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Original Article

Genetic diversity of *Lecanicillium fungicola* causing button mushroom dry bubble disease using ISSR markers and evaluation of its sensitivity against essential oils and selected fungicides

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ABSTRACT

Dry bubble disease, caused by *Lecanicillium fungicola*, is a major challenge in button mushroom cultivation worldwide. This study examined the genetic diversity of *L. fungicola* isolates collected from various regions of Alborz, Isfahan, and Khuzestan provinces. Ten ISSR primers were employed to assess genetic diversity among the isolates. A total of 224 amplified fragments were obtained, with a similarity coefficient of 0.42 to 0.82 among isolates. Based on a similarity coefficient of 67%, the isolates were categorized into six groups. The average Polymorphism Information Content (PIC) was 0.772, with the highest PIC observed at 0.888, indicating a polymorphism rate of 96.8%. Furthermore, the efficacy of six commercial fungicides (including Trifloxystrobin, Copper Oxide, Iprodione + Carbendazim, Penconazole, Neem, and Carbendazim) as well as four plant essential oils (Thyme, Peppermint, Eucalyptus, and Rosemary) on mycelial growth of the isolates was assessed at various concentrations using the disc-diffusion method. The results revealed varying levels of sensitivity to fungicides, with the isolate exhibiting the highest growth rate also showing high sensitivity to the fungicides. Among the tested fungicides, Iprodione + Carbendazim exhibited the highest inhibition. Peppermint Essential oil at a concentration of 0.8 ppm had the highest mycelial growth inhibition. This study provides valuable insights into the genetic diversity of *L. fungicola* isolates, their various level of sensitivity to fungicides, and the potential use of essential oils in disease management. It offers a foundation for future research on resistance evolution and integrated disease control strategies.

KEYWORDS

Agaricus bisporus, Molecular marker, Chemical control, Plant extract.

INTRODUCTION

Agaricus bisporus (Lange) Imbach, commonly known as the button mushroom, holds a significant position in Iran's edible mushroom market, ranking fourth among 70 countries globally and accounting for approximately 11% of the total demand for *A. bisporus* (Mohammadi Goltapeh 2001, Singh et al. 2020). Dry bubble disease, caused by *Lecanicillium fungicola* (Preuss) Zare and Gams, poses a significant threat to *A. bisporus* cultivation worldwide. If left uncontrolled, this disease can decimate entire mushroom crops within two to three weeks, resulting in substantial losses for mushroom growers. In Europe alone, the annual economic impact of this disease is estimated to exceed 300 million dollars (Chrysayi-Tokousbalides et al. 2007, Caitano et al. 2020). The symptoms of dry bubble disease vary, including the appearance of round, undifferentiated lumps, crooked and cracked bases, and spotted caps, and the appearance of symptoms depends on the stage of infection. Additionally, under rare circumstances, amber drops may form on the caps. The

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spores of *L. fungicola* can survive in compost for over a year, and under dry conditions for about 7-8 months (Largeteau and Savoie 2008, Gea et al. 2014). Factors such as high humidity, elevated carbon dioxide levels, and increased temperatures facilitate the rampant spread of this disease (Fletcher and Yarham 1976). In Iran, dry bubble disease, which is caused by *L. fungicola. var fungicola* is responsible for the main cause of *A. bisporus* deformities, accounting for approximately 80% of cases (Memarzadeh et al. 2020).

Chemical interventions, although limited due to *Lecanicillium* resistance to benzimidazole-based fungicides, have seen some advancements. Chlorothalonil fungicide and natural compounds like Trilogy have shown promise in disease management. Another fungicide used is Acord, however, caution must be exercised to prevent the development of fungicide resistance and minimize environmental impact (Mehrparvar et al. 2016, Zaker 2016). Fungicides, depending on their half-life, can have adverse effects such as leaving toxic residues on humans and other organisms, contributing to environmental pollution, and accumulating in the food chain. Various fungicides are used to combat this disease, including Benlate, Bavistin, Rovral, Topsin-M, Daconil, Acord, Mertect, Blitox, Dithane Z-78, Clorox, and Flint. However, these fungicides are prohibited in the European Union due to the resistance of the disease agents to them and their recognized hazards to human health (Chrysayi-Tokousbalides et al. 2007). In contrast, some fungicides like thiophanate methyl and chlorothalonil are still utilized in Poland. Canada permits the use of carbendazim, thiophanate methyl, dithiocarbamate, and chlorothalonil, while in the United States, chlorothalonil and thiabendazole are commonly used (Anonymous 2021a, 2021b). The banning of benomyl in 2002, the decrease in registered fungicides permitted in *A. bisporus* cultivation, the widespread resistance *of L. fungicola* to methyl benzimidazole carbamates, and the diminishing efficacy of fungicides against *L. fungicola* have led to increased interest in using safer alternatives, especially plant essential oils to control this fungus (Chrysayi-Tokousbalides et al. 2007). The discovery and utilization of plant essential oils and pure plant extracts as fungicides offer several benefits, such as the discovery of effective chemical structures, addressing resistance issues, diverse modes of action, reducing health and environmental risks, and volatility. Thyme oil has been demonstrated to be effective in preventing blistering disease when applied before conidial germination. Additionally, when applied post-infection, essential oils containing phenolic compounds (Thymol, Carvacrol, and Simol) can prevent further fungal growth and disease development. However, these essential oils may lack selectivity as they also inhibit the growth of *A. bisporus* mycelium (Geösel et al. 2014). Two *L. fungicola* varieties, *L. fungicola. var fungicola* and *L. fungicola var aleophilum* have been reported to cause the disease, but they have similar morphological characteristics, making their identification only based on morphological features difficult. Phylogenetic studies using ITS-rDNA sequences were used to differentiate these two *Lecanicillium* species (Zare and Gams 2008). Random Amplified Polymorphic DNA (RAPD) and growth at 30°C are also suggested. (Zare and Gams 2008, Zied et al 2015). Bonnen and Hopkins (1997) studied the DNA polymorphisms by RAPD analysis, and the isolates were divided into four groups and limited correlation was found between this grouping, fungicide response and geographical origin. To find the interspecies differences, primers of Inter Simple Sequence Repeats (ISSR), are used in genetic diversity analysis and DNA fingerprinting (Idrees and Irshad 2014).

The objectives of this study are (1) to evaluate the genetic diversity of *L. fungicola* isolates using the Inter-Simple Sequence Repeat (ISSR) marker, and (2), to determine the sensitivity of selected isolates to six commercial fungicides and four plant essential oils by disc-diffusion method.

MATERIALS AND METHODS

Sample collection and isolation

A total of 21 isolates of *L. fungicola* were collected and identified based on morphological and molecular characters in a previous study (Memarzadeh et al. 2020). A mixture of 200 g of button mushroom caps and 500 mL of sterile distilled water was filtered using filter paper, and 20 ml add to one liter of PDA medium. Additionally, a medium consisting of 40 g of chopped button mushroom caps, 200 g of potatoes, 20 g of agar, and 20 g of dextrose in one liter of water was used and the cultures incubated at 22 °C for 10–20 days., resulting in enhanced growth compared to other media (Siwulski et al. 2011). Characteristics of the used isolates are shown in Table 1. Each sample had 4 replicates, and a one-way analysis of variance (ANOVA) test was performed. The cultures were incubated for seven days, after which the diameter of the fungal mycelium was measured using a standard ruler. For spore measurement, 1 mL of sterile distilled water was added to the surface of each culture to dislodge the spores. The suspension was serially diluted, and 30 spores from each treatment were selected and measured.

DNA extraction and PCR amplification

Genomic DNA extraction was performed using the cetyltrimethylammonium bromide (CTAB) method of Murray and Thompson (1980) with minor modifications (not adding β-mercaptoethanol in the extraction buffer). The quality and quantity of the extracted DNA were evaluated using a Nanodrop spectrophotometer.

A total of 10 ISSR primers were used in this study (Table 3). Each PCR reaction mixture contained 5 µl of a ready master mix (AmpliQon, Denmark), 1 µl of sterile distilled water, 1 µl of primer, and 3 µl of DNA (at a concentration of 50 ng/µl) in a final volume of 10 µl. The PCR amplification conditions were chosen according to the recommendations provided by the primer manufacturer (Metabion International AG)

Table 1. Characteristics of *Lecanicillium fungicola* isolates used in this study (LSD≈2 α=5% for growth rate).

Isolate Code	Location	spore size (µm)	growth rate (mm/7days)
D2	Isfahan- Jarghovyh-Yekta Sepahan Farm	2×7	*52 c
H5	Isfahan- Fereydunshahr	2×6	10 l
H6	Isfahan- Fereydunshahr	2×6	64 a
H8	Isfahan- Fereydunshahr	2×7	58 b
E2	Karaj, Alborz	2×7	43 de
I2	Khuzestan- Dezful- Jolgeh Dez	2×12	35 g
I5	Khuzestan- Dezful- Jolgeh Dez	2×12	38 f
I8	Khuzestan- Dezful- Jolgeh Dez	2×12	35 g
I9	Khuzestan- Dezful- Jolgeh Dez	2×9	25 j
I11	Khuzestan- Dezful- Jolgeh Dez	2×8	20 k
I17	Khuzestan- Dezful- Jolgeh Dez	2×5	42 e
I18	Khuzestan- Dezful- Jolgeh Dez	2×15	44 d
I22	Khuzestan- Dezful- Jolgeh Dez	2×10	30 i
I24	Khuzestan- Dezful- Jolgeh Dez	2×8	30 i
I29	Khuzestan- Dezful- Jolgeh Dez	2×11	32 hi
I30	Khuzestan- Dezful- Jolgeh Dez	2×8	25 j
I37	Khuzestan- Dezful- Jolgeh Dez	2×10	34 gh
I38	Khuzestan- Dezful- Jolgeh Dez	2×11	38 f
I43	Khuzestan- Dezful- Jolgeh Dez	2×10	20 k
I47	Khuzestan- Dezful- Jolgeh Dez	2×5	30 i
I56	Khuzestan- Dezful- Jolgeh Dez	2×5	25 j

* Each column data followed by the same letter did not differ significantly (P≤0.05)

Table 2. The common and commercial names of fungicides with the specific doses used in the study.

Commercial name of fungicide	The Dose used (mg/l)	Formulation	Common name
Rovral-TS	50	52.5% WP	Iprodione+ Carbendazim
Derosal	200	60% WP	Carbendazim
Flint	50	50% WG	Trifloxystrobin
Topas	20 (ml/l)	20% EW	Penconazole
Nordox	375	75% WG	Copper oxide
NEEM	3(ml/l)	0.03%	*Azadirachta indica*

(Table 3). The thermo cycling conditions consisted of an initial denaturation step at 94 °C for two min, followed by 35 cycles of 94 °C for 45 s, annealing at the specific temperature for 45 s, extension at 72 °C for 120 s, and a final extension step at 72 °C for 10 min. PCR amplicons were separated and visualized on a 1% (w/v) agarose gel stained with Ethidium bromide and the sizes of amplicons were determined using a 100 bp DNA ladder (Pishgam, Iran).

Statistical analyses

For ISSR analysis, the numbers 1 and 0 were assigned to denote the presence or the absence of a band, respectively (Fig. 2), and a binary matrix was

Table 3. Characteristic of ISSR primers used in the genetic diversity of *Lecanicillium fungicola* analysis.

Number	Name	ISSR Primer repeats	Annealing Temperature (^{0}C)	GC content %	PIC value	ML*	PL**	TL***	PL%	Reference
1	808	$(AG)_8C$	50	53%	0.7422	0	29	29	100	(Mitina et al. 2017, Sunaryo et al. 2020)
2	818	$(CA)_8G$	50	53%	0.8808	1	21	22	95.5	(Mitina et al. 2017, Sunaryo et al. 2020)
3	809	$(AG)_8G$	50	53%	0.7425	1	22	23	95.6	(Mitina et al. 2017, Sunaryo et al. 2020)
4	B	$(AG)_8TG$	52	50%	0.7611	0	23	23	100	(Mitina et al. 2017, Sunaryo et al. 2020)
5	C	$(AG)_8CG$	54	55%	0.6768	2	23	25	92	(Mitina et al. 2017, Sunaryo et al. 2020)
6	D	$(AG)_8$	46	50%	0.8567	0	17	17	100	(Mitina et al. 2017, Sunaryo et al. 2020)
7	AA3	$(AG)_8TA$	50	44%	0.8881	0	23	23	100	(Toledo et al. 2019)
8		$(GA)_8GG$	54	55%	0.6928	0	19	19	100	(Baysal et al. 2011)
9		$GAGG(AG)_6G$	52	59%	0.6769	1	15	16	93.7	(Levi et al. 2005)
10	889	$AGTCGTAGT(AC)_7$	61	48%	0.801	2	23	25	92	(Mitina et al. 2017, Sunaryo et al. 2020)
					0.772	7	215	222	96.8	

* Monomorphic Loci (ML), ** Polymorphic Loci (PL), *** Total loci (TL)

constructed. This data was inputted into Excel software and subsequently transferred to the NTSYS pc. v2.02. software. Using the JACARD similarity matrix and the UPGMA algorithm, a dendrogram was constructed. The average Polymorphism Information Content (PIC) was also measured.

In vitro assay of selected fungicides against mycelial growth of *L. fungicola* isolates

The sensitivity of twenty-one isolates of *L. fungicola* and one isolate of *A. bisporus* was evaluated against six fungicides (Table 2) using the mycelium inhibition method in PDA culture medium. The recommended concentration of each fungicide (Table 2), was prepared by adding an appropriate amount of fungicide to the cooled molten PDA, agitating gently to become homogenized, and allowing it to solidify. Then, mycelial disks (five mm diameter) of actively growing fungal isolates were cut using a sterile cork borer and aseptically placed upside down at the center of each Petri plate. In the control, agar disks without fungicide were used. The test was conducted at a temperature of 22 ± 2 °C. The fungicides assessed are listed in Table 2.

The inhibition percentage of radial growth rate was calculated using the formula: $MI = \frac{Mc-Mt}{Mc} \times 100$, where MI is the mycelial inhibition percentage, Mc is the colony diameter in the control, and Mt is the colony diameter in the treatment (Dianez et al. 2018).

The experiment was conducted in four replicates using a completely randomized two-factorial design (Chrysayi-Tokousbalides et al. 2007).

Sensitivity test against essential oils

Four essential oils, including Thyme, eucalyptus, rosemary, and peppermint, were obtained from Barijessence Company, Kashan, Iran. A five mm paper disk was placed in the center of each petri dish, and up to eight µl of essential oils (according to paper disk capacity), along with Tween 80 was applied (Dianez et al. 2018). For Thyme and eucalyptus essential oils, the concentrations of 0.7, 0.85, 1, and 1.2 ppm, and for rosemary and peppermint concentrations of 0.3, 0.45, 0.6, and 0.8 ppm were tested. The inhibition percentage of radial growth rate was calculated as described for essential oils. Each Petri dish had an internal volume of approximately 20 mL, and tested concentrations were calculated based on the headspace volume. To adjust the concentration, Tween 80 was used. A prepared suspension was applied to a 5 mm paper disk, which was then attached to the lid of the Petri dish (due to the wetness of the disk). Subsequently, a 5 mm mycelial plug was placed at the center of the Petri dish. The dishes were sealed with Parafilm and incubated upside down at 22 °C. For the untreated control groups, 8 mL of Tween 80 was used. Measurements were taken until the group reached the endpoint, at which point the experiment was terminated. The statistical significance of the data was evaluated using a three-factorial random design method.

RESULTS AND DISCUSSION

The mycelial growth of the isolates exhibited several forms such as dense mycelia attached to the agar surface (Fig. 1. A), dense aerial mycelia (Fig. 1. B), and aerial mycelial growth (Fig. 1. C). Notably, fluffy aerial mycelia were observed in old samples, likely due to prolonged storage and multiple cultivations in the culture medium (Bonnen and Hopkins 1997).

The analysis of comparing colony rate and spore size in terms of genetic diversity revealed no morphological correlation in all isolates within species (Table 1).

Ten ISSR primers produced 222 polymorphic bands (Fig. 2) and the isolates were categorized into six groups with 67% similarity coefficient (Fig. 3).

The average Polymorphism Information Content (PIC) was 0.772, with the highest PIC observed at 0.888 for primer 7 and the lowest at 0.676 for primer 5, indicating a polymorphism rate of 96.8% (Fig.4).

Fig. 1. Three forms of *Lecanicillium fungicola* growth in PDA+ *Agaricus bisporus* A: dense mycelia attached to the agar. B: dense aerial mycelia. C: aerial mycelia.

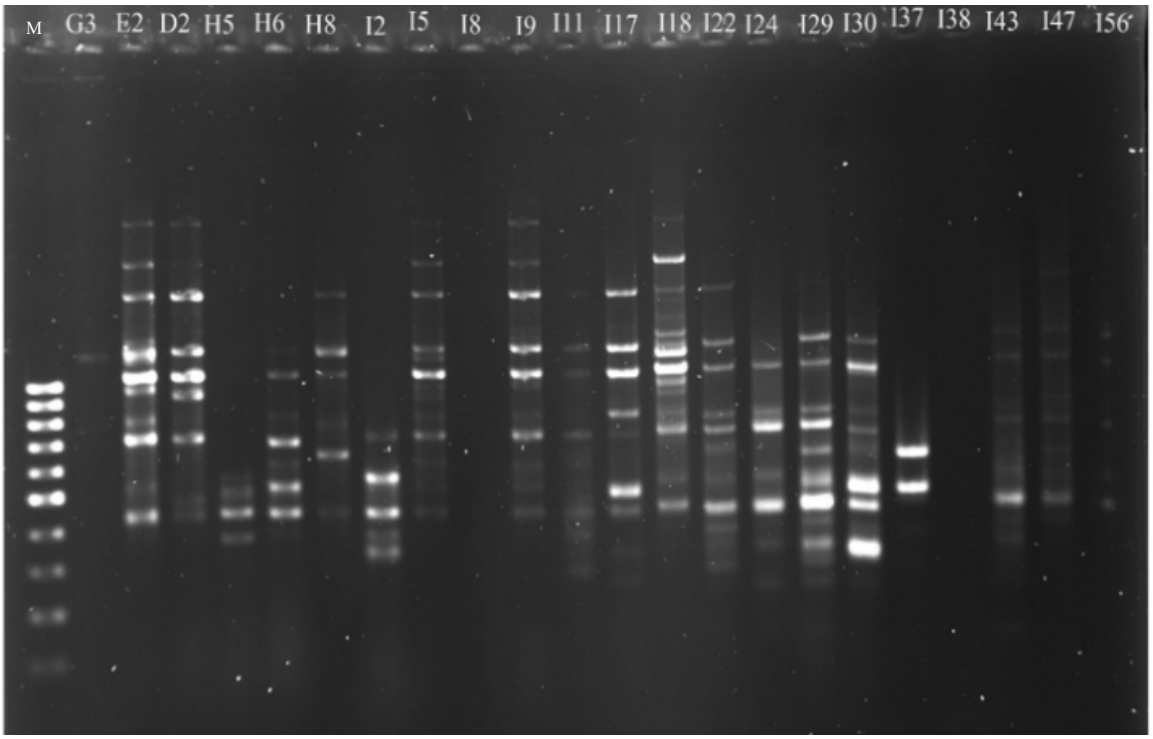

Fig. 2. The ISSR amplification pattern obtained from 21 isolates of *Lecanicillium fungicola* with the 808 [(AG)8C] primer. M: 1 kbp ladder (Sample G3, *Mycogone perniciosa*, was used as outgroup).

Notably, fast-growing isolates (H6, 47I, 56I, 37I, I29, I30) are closely positioned together. The tree, drawn with a Dice similarity coefficient and UPGMA algorithm, exhibits a correlation coefficient of r = 0.96377, indicating high accuracy (Fig. 3). Previous studies also found no relationship between colony growth rate, colony color, and physiological characteristics using the UPR markers (Largeteau et al. 2006, Largeteau et al. 2008, Mehrparvar et al. 2012, Singh et al. 2018). Mehrparvar et al. (2012), indicated that three out of 10 tested primers gave higher polymorphism, whereas in this study, all ten tested primers produced high polymorphisms. Bonnen and Hopkins (1997), using RAPD marker analysis, divided *L. fungicola* isolates into four groups, and the groups were correlated with geographical region and resistance to benomyl fungicide.

In vitro assay of selected fungicides and essential oil

Remarkable symptoms observed in the fungal colonies were a decrease in growth initially when exposed to the Flint fungicide. However, after approximately three days, the growth increased to the extent that the colonies no longergrown on the culture medium's surface, but rather grew almost attached to the Petri lid. A similar pattern was observed with copper oxide fungicide. Initially, the fungus around the primary colony grew normally, but its growth became fluffy after four days. Additionally, pinking of the colony occurred when exposed to Topas and Neem fungicides (Fig. 5).

Among the tested doses, the most effective fungicide was Rovral-TS (Fig. 5B), while Neem was the least effective, even causing an increase in growth rate. However, Neem effectively controlled some isolates and showed no impact on the button mushroom (Fig. 5F). Due to the lack of residue from this fungicide, it may be worthwhile to study it as there have been reports of successful disease control with it (Anonymous 2022). It is important to note that the low concentration of Neem used in this study was significantly different from the commercially (Trilogy) available fungicide concentration.

However, Topas, Neem, Nordox, and Flint fungicides initially reduced fungal growth, but the measurement was not conducted as the control did not reach the edge of the Petri dish. The lack of control may be attributed to fungicide loss and oxidation. Nordox fungicide showed the highest standard deviation (SD) in controlling isolates, inhibiting more than 75% of some isolates (Fig. 6). The fungicide Nordox was not effective at the tested concentration, although it completely controlled the fungus *A. bisporus*, it showed poor control over *L. fungicola*, and in some cases, it even promoted growth (Fig. 5E).

Previously, Derosal showed good efficacy in controlling this disease. However, signs of reduced sensitivity to this fungicide were observed in some isolates, with isolate E showing the most significant reduction in sensitivity. Isolate H6, which was generally sensitive to most fungicides, remained sensitive to Derosal (Fig. 5D).

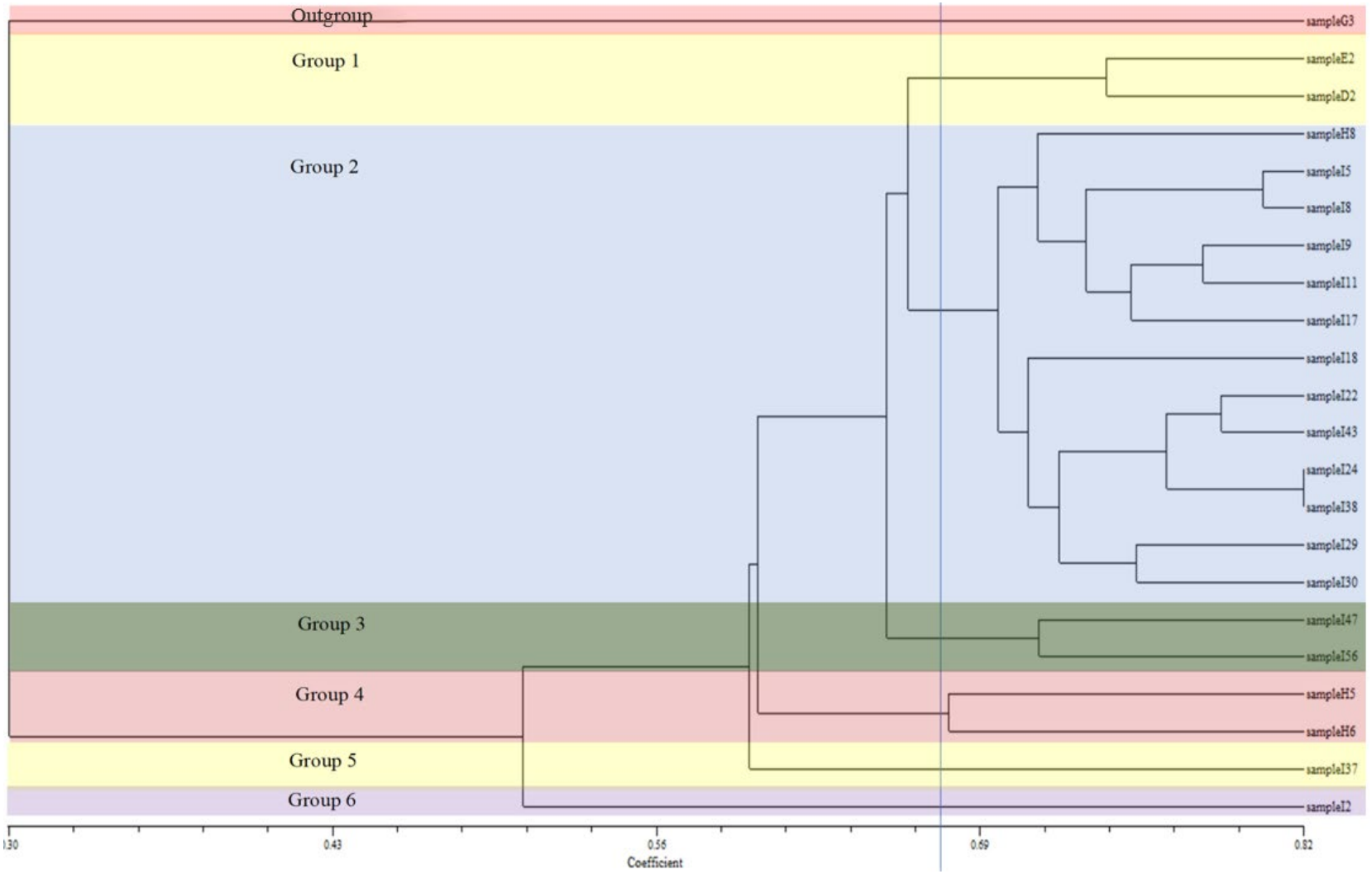

Fig. 3. Dendrogram generated by UPGMA-based cluster analysis of 10 ISSR markers for *Lecanicillium fungicola* isolates (Sample G3, *Mycogone perniciosa*, was used as outgroup).

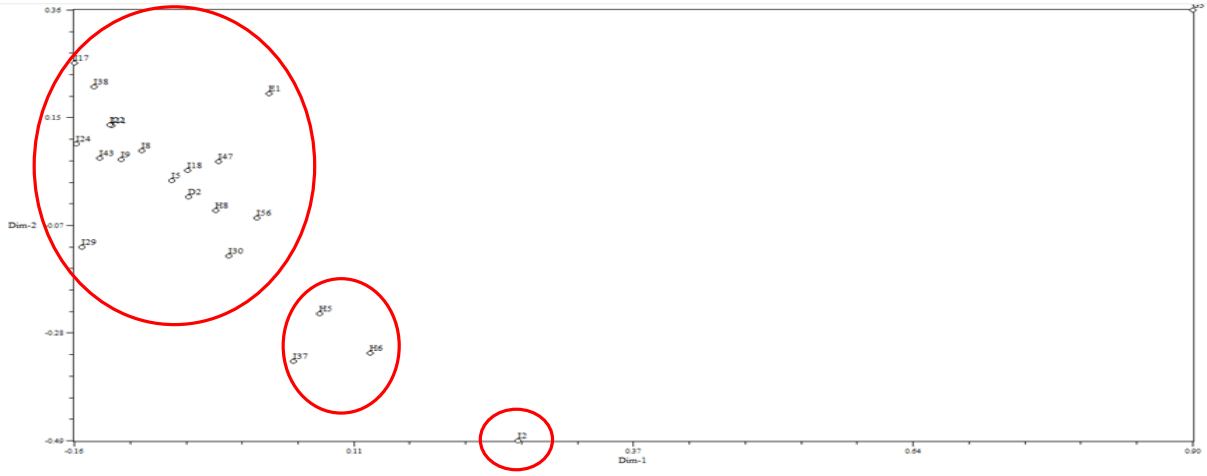

Fig. 4. The two-dimensional plot view of principal component analysis (PCoA) of 21 *Lecanicillium fungicola* isolates studied in this study using NTSYS version 2.02e.

Flint, which attracted attention due to its lower toxicity for humans compared to other fungicides. It showed good control over some isolates, but its negative effects on the button mushroom, so suggests that further studies should be conducted in mushroom cultivation halls (Fig. 5C).

Topas was selected because of its lower toxicity on humans, it showed good control over some isolates as it has not yet been evaluated in mushroom cultivation halls (Fig. 5A).

Isolate 56I, which was generally less sensitive to most fungicides, showed sensitivity to Derosal and Rovral-TS (Fig. 5). Isolates 24I and 38I, when classified using ISSR, were grouped closely together. Comparing their sensitivity to the fungicides, they showed similar reactions, except for Nordox where a significant difference was observed. Other than that, there was no significant difference in their responses to the fungicides (Fig. 5).

The closest ISSR correlation to the fungicides was for Derosal, although it was not completely the same. Therefore, it cannot be conclusively stated, as in the Bonnen and Hopkins (1997) study, that the DNA molecular markers profile directly correlates with the fungicide response (Figs. 3 and 5D)

One of the reasons for the lack of efficacy of some fungicides might be due to the slow growth of certain isolates, which could have caused the fungicides to degrade or oxidize, reducing their effectiveness. The half-lives of the fungicides are as follows: Nordox, Topas and Derosal: 14 days. Flint: 3 days. Rovral-TS: 20 days and Neem: 2 days

All tested fungicides exhibited significant differences at a specific dose with α=5%. The effective doses (ED50) of various fungicides against *L. fungicola* that were previously measured by Navarro and Tello (2005), Chrysayi-Tokousbalides et al. (2007), Grogan (2008), Mehrparvar et al. (2013), Bhat et al. (2017), Singh and Lal (2019) showed that the isolates' susceptibility was decreased. Given the resistance of isolates to fungicides, it becomes imperative to utilize strains that are resistant to dry bubble disease (Gea et al. 2021).

Highlighting awareness of the adverse effects of chemical pesticides and the presence of fungicide residual in fresh button mushrooms has increased interest in evaluating various safer alternatives for controlling *A. bisporus* diseases, particularly dry bubble disease.

Essential oils exhibited significant differences with α=5%., the peppermint essential oil being the most effective. Also, contrary to the earlier findings, thyme essential oil did not effectively control the fungus, even at a high concentration of 1.2 ml/L. This discrepancy could stem from variations in the isolates' resistance, differences in the essential oil's active ingredients, or variations in the extraction method (Fig. 7).

Another point is that the Isfahan H6 isolate, which showed high sensitivity to fungicides, is also classified as highly sensitive to essential oils. However, it is important to note that this difference is not statistically significant (Fig. 7).

In the studies by Allahyari et al. (2021), they observed that the flavor of plant oils remained on the mushrooms and, in some cases, caused spots on the button mushrooms. However, this was tested through spraying, not through fumigation. If a fumigation method is used, similar to the cultivation environment, it might prevent spots or flavor alterations. Unlike Dianez et al. (2018) and Allahyari et al. (2021), Thyme which was ineffective, it is possible that the plant pathogen's sensitivity to chemicals and plant-derived substances may vary, influencing the overall control of the disease.

The button mushroom isolate, except when exposed to peppermint oil, showed higher sensitivity compared to other *L. fungicola* isolates. However, according to the research by Allahyari et al. (2021), this inhibition might not occur in mushroom cultivation environments. This is because fungicides or essential oils are used in the

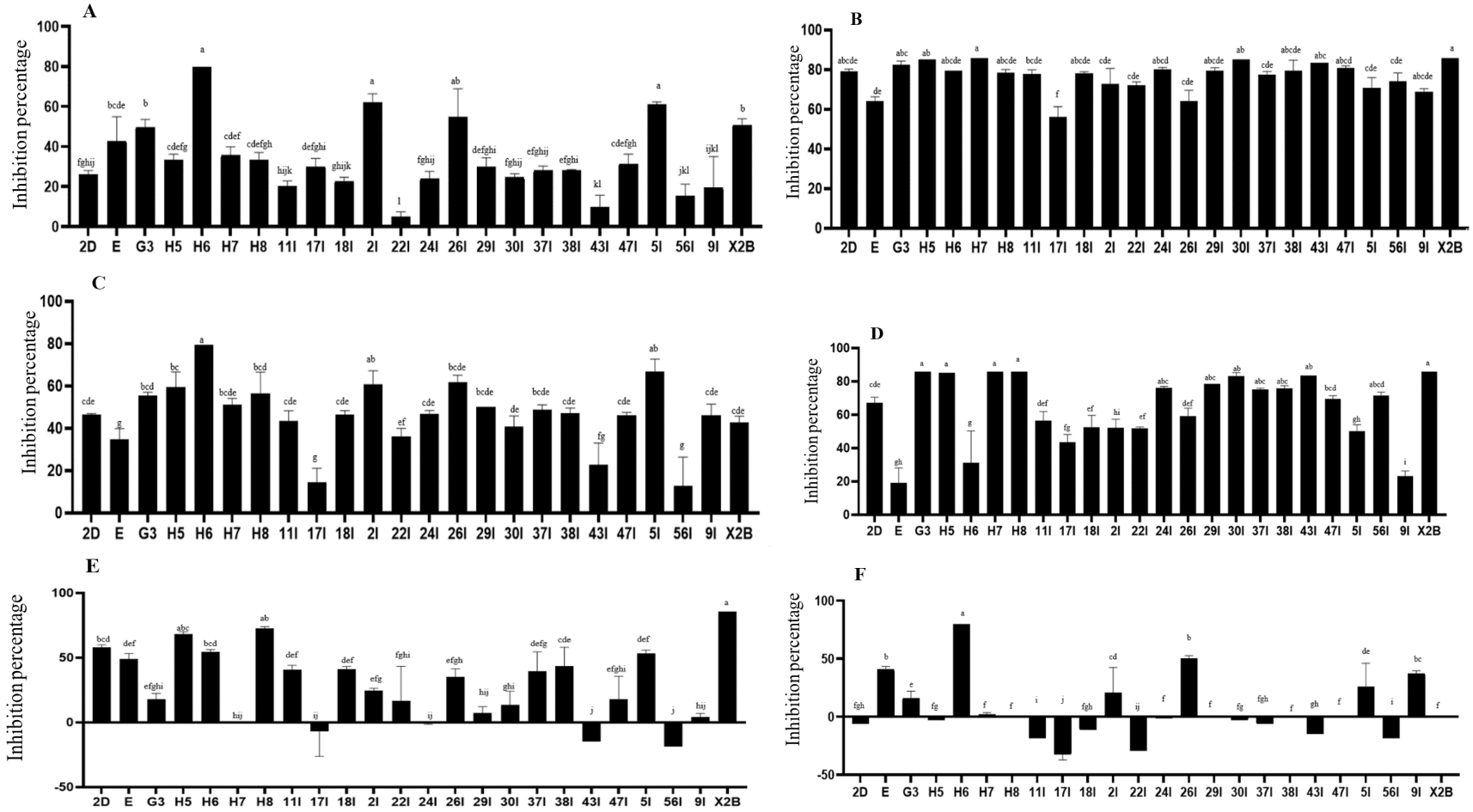

Fig. 5. Percentage of fungicide inhibition in different isolates of *Lecanicillium fungicola* (G3 Representative isolate of *Mycogone perniciosa* and X2B Representative isolate of *Agaricus bisporus*) A: Topas, B: Rovral-TS, C: Flint, D: Derosal, E: Nordex, F: NEEM (each column data followed by the same letter did not differ significantly (P≤0.05)).

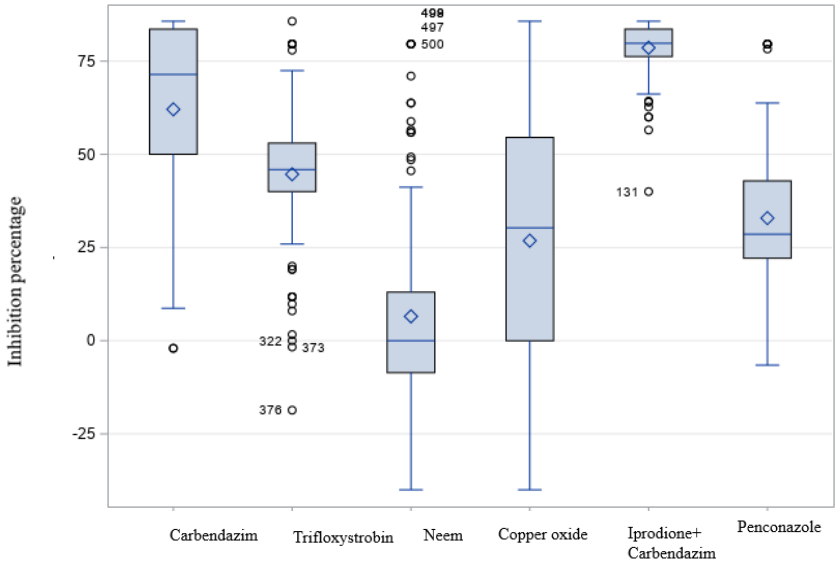

Fig. 6. The percentage of inhibition of different fungicides and their standard deviations.

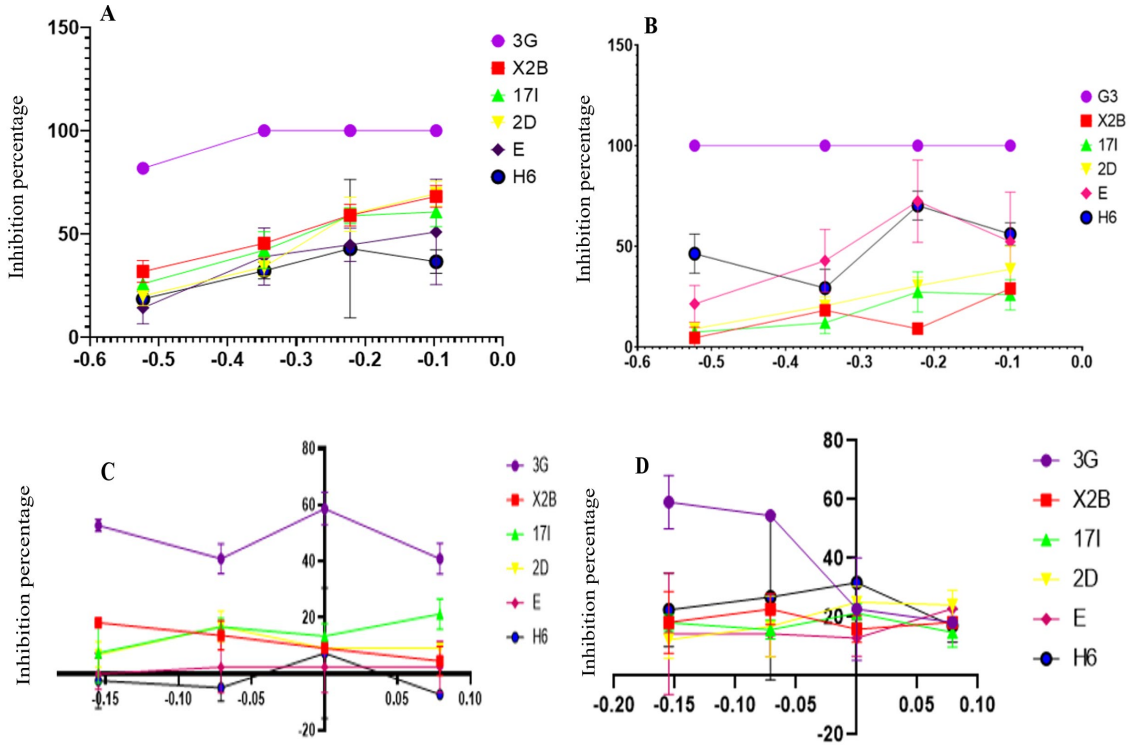

Fig. 7. Percentage of Essential oil inhibition in different isolates of Lecanicillium fungicola (G3, Representative isolate of *Mycogone perniciosa* and X2 B, representative isolate of *Agaricus bisporus*). A: Peppermint, B: Rosemary, C: Thyme, D: Eucalyptus.

later stages of growth (after cap formation) and not in the early stages of button mushroom growth, so their control effects on the button mushroom are less pronounced.

The X2B isolate, which is a button mushroom, exhibited high sensitivity to chemical fungicides (Fig. 5 and Fig. 7). This might explain the lack of resistance observed in cultivated button mushrooms and the absence of mutation in *A. bisporus* against fungicides. Nevertheless, mushrooms exhibited the least inhibition among essential oils, underscoring the potential of plant-based essential oils.

Agaricus bisporus isolate displayed greater sensitivity as compared to *L. fungicola* isolates, particularly when exposed to peppermint essential oil. However, according to Allahyari et al. (2021), this inhibition may not occur in mushroom cultivation halls. The discrepancies in results can be attributed to several factors. Firstly, while in the test, the essential oil was mixed directly with the culture medium, in the experiment, it was fumigated within the culture medium. Furthermore, variations in growth area, growth conditions, and differences in geographical sites of various plant species collections further contribute to the variability observed in the effectiveness of plant essential oils. Several studies have highlighted the potential use of plant essential oils in combating dry bubble disease. For instance, Santos et al. (2017) examined the inhibitory effects of cinnamon, eucalyptus, lemon balm, oregano, clove, and thyme essential oils both *in vitro* and *in vivo* against *L. fungicola* isolates, and found that the cinnamon, thyme, and clove essential oils were more effective in inhibiting mycelial growth.

Research indicates that the use of fungicides has exerted a unique selection pressure, eliminated sensitive pathogen populations and genetically homogenized the remaining agents of the pathogen. Based on worldwide findings documenting decreased pathogen sensitivity to different fungicides across multiple years. (Bonnen and Hopkins 1997, Zare and Gams 2008, Berendsen et al. 2010, Mehrparvar et al. 2012, Rokni et al. 2019, Rokni et al. 2020).

CONCLUSSION

Dry bubble disease caused by *L. fungicola* remains a dominant issue in Isfahan, Karaj, and Dezful, leading to deformities, cracking, brown spots, and undifferentiated tissues in button mushrooms. Without control measures, up to 60% damage to cultivation may occur. The genetic diversity of the fungus was explored using ISSR markers, revealing isolates grouped into six clusters, with potential genetic diversity observed in Dezful isolates. The presence of isolate I2 separately suggests potential variation, although morphological classifications did not yield significant differences among isolates.

Given the harmful nature of this disease, the use of fungicides in button mushroom cultivation halls is prevalent, potentially contributing to the fungus's resistance to fungicides. However, in recent years, awareness of the detrimental effects of chemical fungicides and their residues in *A. bisporus* has led to increased use of both fungicides and essential oils, which may enhance the resistance of *A. bisporus* to pathogens. To combat this disease in the laboratory, four essential oils were evaluated on several representative samples, with peppermint Essential oil at a concentration of 0.8 ppm demonstrating the highest inhibition among thyme, rosemary, and eucalyptus essential oils.

Notably, the Isfahan H6 isolate, characterized by high sensitivity to fungicides, also demonstrated significant sensitivity among essential oils, even though not significantly different. The X2B isolate, representative of *A. bisporus*, displayed high sensitivity to chemical fungicides, potentially explaining the button mushroom's lack of resistance and mutation to fungicides. However, the *A. bisporus* exhibited minimal inhibition among essential oils, underscoring the potential efficacy of essential oils.

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AUTHOR CONTRIBUTION

F. Memarzadeh: Data collection, Formal analysis, Investigation, and writing original draft., B. Sharifnabi: Conceptualization, Project administration, Resources, Supervision, Validation, Writing original draft, review and editing, R. Eslamizadeh: Validation, Resources, Data collection, Investigation and supervision.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author upon request.

DECLARATION

The authors declare no conflicts of interest.

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ETHICS APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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تنوع ژنتیکی قارچ *Lecanicillium fungicola* عامل بیماری حباب خشک قارچ دکمه‌ای با استفاده از نشانگرهای ISSR و ارزیابی حساسیت آن نسبت به اسانس‌های گیاهی و قارچ‌کش‌های منتخب

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چکیده

بیماری حباب خشک که توسط *Lecanicillium fungicola* ایجاد می‌شود از مشکلات اصلی در کشت و پرورش قارچ دکمه‌ای در سراسر جهان می‌باشد. در این مطالعه تنوع ژنتیکی ۲۱ جدایه *L. fungicola* که از مناطق مختلف استان‌های اصفهان، البرز و خوزستان جمع‌آوری شده بودند بررسی شد. DNA ژنومی به روش CTAB استخراج و ۱۰ آغازگر ISSR برای تعیین چند شکلی جدایه‌ها بکار برده شد. در مجموع، ۲۲۴ باند تکثیر شده‌ی حاصل از ۱۰ آغازگر ISSR بدست آمد که ضریب تشابه بین جدایه‌ها از ۰/۴۲ تا ۰/۸۲ متغیر بود. جدایه‌ها براساس ضریب تشابه ۶۷ درصد در شش گروه قرار گرفتند. میانگین محتوای اطلاعات چند شکلی (PIC) ۰/۷۷۲ بود و بالاترین آن ۰/۸۸۸ به دست آمد که نشان دهنده نرخ چندشکلی ۹۶/۸ درصد است. علاوه بر تحلیل ژنتیکی، حساسیت جدایه‌ها به قارچ‌کش‌های مختلف از جمله فلینت، نوردوکس، رورال تی اس، توپاس، نیم و دورسال نیز بررسی شد. همچنین، اثر بخشی چهار روغن گیاهی شامل آویشن، نعناع فلفلی، اکالیپتوس و رزماری در غلظت‌های مختلف با استفاده از روش دیسک‌دیفیوژن ارزیابی گردید. نتایج نشان داد که حساسیت جدایه‌ها به قارچ‌کش‌ها متفاوت است، به طوری که جدایه‌ای که بیشترین نرخ رشد را داشت، بیشترین حساسیت را به قارچ‌کش‌ها نشان داد. در بین قارچ کش‌های آزمایش شده، قارچ‌کش رورال تی اس بالاترین مهار را نشان داد. عصاره نعناع فلفلی با غلظت ۰/۸ ppm بالاترین بازدارندگی را داشت. این مطالعه بینش‌های ارزشمندی در مورد تنوع ژنتیکی جدایه‌های *L. fungicola*، مقاومت متفاوت آنها به قارچ‌کش‌ها و استفاده احتمالی از روغن‌های گیاهی در مدیریت بیماری ارائه می‌دهد. این تحقیق پایه‌ای برای تحقیقات آینده در زمینه تکامل مقاومت و استراتژی‌های کنترل یکپارچه بیماری‌های قارچ خوراکی دکمه‌ای فراهم می‌آورد.

کلمات کلیدی: *Agaricus bisporus*، نشانگرهای مولکولی، کنترل شیمیایی، عصاره گیاهی

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