Abo-Elyousr *et al.*
Egyptian Journal of Biological Pest Control (2024) 34:11
https://doi.org/10.1186/s41938-024-00776-6

Egyptian Journal of Biological Pest Control

RESEARCH

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Sustainable biocontrol of purple blotch disease in *Allium cepa* L. by biocontrol yeasts, *Pichia kluyveri* and *Filobasidium wieringae*

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Abstract

Background Purple blotch disease is a devastating disease, resulting in significant yield losses in onion. Application of synthetic fungicides is a rapid approach for the control of this disease, but extensive use of synthetic fungicides results in resistance development in pathogen. Thus, it is mandatory to explore alternative approaches to overcome the fungicide resistance challenges. The present study focused on a sustainable biocontrolling approach by using biocontrol yeast. The objective was to develop a management strategy that offers an environmentally acceptable alternative to commonly used fungicides.

Results A total of six pathogenic isolates were obtained from the infected onion leaves, out of all tested isolates, OP-4 recorded as highly virulent with disease incidence (75.2%). *Filobasidium wieringae* and *Pichia kluyveri* drastically reduced the in vitro mycelial growth of *Alternaria porri* (14 and 21 mm, respectively). In the greenhouse experiment, plant inoculated with *P. kluyveri* suspension was the most effective, resulting in considerable reduction (77.1%) in disease severity, before two days of pathogen inoculation. However, *F. wieringae* showed a considerable reduction (84.5%) in disease severity when applied in combination with *P. kluyveri* two days post-inoculation.

Conclusions These findings highlighted the strong biocontrol potential of *P. kluyveri* and *F. wieringae* in managing the purple blotch disease of onion and can reduce the reliance on synthetic fungicides. Further research and field trials should be conducted to optimize the application methods and evaluate the long-term effectiveness of these bioagents.

Keywords Antioxidants, *Alternaria porri*, Indigenous Yeast, *Filobasidium wieringae*, *Pichia kluyveri*

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Background

The onion (*Allium cepa* L.) plant belongs to the *Liliaceae* family, and is one of the most noteworthy and historically significant vegetable crops (Abbas et al. 2019). In onion and garlic crops, reports indicated the presence of more than sixty fungal infections, fourteen bacterial infections, as well as infections caused by nematodes, viruses, and phytoplasma-like microorganisms (Abdel-Hafez et al. 2014). These infections possessed major threat to the annual crop production and storage of onion. Among them, fungal infections are particularly widespread across onion and

garlic-producing regions worldwide, which can lead to substantial crop losses (Ali et al. 2016). Among the various diseases affecting *Allium* spp., including onions, garlic, shallots, leeks, scallions, and chives, the most threatening to onion crops is the destructive fungal pathogen *Alternaria porri*, causing purple blotch disease (PBD) (Abdel-Rahim et al. 2017). The disease primarily affects the leaves and bulbs of the plants, leading to significant yield losses up to 97% (Andersen et al. 2008). PBD thrives under specific environmental conditions, such as high humidity (80–90%) and moderate temperatures (25–30 °C), which contribute to the severity of this disease. The impact of PBD on *Allium cepa* highlights the importance of implementing the effective disease management strategies, including the use of synthetic fungicides, resistant cultivars, proper sanitation practices and controlled environmental conditions under greenhouse production to mitigate the damage caused by this fungal pathogen.

Considering the environmental and health risks associated with chemical fungicides, it is imperative to seek sustainable and eco-friendly alternatives for managing PBD. The development of integrated disease management approaches, incorporating biological control agents, cultural practices, and resistant cultivars, among other strategies, holds promise in reducing reliance on chemical fungicides and promoting sustainable agriculture. Efforts to address the challenges posed by *A. porri* and PBD require a comprehensive approach, encompassing research, education, and practical implementation of effective management practices. By adopting environmentally friendly strategies, it is possible to minimize the negative impacts of this disease while ensuring the sustainability and productivity of *Allium* spp. crop cultivation (Imran et al. 2023).

Biocontrol applications have shown promising results in disease management. However, it is worth noting that the specific use of yeasts as a treatment for PBD in onions has not been documented previously. Their potential effectiveness against PBD in onions caused by *Alternaria porri* has not been extensively explored or reported. Given the successful use of yeasts in controlling various plant diseases, it would be interesting to investigate their potential application for managing PBD in onions. Further research and experimentation would be necessary to evaluate the effectiveness and feasibility of utilizing yeasts as a biocontrol agent specifically for this disease. Exploring alternative and sustainable methods, such as utilizing yeasts, can contribute to the development of integrated disease management strategies for onions and other crops, potentially reducing reliance on chemical fungicides and promoting environmentally friendly practices (Imran et al. 2022).

The objective of the study was to develop a management strategy that offers an environmentally acceptable alternative to commonly used fungicides. The use of individual yeast strains and combinations aimed to provide effective control of PBD, while minimizing potential environmental risks associated with chemical fungicides. By utilizing these yeast strains, the intention was to offer a sustainable and eco-friendly controlling approach for this particular disease in onions.

Methods

Isolation of the pathogen

In order to isolate the pathogen of PBD of onion, some regions of the Assiut Governorate in Egypt were frequently visited during the year 2021–2022 for the prevalence of PBD on onion plants. The symptoms observed included the small water soaked lesions (2–3 mm diameter) with irregular purple spots. The casual pathogen of PBD was isolated from infected leaves and identified as reported by Simmons (2007). Briefly, the samples of infected leaves showing blotch symptoms were collected and preserved in sterile polythene bags, incubated in an ice box and transferred to the Plant pathology laboratory of Assiut University, Egypt. The leaves were segmented after removing other impurities and rinsed thrice with sterile distilled water. Subsequently, the segmented leaves were disinfected under aseptic conditions with 3% sodium hypochlorite (NaOCl) solution for 3–5 min, followed by disinfection with 70% ethanol solution for 50 s and then rinsed thrice with sterile distilled water, followed by moisture drying on sterilized Whatman filter paper (No 1) (Macherey–nagel GmbH & Co.KG Germany). Thereafter, potato dextrose agar (PDA) (HiMedia Lab. Pvt.Ltd, India) Petri plates (containing streptomycin as 0.05 g/l) were prepared and moisture dried segmented leaves were placed on PDA plates using a direct transfer plating method (Abdel-Rahim et al. 2017). Petri plates were then incubated at 27 ± 2 °C 3–5 days. The germinated mycelial colonies on each plate were observed and purified by transferring the mycelial disk (3-mm) to new PDA plates incubated at 27 ± 2 °C for 5–7 days. Purified cultures were maintained at 4 °C in sterile Potato broth (PD) glass containing glycerol (70%). Preserved cultures were further used for experimentations.

Pathogenicity test

During 2021–2022, pathogenicity tests were performed in the greenhouse of Plant Pathology Department, Assiut University, Egypt. In order to conduct the experiment, seedlings of an onion cultivar "Giza-6"were grown in a small seedling growing tray (50 holes) filled with peat moss (1:3). Seedlings were irrigated and six-week-old seedlings were transplanted to new plastic pots (25 cm)

filled with autoclaved clay soil (3 kg/ pot), followed by the addition (20 ml/ pot) of NPK fertilizer formulation (12:4:6). To prepare the fungal inoculum, all fungal isolates obtained and purified from diseased onion leaves were subcultured on PDA Petri plates for 5–7 days incubated at 25 ± 2 °C. Then, mycelial cultures were carefully collected by adding sterile distilled water (10 ml/ plate) and scraped by bacterial needle thoroughly mixed by a vortex and stored at room temperature under aseptic conditions. The obtained suspension was diluted to 5×10^4 CFU/ml. Subsequently, 12-week-old onion plants were sprayed (30 ml/pot) with the pathogenic fungal inoculum. The onion plants treated with sterile distilled water were subjected as a control. The pots were covered with sterile polythene bags for 48 h to initiate infection. Pots were irrigated as per requirement (every 2 days); temperature and humidity inside the greenhouse were maintained at 20 ± 2 °C during the day and 18 ± 2 °C during the night. Posts were monitored and the PBD symptoms were observed, 20 days post-inoculation (dpi). Subsequently, percentage of disease index (PDI) was calculated by using a reported 0–4 scale disease rating scale: 0 = no disease symptom; 1 = 1–25% several dark purplish brown patches covering leaf area; 2 = 26–50% covering leaf area; 3 = 51–75% streaks covering leaf area; 4 = 76–100% streaks covering leaf area. The disease severity index (DSI) was calculated using the following formula (Abdel-Hafez et al. 2014):

$$\text{DSI \%} = \frac{\text{Total sum of numerical ratings}}{\text{No. of observations} \times \text{Maximum disease rating in the scale}} \times 100$$

In order to validate the Koch's postulates, the fungal pathogen was re-isolated, characterized, and compared to the originally isolated colony cultures. For each fungal isolate, twelve replicates were used and each replicate was carried in nine pots (two seedlings /pot). A highly virulent isolate, exhibiting robust pathogenic symptoms, was selected, identified, and utilized for further experiments.

Morphological and molecular identification of purple blotch pathogen

Morphological and cultural characterization, viz., colony color, texture, and morphology of all purified fungal colony cultures, was observed on PDA Petri plates containing mycelial disk (5 mm) incubated at 27 °C for 7 days in darkness (Imran et al. 2022). However, the conidial size, shape, and beak length were observed on V8 Juice agar and potato carrot agar medium (HiMedia Lab. Pvt. Ltd, India) inoculated with identical mycelial disk incubated at 22 °C with a light period of 8 h light/16 h darkness. Subsequently, the conidia and conidiophores were observed after 1 week fixed with lactophenol picric acid solution and observed under a Binocular Fluorescence microscope (MDS300; KERN Ltd, Chongqing, China). Then, the genomic DNA of the selected highly virulent isolate was extracted and used for the identification by Polymerase Chain Reaction (PCR) amplification of Internal Transcribed Spacer (ITS) region (Berbee et al. 1999), glyceraldehydes-3-phosphate dehydrogenase (GAPDH) (Carbone and Kohn 1999) gene, and translation elongation factor 1-alpha (TEF1) gene (Woudenberg et al. 2013) regions using the specific reported primer pairs. Standard PCR reaction was performed in a thermal cycler PCR (iCycler™) (Thermo Scientific® cyclone 25 (PeqLab, Erlangen, Germany) (Perez et al. 2016) with 50 µl as final volume of PCR reaction mixture containing 1 µl of each primer, 5 µl of 10 × standard Taq reaction buffer, 4 µl 2.5 nM dNTPs (TSINGKE, Beijing, China), 0.5 µl Taq Polymerase, 2 µl template DNA, and 37.51 µl nuclease free water (TSINGKE, Beijing, China). Amplified PCR products were sequenced and the sequences were by Macrogen, Inc., Seoul, South Korea, aligned using BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST) in the public domain of the National Center for Biotechnology Information (NCBI) and identified sequences were submitted in NCBI GenBank under specific accession number.

Isolation of yeast and assessment of antifungal activity against purple blotch pathogen

In order to isolate the native yeasts from onion endophytes, healthy onion leaves were randomly collected in sterile polythene bags labeled and preserved at room temperature under aseptic conditions. Thereafter, sterile cotton swabs were carefully streaked on the onion leaf surface and placed into sterile glass slants containing broth yeast extract peptone dextrose (YEPD, HiMedia Lab. Pvt. Ltd, India) medium (10 ml) supplemented with ampicillin (100 mg/l) and chloramphenicol (50 mg l^{-1}) to inhibit bacterial growth (Peter 2006). Slants were incubated at 28 °C for 24 h on a rotary shaker (140 rpm). Then, the incubated suspension was streaked on YEPD agar medium Petri plates that were incubated at 28 °C for 48 h. Colony characteristic, surface, color, elevation, and margin of growing yeast colonies were observed to study their macroscopic features and morphology of yeast was

observed and compared to those reported by Fernaândez et al. (2006).

In order to evaluate the antifungal potential of yeast, the purified isolates were subcultured on yeast extract agar (YEA) plates incubated at 28 °C for 24 h, whereas the highly virulent pathogenic fungal isolate (OP-4) was subcultured on PDA plates incubated at 27 ± 2 °C for 48 h. Thereafter, a mycelial disk (5-mm) from the edge of actively growing fungal colony was placed face-down in the middle of PDA plates, and subsequently, overnight (24 h old) yeast culture was streaked parallel (both sides of fungal colony) along the sides of growing fungal culture at an equal distance from margin. The PDA plates containing only pathogenic mycelial disk, lacking yeast streaks were the control and incubated at 27 ± 2 °C for 7–10 days until fungal growth covered the entire plate. For each yeast isolate, nine replicates were used and each replicate was carried in four plates. Based on mycelial growth inhibition, the putative yeast isolates exhibiting strong antifungal potential were selected, identified, and used for further experiments.

Identification of antagonistic yeast

Genomic DNA of the selected yeasts strains (exhibiting strong antagonistic potential against blotch pathogen) was extracted by a reported method of Sugita et al. (2003), with slight modifications. In order to perform molecular identification, PCR reaction was performed using D1/D2 region from 26S rDNA. PCR amplificatio was performed using a reported primer pair, viz., NL-1 (5′-GCA TATCAATAAGCGGAGGAAAAG-3′) and NL-4 (5′-GGTCCG TGTTTCAAGACGG-3′) (Hemeda and Klein 1990). The amplified PCR products were analyzed by electrophoresis on a 1% (w/v) agarose gel, stained with Syber Green according to the manufacturer's instructions. Products were sequenced and the sequences were aligned with the available sequence in the Public domain of NCBI using Basic Local Alignment Search Tool (BLAST) analysis for identification. The sequences were submitted to National Center for Biotechnology Information (NCBI) GenBank under specific accession numbers. A phylogenetic tree was constructed with the sequenced products using the neighbor joining algorithm in the MEGA 6X package.

Impact of bioagents on *A. porri* under greenhouse conditions

The experiments were performed in the growing season of 2021–2022 at the Plant Pathology Department, Assiut University, Egypt, Giza-6 onion cultivar. Briefly, seedlings of "Giza-6" onion variety were prepared as previously mentioned (Pathogenicity test section). Six-week-old seedlings were transplanted to plastic pots (25 mm diameter) containing autoclaved clay soil (3 kg/pot). The treatments used under greenhouse conditions were as follows:

T1, Filobasidium wieringae (Y2), T2, *Pichia kluyveri* (Y4), T3, Combination of T1 + T2, T4, Infected control (pathogen) and T5, Healthy control (Sterile distilled water).

In order to prepare the pathogen inoculum, highly virulent isolate (OP-4) was cultured on PDA plates incubated at 27 °C for 5–7 days. Subsequently, pathogenic suspension was prepared by adding sterile distilled water (10 ml) to each Petri plate. The selected yeast strains were cultured on YEA plates incubated at 28 °C. Then, the suspension from overnight yeast cultures was prepared using sterile distilled water (10 ml) and the concentration of suspension was adjusted (1×10^6 spore /ml) using a hemocytometer. After 1 week of transplanting, the onion seedlings were sprayed (30 ml pot^{-1}) with the suspension of pathogen (*A. porri*) and pots were covered with sterile polythene bags for 48 h. Then, the polythene bags were removed and the suspension of yeast of each treatment was sprayed. Plants inoculated with an equal volume of sterile distilled water were subjected as the healthy control group, whereas the plants treated with the suspension of the pathogen only were subjected as infected control treatment. The treatments were inoculated with two groups, i.e., one group was inoculated with pathogen and then 24 h later, was treated with the previous treatments, whereas the other group was treated with previous treatments and 24 h later was inoculated with pathogen. For each of the treatment, nine replicates were used and six pots (2 seedlings pot^{-1}) were subjected as a replicate. Experiment was performed in a complete randomized design. The temperature and humidity were maintained in the greenhouse where plants were irrigated as per requirement as mentioned previously. Purple blotch symptoms on onion plants were observed and disease severity was measured, fifteen days after the inoculation of pathogen.

Statistical analysis

The experimental data underwent analysis using a two-way ANOVA design through Statistix software (version 8.1, Analytical Software, Tallahassee, FL). Subsequently, the least significant difference (LSD) test was conducted to compare the means of disease severity and disease incidence. All presented data are expressed as mean ± standard deviation (SD).

Results

Isolation and characterization of purple blotch pathogen

A total of six isolates were obtained from the infected onion leaves. Morphological characterization under

microscope showed that all isolates belong to *Alternaria*. Further, the pathogen, cultured on Potato dextrose agar (PDA) medium plates incubated at 25 °C, exhibited aspecific colony morphology with dark brown to black appearance in color with diameter ranging from 5.6 to 8.3 cm. The hyphae were thin, hyaline (translucent), septate (having cross walls), and showed a radiating or radial pattern. The conidia, or asexual spores, were observed as single and elliptical in shape. The length of conidia ranged between 15 and 65 µm with the average width 10–20 µm. The vertical septations in conidia were about 1–4, while horizontal septations were 2–7 with beak length 8–16 µm appeared straight or curved and possessed 1–3 longitudinal septa and 7–9 transverse septa. The elliptical body of the conidia tapered to form a beak-like structure. Mature colony culture of the pathogen was initially white which later on, turned to dark brown to black appearance in color. This change in colony color showed a distinguishing feature of the growth of pathogen. These observations provided valuable insights into the morphological characteristics of the pathogen mainly responsible for PBD in onion.

Validation of Koch's postulates and pathogenicity test

To validate the Koch's postulates, re-isolation of purple blotch pathogen from onion leaves (artificially inoculated) showed high similarity with the originally isolates as colony morphology and their microscopic characteristics were identical. The artificially infected leaves showed prominent typical purple blotch disease symptoms on onion plants as distinct visual characteristics were observed. The infected leaves demonstrated deep, oval-shaped lesions and surrounding tissues of the lesions appeared light brown, encircling the core of the lesions, which varied in color from brown to purple. The level of virulence of these isolates was assessed on Giza 6 onion cultivar. The results clarified that the isolate OP-4 showed that the highest disease incidence as disease severity was significantly higher (75.2%) than other isolates and was the most virulent among all the isolates (Fig. 1). However, all isolates were pathogenic and showed significant purple blotch symptoms on onion plants. These results demonstrated a range of disease incidence from 15.5 to 75.2% (Fig. 1). Molecular identification of highly virulent isolate "OP-4" by PCR amplification was performed and the length of obtained sequence was about 640 bp and the BLAST analysis performed in the public domain of NCBI showed the highest similarity (100%) to *Alternaria Porri* (accession no. LC440611.1), and therefore, the highly virulent isolate OP-4 was identified as *Alternaria porri* and the sequence was submitted to NCBI GenBank under the accession number KR811362.1. The phylogenetic tree of closely related taxa of this specie was constructed (Fig. 2).

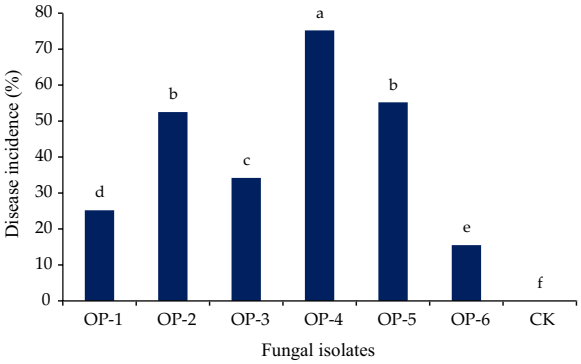

Fig. 1 Pathogenicity test of six *Alternaria porri* isolates on Giza-6 onion cultivar. Values followed by different letters indicate that means are significantly different from each other according to Fisher's least significant difference (LSD) test at $p \leq 0.05$

Isolation and in vitro killer potential of yeast against purple blotch pathogen

Six yeast isolates were obtained from healthy onion leaf samples. All yeast isolates demonstrated antagonistic activity against *A. porri* strain as significant mycelial growth was inhibited by all yeast isolates. However, yeast isolates Y2 and Y4 demonstrated significant reductions (79 and 77%, respectively) in the mycelail growth (14 mm and 21 mm, respectively), followed by the control (90 mm) and other isolates (Fig. 3). However, the myelial growth was relatively lower by other isolates Y1, Y3, Y5, and Y6 (31 mm, 43 mm, 53 mm, and 55 mm, respectively) than control, but significantly higher than Y2 and Y4 strains which showed strong inhibition of *A. porri*.

The two yeast strains, Y2 and Y4, demonstrated promising mycelial growth reduction. Thus, the PCR amplification analysis of these two yeast strain showed high resemblance to reported strains. The BLAST analysis of the obtained sequence of Y2 strain showed high similarity (94.55%) with *Filobasidium wieringae* (accession no. KX078415.1), and therefore, Y2 strain was identified as *Filobasidium wieringae* and the sequence was submitted to NCBI GenBank under the accession number OM990844. However, the BLAST analysis of the obtained sequence of Y4 strain showed 100% similarity with *Pichia kluyveri* (accession no. OL 614155; DQ104732), and therefore, Y4 strain was identified as *Pichia kluyveri* where the sequence was submitted in NCBI GenBank under the accession number MT582191. Phylogenetic tree of both strains was constructed (Fig. 4A, B) to understand the relationship of the identified isolates with other reported species.

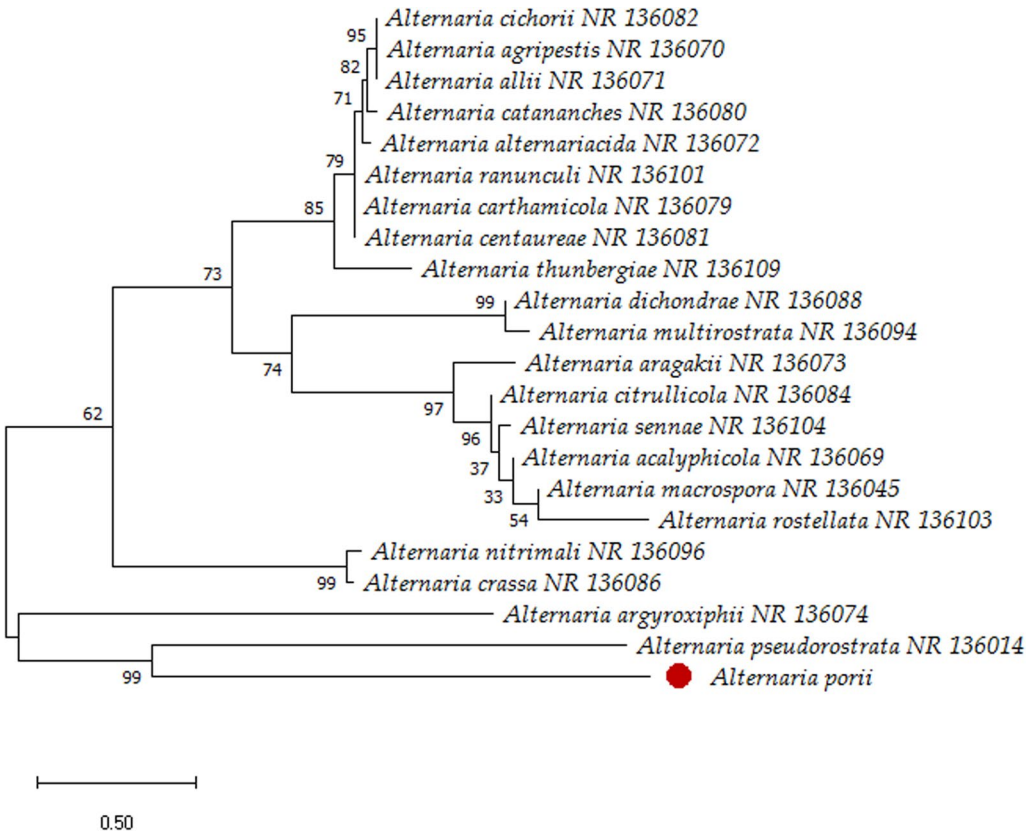

Fig. 2 Semistrict concurrence of most-parsimonious trees based on retrieved *Alternaria porri* sequence data from NCBI public domain. This tree was compatible overall in highly supported lineages to the Bayesian 50% majority-rule consensus tree. Jackknife frequencies (10,000 replicates) are shown above each node. Red color indicates the relationship of identified isolate (*A. porri*) of present study

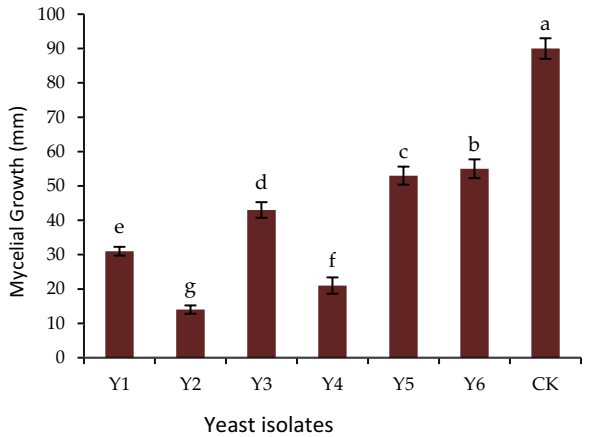

Fig. 3 In vitro evaluation of indigenous yeast isolates as antagonists; against mycelial growth inhibition of *Alternaria Porri*. Values followed by different letters indicate that means are significantly different from each other according to Fisher's least significant difference (LSD) test at $p \leq 0.05$. CK-Control (only pathogen)

In planta assay

In greenhouse experiments, the efficacy of selected yeasts *P. kluyveri* (Y4) and *F. wieringae* (Y2) alone as well as in combination against PBD in onions was assayed and the application of indigenous yeasts demonstrated significant reduction in the disease severity. The disease severity index (DSI) in the plants treated with *F. wieringae* after the application of pathogen was relatively low 25.1%, which was almost constant in other group (Table 1), whereas the disease severity index (DSI) in the plants treated with *P. kluyveri* after the application of pathogen showed reduction, (15.1%) than other group (17.2%). The application of both treatments (Y2+Y4) showed considerable reduction in both groups even before treatments (18.6%) and after treatment (11.7%) with pathogen, followed by control in both groups (Table 1). These results indicated that the combined application of yeast strains (Y2+Y4) strongly reduced the disease severity than alone which conferred competitive potential of these strains against *A. porri*.

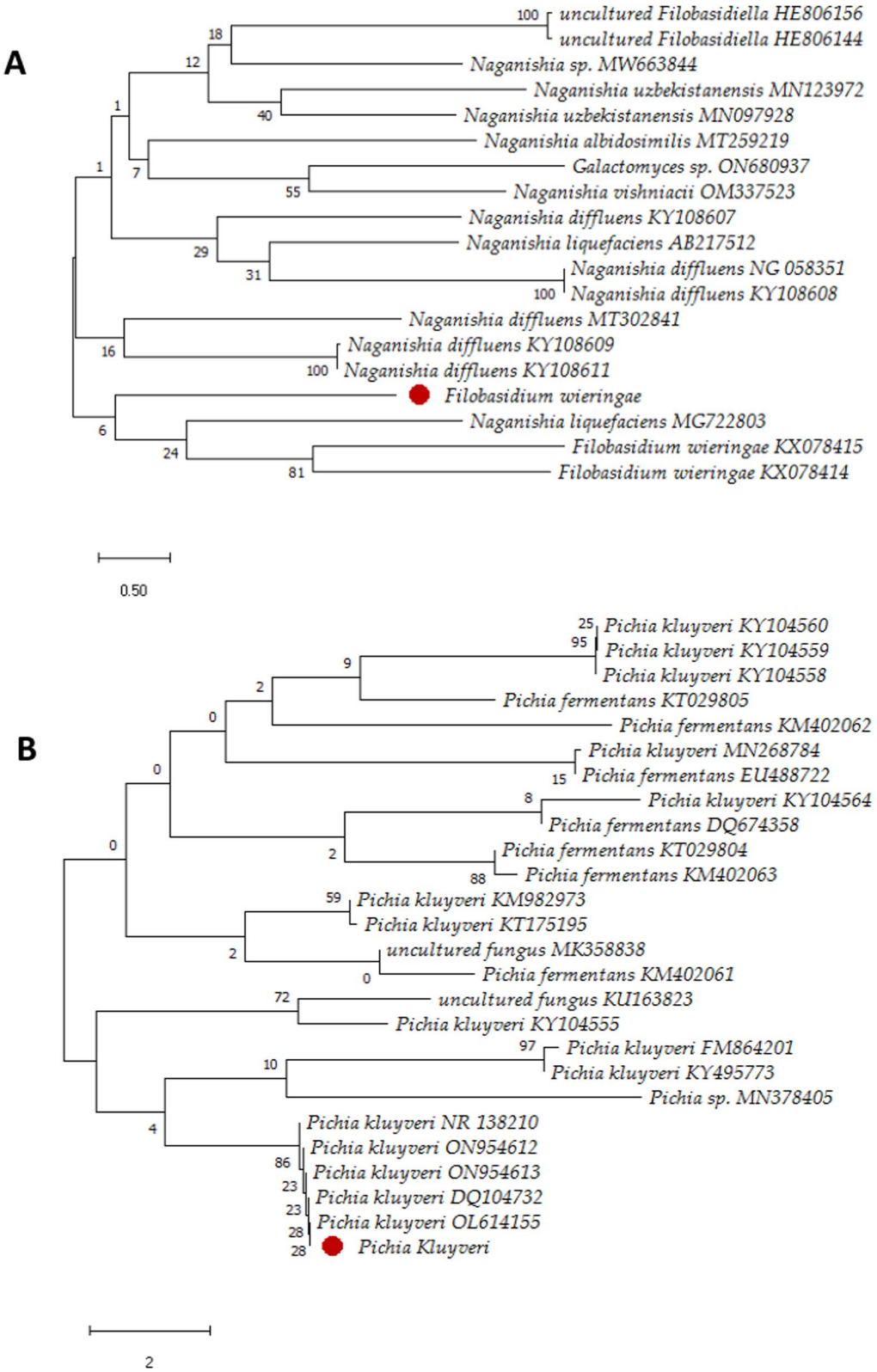

Fig. 4 Semistrict concurrence of most-parsimonious trees based on retrieved yeasts sequences data from NCBI public domain **A** *Filobasidium wieringae* **B** *Pichia kluyveri*. This tree was compatible overall in highly supported lineages to the Bayesian 50% majority-rule consensus tree. Jackknife frequencies (10,000 replicates) are shown above each node. Red color indicates the relationship of identified yeast isolate of present study

Table 1 Effect of various treatments on the disease severity index (DSI) of onion purple blotch disease under greenhouse conditions after inoculation with *Alternaria porri* (PO-4)

Treatments	Disease severity index (DSI%)	
	Before inoculation (G-I)	After inoculation (G-II)
Filobasidium wieringae (Y2)	25.2[b]	25.1[b]
Pichia kluyveri (Y4)	17.2[c]	15.1[c]
Y2 + Y4	18.6[c]	11.7[d]
CK	75.1[a]	75.5

Values in the same column followed by different letters indicate that means are significantly different from each other according to Fisher's least significant difference (LSD) test at $p \leq 0.05$. CK-Control. Group I (G-1) refers to the application of treatments 24 h before inoculation of pathogen on onion plants; Group II (G-II) refers to the application of treatments 24 h after inoculation of pathogen on onion plants

Discussion

In the present investigation, the isolates of *A. porri* showed typical symptoms of PBD on Giza 6 onion plants in a greenhouse experiment. These results revealed that all isolates showed capability to infect the onion plants and caused disease. Therefore, the severity of the disease varied among the isolates but considerable level of virulence was recorded. The findings of this study are aligning with reported studies (Abdel-Rahim et al. 2017) where *A. porri* caused PBD, showing typical symptoms. The pathogenicity of *A. porri* was believed to be influenced by various secondary metabolites produced by the fungus, including altersolanol A, alterporriol, macrosporin, erythroglaucin, and tentoxin (Jijakli et al. 1993). These secondary metabolites may influence the pathogenicity of this fungus. Furthermore, the genes responsible for the pathogenicity of *Alternaria* encode for a range of physiological factors, including toxins and enzymes involved in signal transduction and cell wall degradation. It is worth noting that different *Alternaria* species produce diverse types of toxins, although some species may share similar toxin precursors during their biosynthesis (Kareem et al. 2021).

In the present study, the endophytic yeasts *Pichia kluyveri* and *Filobasidium wieringae* were isolated from asymptomatic healthy onion leaves. They were identified at molecular level obtaining accession numbers OM990844 and MT58219. Glushakova and Kachalkin (2017) reported that the *Pichia kluyveri* and *Filobasidium wieringae* were found in many countries associated with different sources, e.g., *Malus domestica* and *Pyrus communis* fruits, grapes (Cioch-Skoneczny et al 2020).

Understanding the pathogenicity mechanisms and secondary metabolites produced by *A. porri* can provide valuable insights into the disease development and aid in the development of management strategies against PBD. Further research on the genetic and biochemical aspects of this pathogen will contribute to a comprehensive understanding of its virulence and potential targets for disease control. The persistence and impact of *A. porri* and PBD on *Allium* spp. crops have posed significant challenges for farmers and the scientific community, resulting in substantial crop damage and reduced output. Therefore, effective management strategies are crucial to mitigate the devastating yield losses associated with this disease. Various researchers have explored chemical methods to control onion purple blotch (Abdel-Rahim et al. 2017). Biological control is an emerging approach, which is considered as an effective and eco-friendly way for the control of plant diseases. Various bacterial and fungal endophytes along with synthetic inducers, essential oils, and inorganic compounds have been widely reported and used against a variety of plant pathogens (Camacho-Luna et al. 2021). These approaches not only reduced the use of chemical fungicides, but also supported the beneficial soil microbiota (Imran et al. 2023). Indeed, yeasts have been emerged as an effective biocontrol agent for various plant diseases, targeting pathogens such as powdery mildews, gray mold, bacterial fruit blotch, brown rot in apple fruits, and gray and white mold of snap beans (Lahlali et al 2022).

The results present here showed that the efficacy of selected yeasts *P. kluyveri* (Y4) and *F. wieringae* (Y2) alone as well as in combination reduced the disease index of PBD in onions, these results maybe support by results of Tyagi et al. (2008), observation of reducing *A. porri* infection by 79% by collectively using isolates of *Aureobasidium pullulans, Cryptococcus luteolus, Sporobolomyces roseus* and *Penicillium* spp. Further, it was reported that *Trichoderma harzianum* alone significantly reduced purple blotch disease in onion crop to about 60–80% (Prakasham and Sharma 2012). In the biocontrol of plant pathogens through yeasts, various mechanisms come into play. These include the production of toxins; volatile organic compounds, release of lytic enzymes, competition for space and nutrients, mycoparasitism and stimulation of plant immunity (Di Francesco et al. 2020).

Conclusions

The findings of the present study provided compelling evidence supporting the use of two yeast strains (*P. kluyveri* and *F. wieringae*) as a potential bioagent individually and in combination as an economical and environmentally friendly approach for controlling PBD in onions. The results showed that the combinations of the two yeasts were more effective in all experiments. By reducing reliance on chemical fungicides, farmers can

mitigate the negative environmental impacts and potential health risks associated with their use, future studies should focus on the study of both strains on different pathogens, as well their ability to reduce disease under field conditions.

Abbreviations

PBD Purple blotch disease
PDA Potato dextrose agar
SD Standard deviation
LSD Least significant difference

Acknowledgements
This research work was funded by Institutional Fund Project under grant no "IFPIP: 1672-155-1443." The authors gratefully acknowledge technical and financial support provided by the Ministry of Education and King Abdulaziz University, DSR, Jeddah, Saudi Arabia.

Author contributions
KAMA and NMAS were involved in conceptualization, methodology, formal analysis, writing—original draft. AMKA, MI and IRA helped in supervision, review & editing. MA assisted in conceptualization, formal analysis IRA, review & editing, formal analysis, review& editing.

Funding
This research work was funded by Institutional Fund Project under grant no "IFPIP: 1672-155-1443." The authors gratefully acknowledge technical and financial support provided by the Ministry of Education and King Abdulaziz University, DSR, Jeddah, Saudi Arabia.

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable. This manuscript is in accordance with the guide for authors available on the journal's website. Also, this work has not been published previously and is approved by all authors and host authorities.

Consent for publication
Not applicable.

Competing interests
No potential conflict of interest was reported by the authors.

Received: 17 December 2023 Accepted: 3 February 2024
Published online: 07 February 2024

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