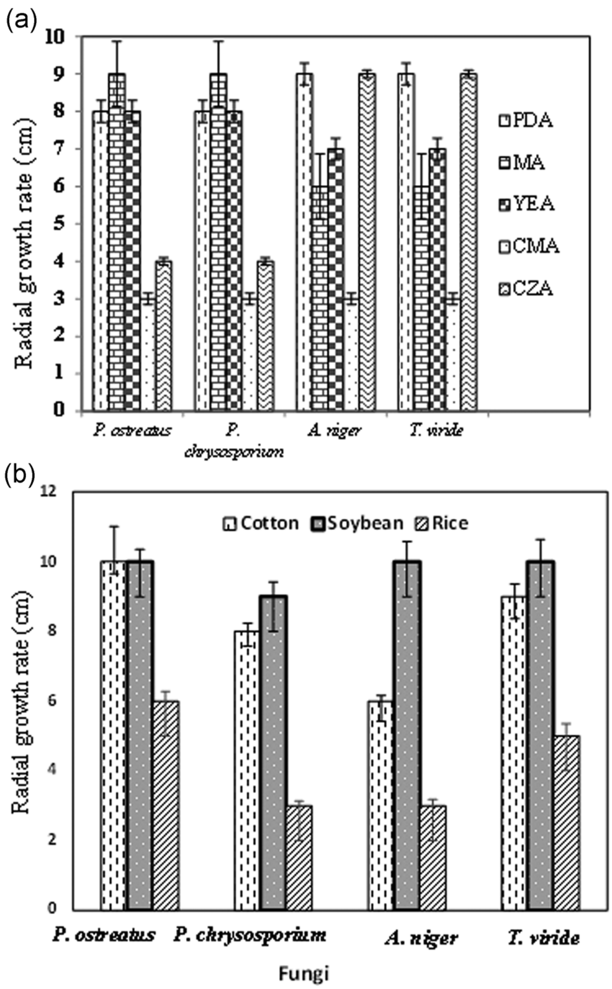

FIGURE 2 (a) The radial growth rate of four studied fungi on different culture media after 20 days: potato dextrose agar (PDA), malt agar (MA), yeast extract agar (YEA), corn meal agar (CMA), and Czapeck Agar (CZA.) (b) Radial growth (±*SE*) of fungi (cm) on different crop residues

each month of the year. Finally, the monthly temperature patterns of the study areas were compared with the range of minimum, optimal and maximum temperatures obtained for the studied fungi in Experiment III.

3 | RESULTS

3.1 | Growth of studied fungal species under different culture media

The culture media had a significant effect on the radial growth of the fungi colonies. Irrespective of the species, growth was always high in PDA and very low in CMA (Figure 2a), while MA, YEA, and CZA led to differential performances among species. Radial growth in *P. ostreatus* and *Phanerochaete chrysosporium* was high for MA and YEA and low for CZA. By contrast, *A. niger* and *T. viride* exhibited higher growth for CZA than for MA and YEA. Since all four species showed good growth in PDA, this culture medium was adopted for the growth of studied fungal species to pH and temperature experiments.

3.2 | Growth of studied fungal species in different crop residues

The type of plant residues also affected significantly radial growth of the studied fungi (Figure 2b). Regardless of the species, soybean residues always led to high growth rates (>8.9 cm), and the lowest ones were systematically found for those of rice (<6.3 cm). Cotton residues resulted in similar growth than those of soybean for *P. ostreatus*, *T. viride*, and *P. chrysosporium* but significantly lower for *A. niger*.

All four fungi were able to reduce significantly the C/N ratio in relation to the noninoculated control, irrespective of the residue (Figure 3). In soybean, *A. niger* was the most efficient (lowest C:N) and both *P. ostreatus* and *P. chrysosporium* the least. By contrast, *P. ostreatus* and *P. chrysosporium* were the fungal strains leading to a lowest C:N in cotton residues. For rice residues, no statistical differences were found among the fungi. The amount of nitrogen in fungal-treated cotton residues increased about four times compared with the control and in other studied residues increased twice as much as the control. On the contrary, no significant differences in nitrogen content were found for soybean residues when the control, *P. ostreatus* and *P. chrysosporium* samples were compared.

3.3 | Growth of studied fungal species to pH

The growth of the four studied fungi decrease with pH, showing the highest values for the lowest of the five pH levels tested (i.e., 4). Radial growth measurements were linearly correlated with pH with determination coefficients ranging from 0.83 to 0.94 (Figure 4). Resulting regression lines indicate that the radial growth of *P. ostreatus*, *P. chrysosporium*, *T. viride*, and *A. niger* decreases by 0.7, 0.46, 0.28, and 0.24 cm per unit of increase in pH (within the studied range of pH). This suggests that *P. ostreatus* was the most sensitive species to changes in the acidity of the media. The coefficients of the fitted equations and determination coefficient ($Y = a - bX$) for the studied fungi shown in Table 1.

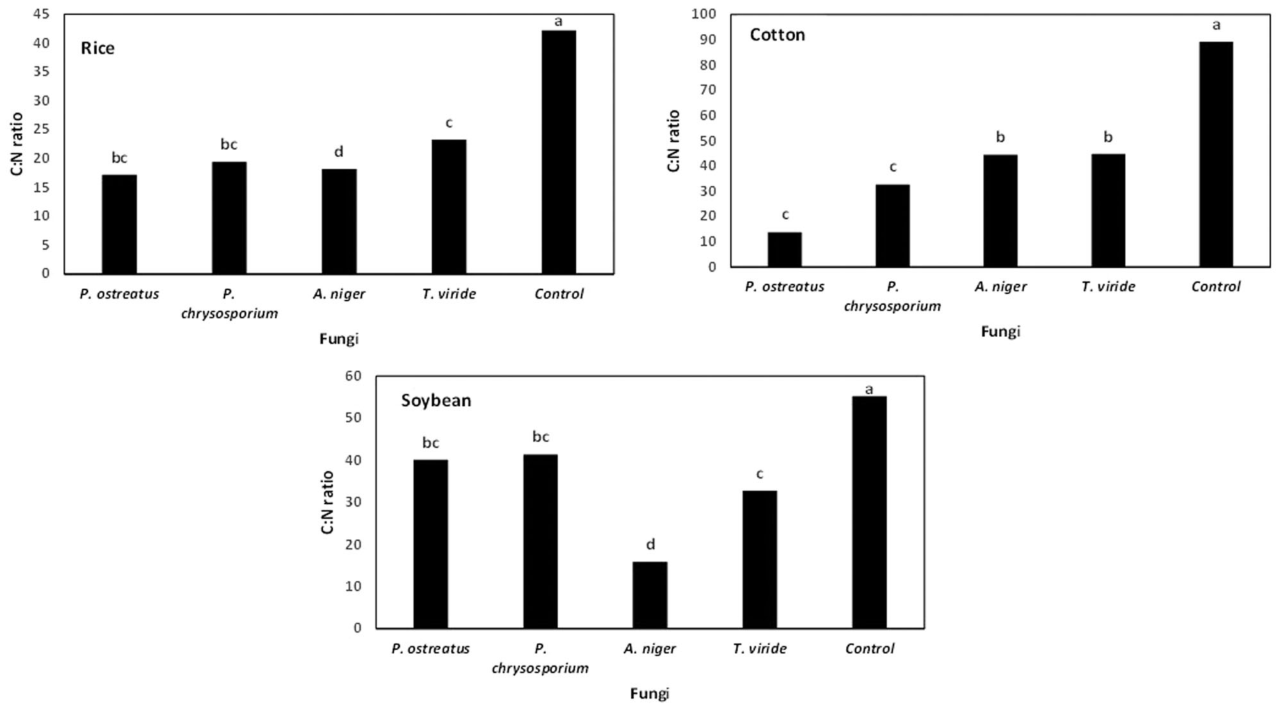

FIGURE 3 Comparison of carbon to the nitrogen ratio of fungus-free and fungus-infected crop residues

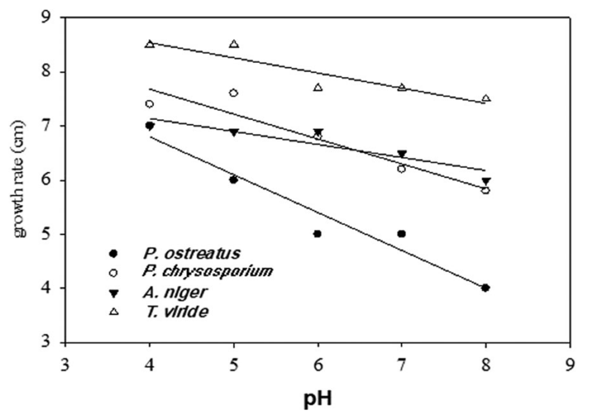

FIGURE 4 The radial growth of *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, *Aspergillus niger*, and *Trichoderma viride* in potato dextrose agar in different pHs after 20 days

There were no significant differences between plant residues concerning pH, with cotton, soybean, and rice exhibiting values of 6.74, 7.51, and 7.15, respectively.

3.4 | Growth of studied fungal species to temperature

Temperature affected drastically relative radial growth of the four fungal species with responses fitting well to a triangular function (Figure 5). The fitting helped to quantify the cardinal temperatures for each species. Statistically, no significant differences were found for any of the estimated cardinal temperatures. Base temperatures ranged from 6.2°C to 7.7°C, minimum and maximum optimal temperatures were 24.3°C and 26.4°C, and

TABLE 1 Estimated cardinal (base = T_b, optimum = T_o, and ceiling = T_c) temperatures of *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, *Aspergillus niger*, and *Trichoderma viride* using segmented model

Fungi	T_b	T_o	T_c	R^2
P. ostreatus	6.18 (1.66)[a]	24.31 (1.51)	40.16 (1.64)	93%
P. chrysosporium	6.66 (1.42)	26.01 (1.35)	39.28 (1.18)	95%
A. niger	7.60 (2.04)	26.43 (1.99)	38.80 (1.64)	90%
T. viride	7.75 (2.58)	24.28 (2.66)	38.38 (2.43)	83%

[a]Standard error.

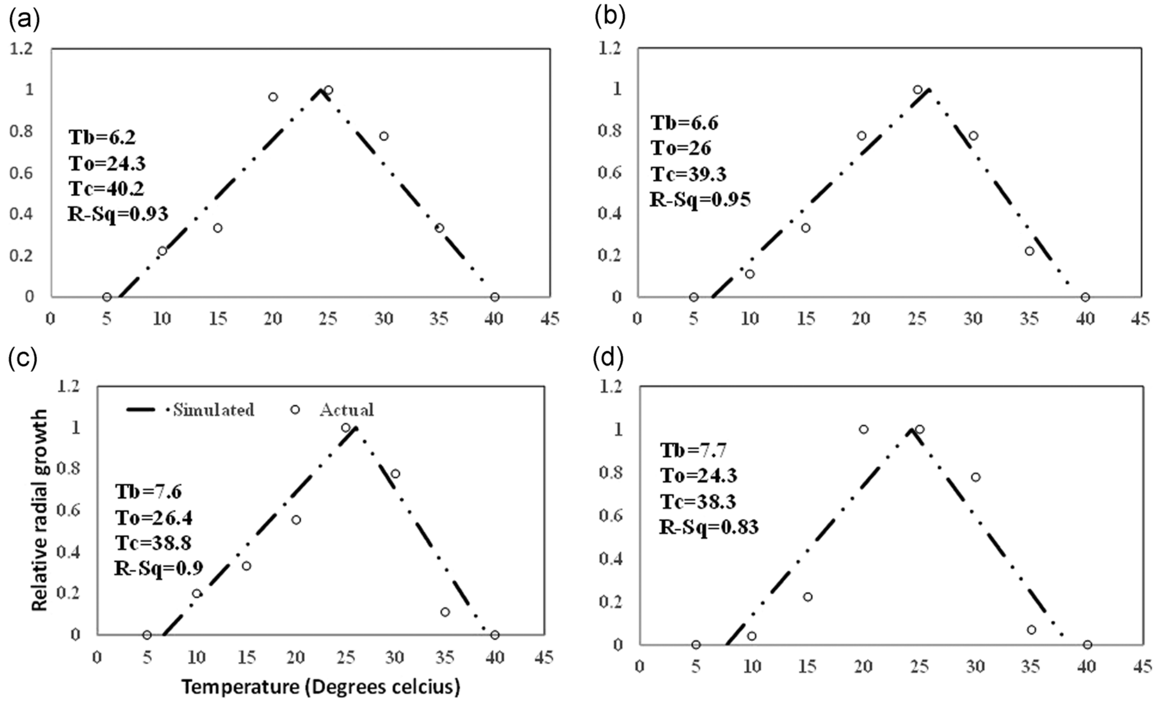

FIGURE 5 The segmented models fitted to the amount of relative radial growth of fungal hyphae (cm) against constant temperatures (with 5°C intervals) for *Pleurotus ostreatus* (a), *Phaerochaete chrysosporium* (b), *Aspergillus niger* (c), and *Trichoderma viride* (d). Points are observed and lines are predicted values. Estimated cardinal (base = T_b, optimum = T_o, and ceiling = T_c) temperatures are included in chart areas

ceiling temperatures were in the range from 38.4°C to 40.2°C.

3.5 | Spatial characterization of the suitability of Golestan cropping areas for using the studied fungi

The outputs of the OBLI method estimated that cotton, soybean, and rice crops covered, respectively, 15.2×10^3, 28.1×10^3, and 57.0×10^3 ha in Golestan province in the 2017–2018 growing season (Figure 6). The highest area under soybean cultivation belonged to the three counties of Gorgan, Aliabad, and Kordkooy (central zone of the province). These lands are mostly located around the main Gorgan-Mashhad road. The largest area under cotton cultivation belonged to Gonbad and Aqqala. Rice was also distributed in other parts of the Golestan province, except for the eastern parts. The κ and general accuracy coefficients were 0.8 and 86.3, respectively, supporting the reliability of these results. Temperature regimes and soil pH were characterized specifically for these crop layers.

Our analysis revealed that soil pH was in the range from 7 to 8 for about 96% of the 2618 points sampled within the study area (Figure 7). Thus, minimal differences were found when comparing both the three crop layers and the points within each crop layer.

The spatial variability in minimum and maximum monthly temperatures within the study area is depicted by the box plots of Figure 8 for each crop layer. Differences among crop layers were generally small. Minimum monthly temperatures were usually below 5°C from December to February and above 20°C in July and August showing limited spatial variability (average coefficient of variation of 3%). Spatial variability was higher for the maximum monthly temperatures (average coefficient of variation of 17.9%), particularly around the summer. Values ranged from 1.02°C to 1.21°C in January and from 40.7°C to 42.2°C in July. Cardinal temperatures are also depicted in Figure 8. Minimum monthly temperatures during the winter were consistently below the estimated base temperatures, irrespective of the fungal species and crop layer. Maximum monthly temperatures never exceeded the ceiling temperature, except for some points in the cotton cropping area during the summer.

Table 2 shows the crop calendar (start and end of planting and harvesting) for the three crops of cotton, soybean, and rice in the study area [31]. As shown in the table, the cultivation calendar of these three crops starts

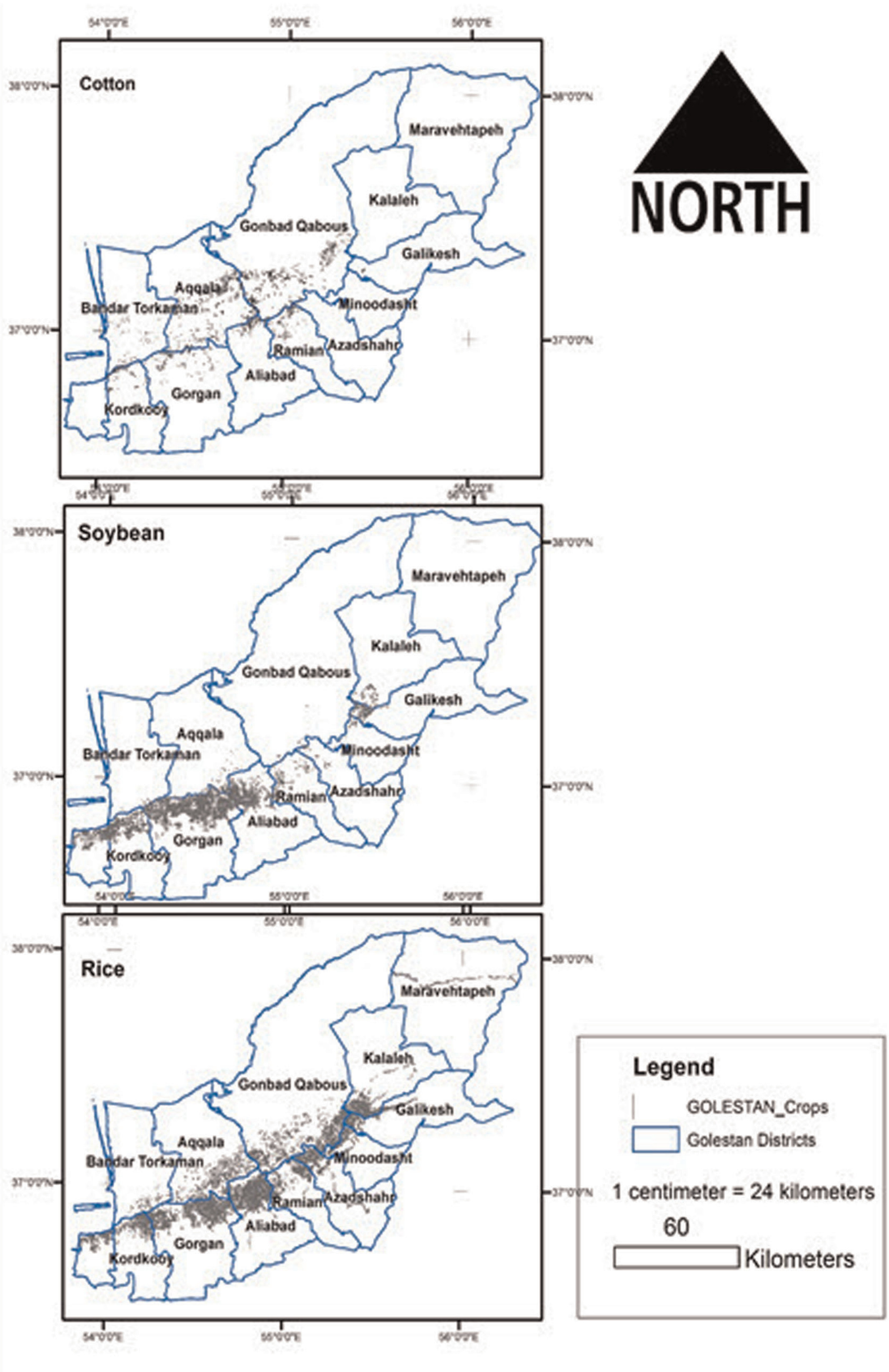

FIGURE 6 Detected land area under cultivation of cotton, soybean, and rice in Golestan province using the OBLI method

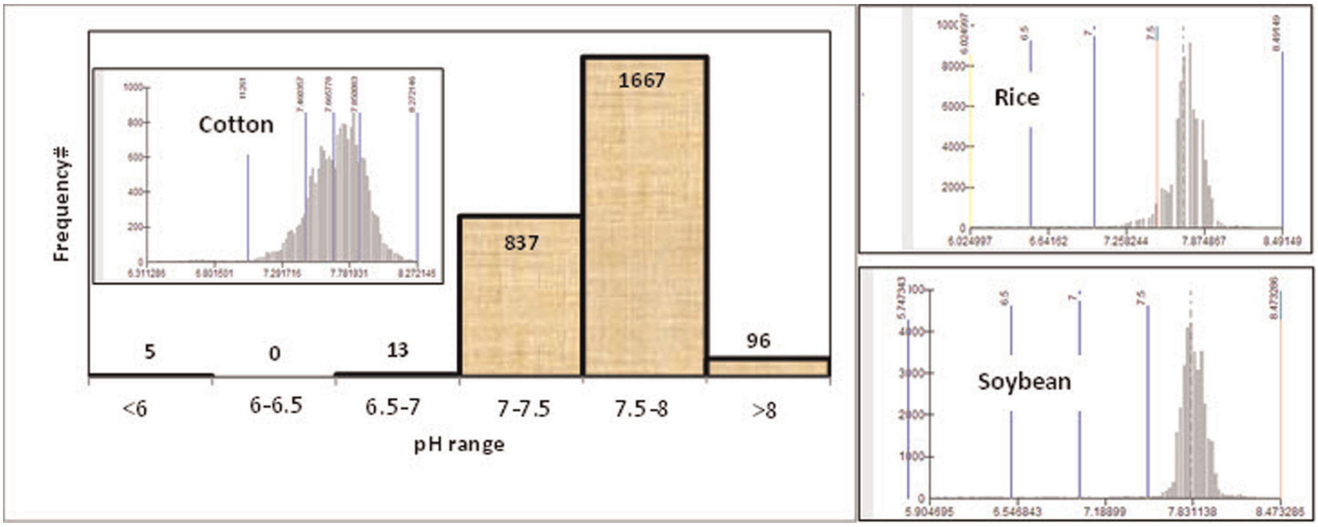

FIGURE 7 The frequency diagram of pH values from 2618 measured points in the agricultural lands of Golestan province. The diagrams shown in the small panels for each crop show the frequency distribution of the value of the pixels of the interpolated pH layer using the inverse distance weighted method (*X*-axis: pH values for pixels, *Y*-axis: frequency of each pH value)

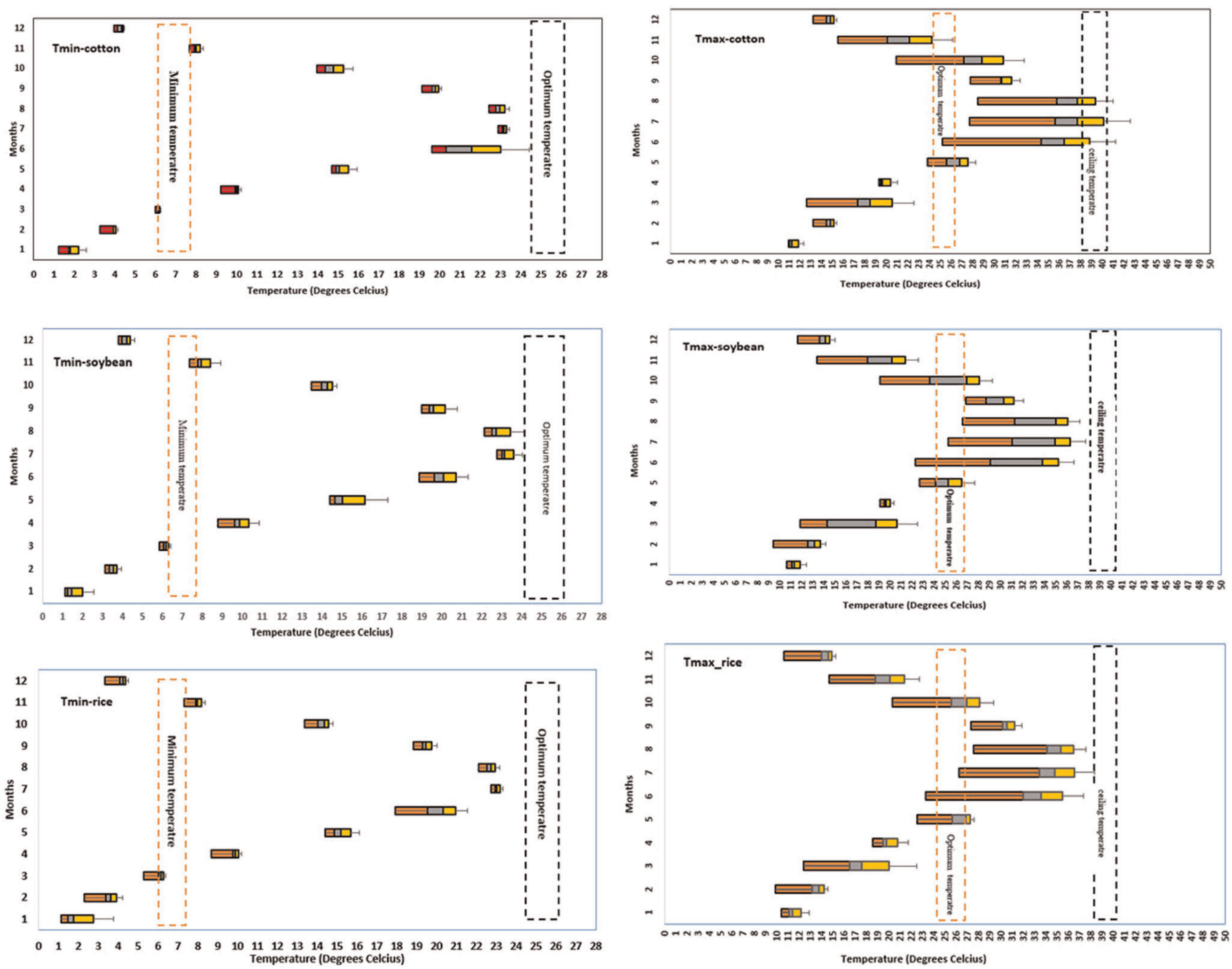

FIGURE 8 The range of minimum and maximum temperature changes during the 12 months of the year in cotton, soybean, and rice farms (box plots) and the range of cardinal temperatures obtained for the four fungi studied

TABLE 2 Sowing and harvesting calendar for three studied crops in Golestan province

Crop		From	To
Rice	Sowing date	4 May	3o June
	Townships	All	All
	Harvesting date	22 August–5 September	6–21 October
	Townships	Aqqala, Bandar Torkaman, Gonbad Qabous, Gomishan	Other townships
cotton	Sowing date	12 April	30 June
	Townships	Gorgan	Aqqala
	Harvesting date	11 August	5 September
	Townships	Gomishan	Kalaleh, Azadshahr, Galikesh
Soybean	Sowing date	20 April	20 July (late)
	Townships	Kordkooy	Gorgan, Minoodasht, Aliabad, Galikesh, Ramian
	Harvesting date	Early October	Mid-November
	Townships	Minoodasht, Kordkooy, Bandar Gaz	Aliabad, Gonbad Qabous

Note: Dates refer to early and late sowing dates in 12 townships of the province.
Source: Adopted from Kamkar et al. [31].

from April 12 for cotton in the Gorgan township and ends in mid-November with the harvesting of soybean in Minoodasht, Kordkooy, and Bandar Gaz townships. On the other hand, the results show that the minimum temperatures in December, January, and February are outside the cardinal temperature of these fungi. Therefore, the establishment of these fungi is not possible immediately after the harvest. Consequently, these fungi can be added to the soil in the next year so that both the residues of the autumn crops (wheat, canola, barley, and fababean) and previous summer crops (studied crops) are decomposed.

4 | DISCUSSION

Previous studies have shown that many fungi can grow under different environmental conditions on different lignocellulose materials [32,33], but pH, temperature and the substrate strongly modulate several processes like germination or mycelial growth [34–36]. These fungal species play a key role as decomposers of plant residues that contribute to carbon and nutrient cycling in ecosystems [37]. If some of these fungi can improve the turnover of crop residues, then, their introduction in agricultural systems can be envisaged as a possible avenue for reducing their dependence on chemical fertilizers to maintain high yields.

In this study, *P. ostreatus* and *P. chrysosporium* performed the best in the MA medium (no significant difference with the two culture media PDA and YEA). *P. ostreatus* can grow better in a culture media with 9% glucose and 5% peptone [38]. Therefore, it seems that the fungus grows better in substrates with higher sugar and starch content. By contrast, the MA medium did not lead to such satisfactory performances for *A. niger* and *T. viride* (Figure 2). High growth rates were found in PDA for the four fungal species, so such conditions were considered for the growth of studied fungal species to pH and temperature experiments (Experiments III and IV).

Penicillium, Aspergillus, and Trichoderma settle rapidly on wheat and corn residues [39]. Also, they reported that bacterial and actinomycete populations were significantly more on soybean residue compared with corn and wheat residues. According to the same study, the fungal species that can establish and operate widely have a higher competitive advantage over the years. For example, *P. ostreatus* has high decomposition power and it can act on wood and dead residues [40]. In this study, the best function of *P. ostreatus* was observed on cotton (without significant differences with *T. viride*). *P. chrysosporium* grew well on soybean (without significant differences with cotton), *A. niger* did the best growth on soybean, and its performance differed significantly from that on cotton and rice residues. *T. viride* had a similar function to *P. chrysosporium* (Figure 2b). Besides, we found significant reductions in C:N ratios regardless of the species in relation to noninoculated residues of the three crops, which suggests that the use of these fungi in agroecosystems could improve nutrient cycling. The lowest C:N values for cotton and rice residues were found for *P. ostreatus* while *A. niger* was the most efficient for those of soybean. *P. ostreatus* needs more carbon and less nitrogen, so it can easily grow on materials such as wheat straw, cotton residues, and sawdust that are enriched with a few ingredients such as wheat grain and rice husk [41].

High variability among fungal species is usually found when studying their responses to pH. According to the literature, Pleurotus grows optimally in the pH range from 5.5 to 7.0 [42], but various strains tolerate a wide range of pHs [38,43,44]. In our study, *P. ostreatus* grew in all the range of pH tested in Experiment III (i.e., 4–8), but it showed a clear preference for acidic conditions so that the higher the pH level tested, the lower the growth rates recorded. In the other three species, the maximum growth rates were also found for the lowest pH level (i.e., 4), but the responses were not so sensitive to pH increases as in *P. ostreatus* (Figure 4). Pitt and Hocking [43] and Perrone et al. [44] showed that *A. niger* could tolerate high pHs. For the genus Trichoderma, the responses to pH vary a lot among species. For example, *Trichoderma harzianum* and *Trichoderma virens* have high vegetative growth under acidic conditions (pH 5) while *Trichoderma hamatum* and Trichoderma sp. T (one isolate of Trichoderma isolated from Moghan area soil) display high vegetative growth under alkaline conditions (pH = 8) [11]. In any case, we would like to highlight that the discussion on the fungal responses to pH should consider both aspects related to “optimal functionality” and "establishment feasibility." Our results indicate that the pH of plant residues was close to neutral conditions (pHs from 6.7 to 7.5), which suggests that they should be able to settle and operate on them, albeit their growth would not be the highest. The same applies when considering that, in the Golestan case study, 96% of the sampled points revealed pH values in the 7–8 interval (Figure 7).

Although different mathematical models have been used to describe fungal growth responses to temperature (e.g., [45]), both the patterns and cardinal temperatures observed in Experiment IV were consistent with findings reported in similar works.

For instance, has reported that the optimal temperature for vegetative growth was 25°C for *T. hamatum* and *T. harzianum* and 30°C for *T. virens* and Trichoderma sp.[11]. For *P. ostreatus*, the maximum, optimum, and minimum temperatures for growth were in the ranges of 25–30°C, 16–22°C, and 12–15°C, respectively [46]. In the present study, optimum temperatures were around 24°C for *P. ostreatus* and *T. viride* and around 26°C for *A. niger* and *P. chrysosporium*.

The cardinal temperatures estimated in Experiment IV were used to assess the theoretical suitability of cotton, soybean, and rice cropping areas in Golestan for the growth of the four studied fungi (Figure 8). According to our analysis, the fungi could cope well with the maximum summer temperatures in the area, which rarely exceeded the calculated ceiling temperatures. By contrast, the minimum temperatures over the winter (from December to February) were regularly below the calculated base temperature which could be problematic for the establishment of the fungi. Consequently, if soil moisture and acidity are favorable, these fungi can be added to the soil in early spring so that both the residues of the autumn crops (especially wheat, canola, barley, and fababean) and previous summer crops (studied crops) are decomposed.

In the study area, these three crops are cultivated as summer crops in rotation (mainly after wheat, barley, or canola), and are fully irrigated. Therefore, soil moisture is not restrictive for these fungi and is usually maintained by adjusting the irrigation cycle to the level of field capacity. According to the studied crops calendar, the results showed that due to the improper minimum temperature at least in January, February, and March, the establishment or activity of these fungi immediately after the harvest is impossible. Consequently, these fungi can be added to the soil (including previous crop residue) in the next year or start their function after a stagnation so that both the residues of the autumn crops (wheat, canola, barley, and fababean) and previous summer crops (studied crops) are decomposed. The results also showed that in the lands under cultivation of these three crops, these fungi will not show the best performance, but will not be ineffective. The results confirmed that the pH is not quite restrictive for the establishment and growth of fungi, but it can reduce the amount of fungal radial growth on the residues. The findings also revealed that the pH of the residues changes around neutral pH, which would reduce the radial growth of fungal hyphae with a decreasing slope.

Fungal pathogenicity testing showed that none of the four fungi had a negative and pathogenic effect on rice, cotton, and soybean seeds and seedlings. This shows that the development of these fungi in the soils of Golestan province or increasing their population will not cause any particular problem for crops [47].

Because there is a large amount of residues of these crops in the lands of Golestan province, use or increase their population in the soil can increase the organic matter of the soil by decomposing these residues. In a study to investigate the possibility of biofuel production from crop residues in four watershed basins of Golestan province (covering 37% of the total arable lands in Golestan province), the amount of residues of the main crops of the area were estimated using ground control points and satellite-imagery indices. The total amount of dry residues of soybean, rice, wheat, barley, and canola fields were reported to exceed 1 Mt in total [27]. This amount of residues can be considered as an opportunity to improve soil structure and biochemistry in Golestan province using natural decomposers such as fungi.

It is noteworthy that these fungi are native to most of the soils of Golestan province and do not die in low-temperature conditions, however, its population may not be sufficient in some soils. These fungi stagnate in this condition and as soon as the conditions are right for them to continue their activity, they start to grow again and show their function.

It is necessary to change the attitude toward crop residue management and using natural agents (such as fungi) to manage crop residues as an internal-input source for agroecosystems looks a promising opportunity moving toward more sustainable agriculture and more cleaner production. These crop residues should be managed in such a way that they are returned to the soil instead of being burned (as a large-scale operation in Golestan province) or removed from the field. In this regard, the ability of soil decomposing agents to establish and form colonies in the soil (containing residues), as well as soil conditions (at least in terms of moisture, pH, and temperature), should be considered. In this study, the pathogenicity and temperature and pH responses of four fungi (*P. ostreatus*, *P. chrysosporium*, *A. niger*, and *T. viride*) were investigated, as well as their ability to grow on and decompose crop residues from cotton, rice, and soybean. The four fungi were classified as nonpathogenic, had similar cardinal temperatures, and performed better under acid conditions. Fungal growth differed among the studied crop residues but their presence always led to significant reductions in the C:N ratios, an indicator of their potential as decomposing agents. Examination of long-term climatic data of the study area showed that temperatures are adequate for fungal activity in nine months of the year (from March to November). Overall, our results suggest that the exogenous application of the studied fungi to agricultural soils holds promise for improving turnover and nutrient cycling from crop residues. Further research should explore the potential of the fungi to establish and decompose crop residues under field conditions.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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