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The frequency of occurrence of fungal pathogens associated with water hyacinth (*Eichhornia crassipes*) in Ho Chi Minh City and its harmful effects on plants

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ABSTRACT

Diseased water hyacinth leaves were collected from 5 different ecosystems in Ho Chi Minh City, then followed by fungal single spore isolation. The fungal isolates were morphologically characterized based on their mycelium, fruiting structures, and spores. The frequency of occurrence of each fungal genus was recorded. The pathogenicity of each isolate on water hyacinth was performed at laboratory conditions and the disease severity (DS) was assessed. A most harmful isolate of each fungal genus was tested for its pathogenicity on plants, associated with water hyacinth. The results showed that 106 fungal isolates were discovered, belonging to seven fungal genera, in which Curvularia spp. and Colletotrichum spp. were classified as very frequent groups with 36.80% and 29.24% of occurrence, respectively, while a frequent group of Rhizoctonia spp. (18.87%) and infrequent groups of Trichoderma spp. (5.67%), Helminthosporium spp. (4.72%), Fusarium spp. (2.83%) and Alternaria spp. (1.89%) were observed. The highest disease severity was found in the genus of Colletotrichum spp. (DS 17.2%), followed by Curvularia spp. (DS 12.7%) and Rhizoctonia spp. (DS 7.1%). All tested fungal isolates were infected plants with various levels of incidence from 0% to 100% depending on fungal genera and host plants.

1. INTRODUCTION

Eichhornia crassipes, more often known as water hyacinth, is a type of aquatic plant belonging to the Pontederiaceae family. It is the most successful invasive species in the plant kingdom and a problematic weed that can be found in at least 59 countries throughout the world (Harley et al., 1996; Njoka, 2004). The formation of dense mats by water hyacinth can disrupt in waterway traffic, as well as a deterioration in water quality and an impact on biodiversity (Gopal, 1987). The presence of water hyacinth in an area makes it possible for mosquitoes to flourish, which has a negative effect on human health (Viswam et al., 1989). To combat the issues caused by water hyacinth, several mechanical, physical, and chemical approaches have been implemented. However, these methods all have some disadvantages in practical applications at a large scale. Biological control of water hyacinth is considered an alternative method of interest because of no need to have specialized equipment, long-term effectiveness, no impact on the ecosystem, and labor (Viswann et al., 1989). In the early 1970s, natural enemies were used for the first time to reduce water hyacinth in the United States (Perkins, 1973). Since then, seven species of natural enemies have been imported into 33 countries throughout the world to control water hyacinth (Julien & Griffiths, 1998). Several other fungal species, such as *Cercospora apiaropi* in South Africa (Morris, 1990) and *Alternaria eichhorniae* in India (Nag Raj &

Ponnappa, 1970) have been characterized and successfully used as bio-agents for the management of water hyacinth (Nag Raj & Ponnappa, 1970; Morris, 1990). This is the first report that identification and characterization of fungal isolates used as potential bio-agents conducted to control water hyacinth in Ho Chi Minh City, Vietnam.

2. MATERIALS AND METHODS

2.1. Isolation and characterization of fungi associated with water hyacinth in Ho Chi Minh City

Symptomatic water hyacinth leaves were collected at 5 different ecosystems in Ho Chi Minh City: Dong Nai River, Tan Hoa - Lo Gom Canal, Rach Dua Canal, Rach Chiec River, Ho Dat Lake - Thu Duc. From each location, 40 foliar disease samples were collected and preserved according to the plant disease management method of Shivas and Beasley (2005). Samples were transported to the laboratory in zip bags and stored at 4ºC. Fungal isolates were named according to the location where the sample was collected, for example DN1: Dong Nai River, disease sample number 1.

The WA (20 g agar and 1 liter distilled water) and PGA (200 g potato, 20 g agar, 20 g D-glucose and 1 liter distilled water) were used to isolate the pathogenic fungi. Pathogenic fungi were isolated according to the method of Hurria and Hussein (2018), and they were tested for pathogenicity on water hyacinth leaves under laboratory conditions based on the method of Barnett and Hunter (1972). The evaluation of disease severity (DS) of fungal isolates was done by visualization after 7-days post inoculation (dpi) as previously described (Naseema & Balakrishnan, 2001). Five degrees were used to classify the levels of severity scales, which included 0 = No symptoms, 1 = 1-9% of the leaf area showing symptoms around the inoculum site, 2 = 10-25% of the leaf area showing yellowing or browning, 3 = 26-50% of the leaf area showing symptoms, 4 = 51-100% of the leaf area showing symptoms (Naseema & Balakrishnan, 2001). The disease severity was determined by the following formula: (Sum of all disease rating/(Total no. of rating * maximum disease grade) x 100 (Praveena & Naseema, 2006).

The morphology of fungal mycelium, fruiting structures and spores was characterized and identified as described in previous studies (Gilman, 1957; Barnett, 1960, Ellis, 1976, Holliday, 1993, Aneja, 2007, Domsch et al. 2007). The frequency of occurrence of fungal genera was calculated through the formula: Frequency (%) = (Number of isolates in genus x 100) / Total no. of isolates and classified into three groups including a very frequent group (>20%), frequent group (10%-20%) and infrequent group (<10%) (El-Morsy, 2004).

2.2. Plant damage of fungal isolates associated with water hyacinth

The crops were chosen based on their habitat within the waterbodies and in lowland areas that could be linked to water hyacinth. They included aquatic plants (arrow leaf pondweed (*Sagittaria sagittifolia L.*), water spinach (*Ipomoea aquatica*), yellow burrhead (*Limnocharis flava*), lotus (*Nelumbo nucifera*) and water lily (*Nymphaea rubra*)), cereals (rice (*Oryza sativa*) and maize (*Zea mays* L.)); vegetables (sweet potato (*Ipomoea batatas*), vegetable mustard (*Brassica juncea*) and cucumber (*Cucumis sativus*)); pulses (peanut (*Arachis hypogaea*) and green bean (*Vigna radiata*)).

Fungal isolates belonging to various genera were cultured in petri dishes containing PGA medium for sporulation. After 7–14 days, 10 milliliters of distilled water were added and rubbed with a sterilized cotton swab on the surface of the medium plate. Finally, a filter paper № 9 was used to collect the spore solution, which was then diluted to a concentration of 10^6 spore/mL by distilled water for inoculation. The same processes were done for the genus of *Rhizoctonia*, and 1% fungus solution was used for inoculation (Luan et al., 2020).

Three symptomatic and asymptomatic leaves of each plant, except for rice and maize with six leaves for each plant were used for fungal inoculation. Three positions with the wound that were aligned and evenly spaced were created by using a pin on the back of the right side of each leaf. After that, 200 µL of 10^6 spore/mL fungal solution was added to the three wound sites and three positions without a wound on the left side symmetrically through the main vein. Finally, the fungal fluid was fixed by covering it with cotton wool. Because of small leaf area, three rice and corn leaves with wounds and three without wounds were used. After that, the experimental leaves were transferred to the plastic container and incubated at room temperature for seven days. The proportion of infected leaves in each target population was calculated by the percentage of the disease incidence (DI) as the following formula: DI = Total positions with symptoms / Total inoculated positions) *100.

3. RESULTS

3.1. Isolation and characterization of fungi associated with diseases of water hyacinth in Ho Chi Minh City

By using the method of isolation from disease samples, two hundred fungal isolates were obtained from symptomatic water hyacinth leaves collected from five different locations in Ho Chi Minh City. The results of fungal pathogenicity screening in the water hyacinth showed that 106 fungal isolates caused typical symptoms such as leaf spot, leaf burn, and necrosis compared to the control (Fig. 1A, 1B, 1C, 1D). The symptoms started off as little necrotic spots and then evolved to a leaf blight spreading across the leaf surface and petiole, depending on the fungal isolates. Eventually, sometimes, the spots turned into necrotic lesions. Additionally, re-isolated fungal samples from inoculated leaves showed similar conidial morphology of initial fungal isolates (Fig.2). Based on the morphology, the arrangement, and structure of conidia, fungal isolates obtained in this study were identified and characterized into seven different fungal genera (Table 1).

As shown in Table 1, The result revealed that *Curvularia* spp. and *Colletotrichum* spp. were classified as a very frequent group with 36.79% and 29.25% of total fungal isolates, respectively, meanwhile *Rhizoctonia* spp. with a rate of 18.87% was grouped a frequent genus. Other remaining fungi including *Trichoderma* spp. (5.67%), *Helminthosporium* spp. (4.72%), *Fusarium* spp. (2.83%) and *Alternaria* spp. (1.89%) were defined as an infrequent group (Fig.3). *Curvularia* spp. and *Rhizoctonia* spp. appeared widely distributed over all infested areas of Ho Chi Minh City. *Colletotrichum* spp. and *Helminthosporium* spp. were detected in four of five ecosystems, whereas *Alternaria* spp. and *Fusarium* spp. were found only in Tan Hoa Canal/Rach Dua River and Tan Hoa Canal/Dong Nai River, respectively.

Figure 1. Water hyacinth leaves after 7 days of inoculation. Leaves without symptoms (A), leaves with leaf spot symptoms because of strain HD18 (B), leaf burn because of strain DN6 (C) and necrosis because of strain RC24 (D)

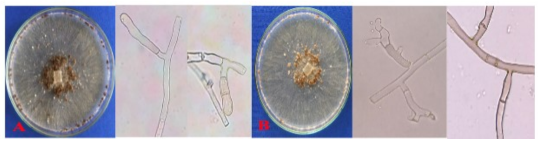

Figure 2. The morphology characteristic of Fungal isolates in the genus *Rhizoctonia* spp.: and the initial isolate (A) and re-isolated isolate (B)

As shown in Table 1, The result revealed that *Curvularia* spp. and *Colletotrichum* spp. were classified as a very frequent group with 36.79% and 29.25% of total fungal isolates, respectively, meanwhile *Rhizoctonia* spp. with a rate of 18.87% was grouped a frequent genus. Other remaining fungi including *Trichoderma* spp. (5.67%), *Helminthosporium* spp. (4.72%), *Fusarium* spp. (2.83%) and *Alternaria* spp. (1.89%) were defined as an infrequent group (Fig.3). *Curvularia* spp. and *Rhizoctonia* spp. appeared widely distributed over all infested areas of Ho Chi Minh City. *Colletotrichum* spp. and *Helminthosporium* spp. were detected in four of five ecosystems, whereas *Alternaria* spp. and *Fusarium* spp. were found only in Tan Hoa Canal/Rach Dua River and Tan Hoa Canal/Dong Nai River, respectively.

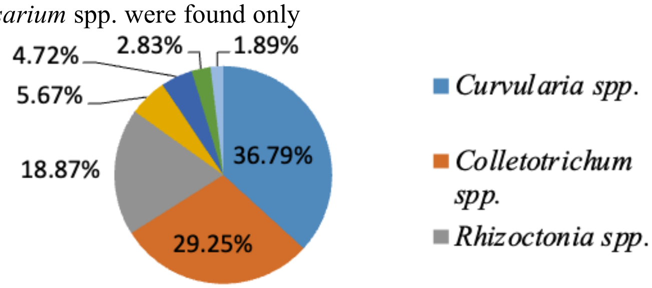

Figure 3. The percentage of occurrence of fungi on the leaves of water hyacinth in Ho Chi Minh City

The Disease severity (DS) of a plant pathogen is an important parameter that is used to measure the level of hyacinth disease. The highest disease grade (4) was observed with one fungal isolate of *Rhizoctonia* spp. (RC24), disease grade (3) was recorded with 5 fungal isolates of *Colletotrichum* spp. and one fungal isolate of *Rhizoctonia* spp. The fungal isolates of remaining genera showed low disease grades (1 or 2). In addition, the disease symptoms induced by the highest DS fungal isolate occurred just only 1-2 days and became more severe until day 7. Other fungal isolates cause mild disease and show symptoms more slowly (after 4 - 5 days) (Figure 1).

Table 1. Fungal isolates of each genus and their disease grades were characterized in this study

STT	***Curvularia* spp.**		***Colletotrichum* spp.**		***Rhizoctonia* spp.**		***Trichoderma* spp.**		***Helminthos-porium* spp.**		***Fusarium* spp.**		***Alternaria* spp.**	
	Isolates	**DG**	**Isolates**	**DG**	**Isolates**	**DG**	**Isolates**	**DG**	**Isolates**	**DG**	**Isolates**	**DG**	**Isolates**	**DG**
1	**RC18**	**2**	**RC37**	**3**	**RC24**	**4**	**HD10**	**2**	**HD37**	**2**	**TH6**	**2**	**TH22**	**2**
2	**HD20**	**1**	**DN24**	**2**	**RD13**	**1**	**DN13**	**1**	**TH24**	**1**	**DN4**	**1**	**RD1**	**1**
3	DN2	1	DN6	3	DN1	1	DN23	2	RC9	2	DN7	1		
4	DN15	1	DN11	2	DN3	2	RC5	2	RC22	1				
5	DN17	1	DN20	3	HD6	1	RC40	2	RD32	2				
6	DN18	1	DN29	3	HD7	2	TH18	1						
7	DN26	1	HD22	3	HD8	1								
8	DN30	1	HD24	3	RD14	1								
9	DN33	1	HD27	3	RD22	1								
10	HD1	1	HD29	3	TH4	1								
11	HD3	2	HD30	1	TH9	1								
12	HD13	1	HD33	2	TH12	1								
13	HD15	1	HD36	2	TH14	2								
14	HD18	2	RC2	2	TH15	1								
15	HD26	1	RC7	3	TH16	2								
16	HD39	1	RC8	2	TH27	2								
17	RC14	1	RC12	3	TH28	1								
18	RC16	1	RC13	3	TH29	1								
19	RC31	1	RC17	2	TH30	3								
20	RC34	1	RC25	1	TH31	1								
21	RD8	1	RC27	3										
22	RD9	1	RC28	3										
23	RD10	1	RC30	1										
24	RD11	1	RC35	1										
25	RD12	1	RC36	2										
26	RD17	1	RC39	3										
27	RD23	2	RD5	1										
28	RD33	2	RD7	2										
29	RD36	1	TH7	1										
30	TH3	1	TH10	2										
31	TH8	1	TH35	2										
32	TH21	2												
33	TH26	1												
34	TH33	2												
35	TH34	2												
36	TH37	1												
37	TH38	1												
38	TH39	2												
39	TH40	1												

Note: DN: Dong Nai River, TH: Tan Hoa Canal, RD: Rach Dua Canal, RC: Rach Chiec River, HD: Ho Dat

The highest disease severity was found in the genus of *Colletotrichum* spp. (DS 17.2%) because of very frequent occurence and high fungal pathogenicity, followed by *Curvularia* spp. (DS 12.7%) and *Rhizoctonia* spp. (DS 7.1%). The fungal isolates belonging to *Trichoderma* spp., *Helminthosporium* spp., *Fusarium* spp. and *Alternaria* spp. showed low disease indexes with DS at 2.8%, 1.9%, 0.9% and 0.7% respectively. One fungal of each fungal genus with the highest disease grade was selected for testing damages on different plants.

In water hyacinth, all tested fungal isolates caused damages with a disease incidence (DI) of 100% in both cases, with and without wound sites, except for the fungal isolate of *Helminthosporium* spp. that showed the DI of 33.3% when inoculating without wound sites.

3.2. The damages of fungi associated with water hyacinth on plants

For the remaining plants, the disease incidence varied from 0% to 100%, depending on the fungal genera. All plants were damaged by *Rhizoctonia* spp. isolates, except for lily when inoculating without wound sites. *Colletotrichum* spp. isolates were harmful for most of tested plants with the DI of 100% in both with and without wound sites, except for peanuts with DI of 11.2% and 0% when inoculating with and without wound sites. The *Trichoderma* spp. isolates caused slight symptoms of leaf spot (Fig.5) with DI of 66.7% in cucumber and DI of 55.6% in lotus when inoculating with wound sites (Table 2).

Table 2. Disease incidence (%) of fungal isolates on tested plants

Type of plants	Wound	*Alternaria* spp.	*Colletotrichum* spp.	*Curvularia* spp.	*Fusarium* spp.	*Helmintho-sporium* spp.	*Rhizoctonia* spp.	*Trichoderma* spp.
		TH22	RC37	RC18	TH6	HĐ37	RC24	HD10
Water hyacinth	K	100	100	100	100	33.3	100	100
	C	100	100	100	100	100	100	100
Arrow leaf pondweed	K	66.7	100	66.7	77.8	88.9	100	0
	C	100	100	88.9	100	100	100	0
Rice	K	0	100	0	66.7	0	100	0
	C	0	100	100	100	0	100	0
Corn	K	0	100	100	100	0	100	0
	C	100	100	100	100	0	100	0
Sweet potato	K	0	100	100	0	0	100	0
	C	88.9	100	100	77.8	100	100	0
Vegetable mustard	K	0	100	22.2	0	0	100	0
	C	0	100	100	0	0	100	0
Cucumber	K	0	100	100	0	88.9	100	0
	C	0	100	100	0	88.9	100	66.7
Water spinach	K	0	100	0	22.2	0	100	0
	C	77.8	100	100	77.8	88.9	100	0
Yellow burrhead	K	77.8	100	33.3	0	100	100	0
	C	100	100	77.8	88.9	100	100	0
Lotus	K	0	100	0	100	0	100	0
	C	100	100	100	100	77.8	100	55.6
Water lily	K	0	100	0	0	0	0	0
	C	0	100	66.7	100	0	100	0
Peanuts	K	0	0	0	0	0	100	0
	C	0	11.2	100	0	0	100	0
Green beans	K	77.8	100	88.9	0	33.3	100	0
	C	100	100	100	0	55.6	100	0

Note: K: treated without wound site, C: treated with wound site

On plants, symptoms of disease caused by different isolates of each fungal genus were irregular leaf burn, necrotic spots, covered broad lesions, and sometimes lesions were colorless. The necrotic zones were clearly surrounded by obvious yellow margins. This characteristic was maintained by most of the signs, for example, the leaves were burned and lost the green color in green beans.

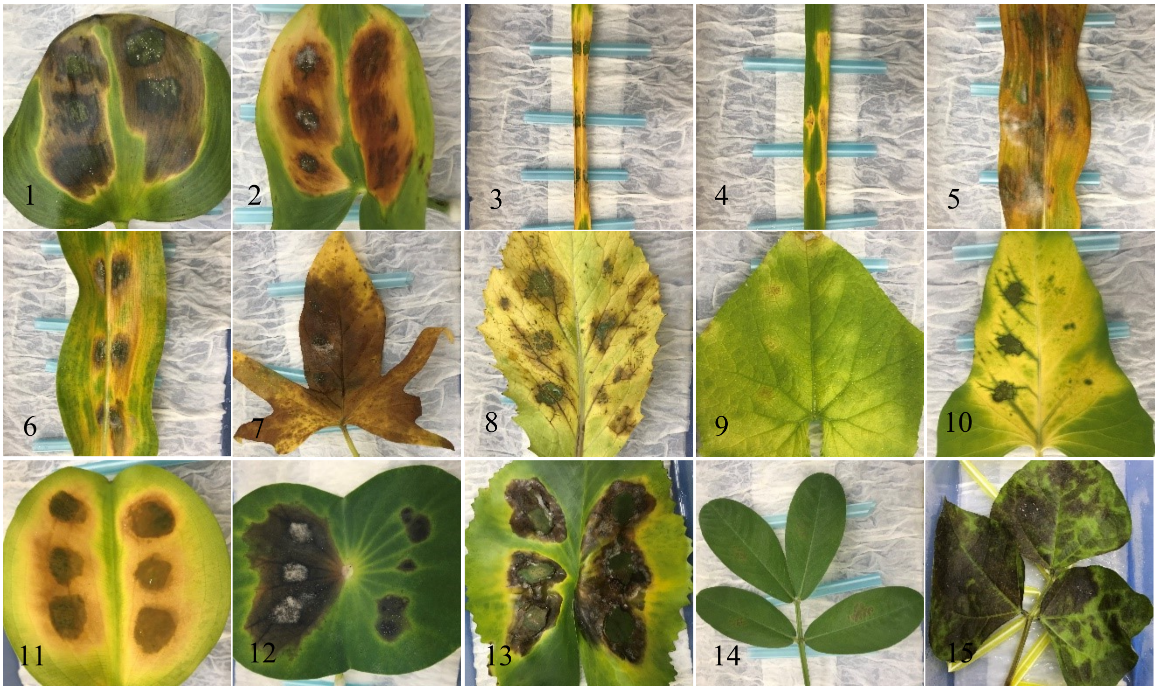

Figure 4. Damages on plants after 7 days post inoculation of RC37 (*Colletotrichum* sp.

Numbers from 1 to 15 correspond to the plants of Water hyacinth (1), Arrow leaf pondweed (2), Rice with wounds (3), Rice without wounds (4), Corn with wounds (5), Corn without wounds (6), Sweet Potatoes (7), Vegetable mustard (8), Cucumber (9), Water spinach (10), Yellow burrhead (11), Lotus (12), Water lily (13), Peanuts (14) and Green Beans (15)

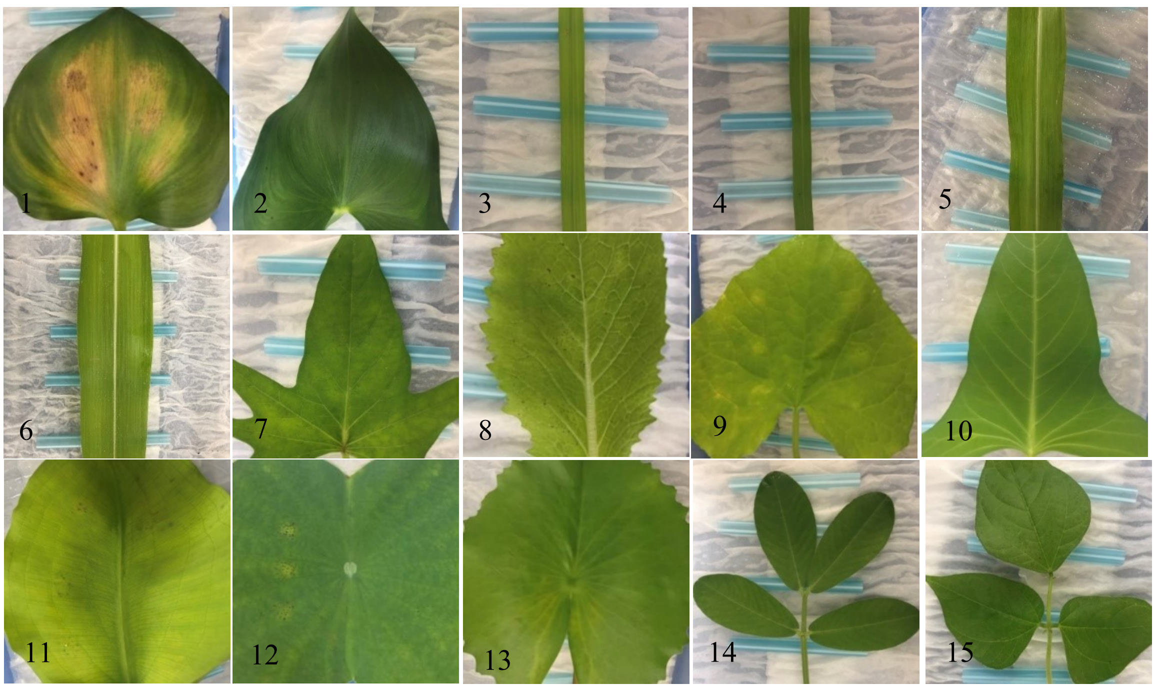

Figure 5. Damages on plants after 7 days post inoculation of HD10 (*Trichoderma* sp.)

Numbers from 1 to 15 correspond to the plants of Water hyacinth (1), Arrow leaf pondweed (2), Rice with wounds (3), Rice without wounds (4), Corn with wounds (5), Corn without wounds (6), Sweet Potatoes (7), Vegetable mustard (8), Cucumber (9), Water spinach (10), Yellow burrhead (11), Lotus (12), Water lily (13), Peanuts (14) and Green Beans (15)

Corn, sweet potato, water spinach, yellow burrhead, lotus, and green beans all showed high levels of disease severity. Arrow leaf pondweed was also affected. The disease severity (DS) in other plants, such as rice, vegetable mustard, cucumber, and water lily, is less severe, and these plants also displayed milder symptoms of the disease. The peanut was only infected when it was inoculated at the wound site by fungal isolate belonging to the genera of *Colletotrichum* spp. and *Curvularia* spp. exhibiting symptoms of a very weak leaf spot.

4. DISCUSSION

The common diseased symptoms of water hyacinth while collecting samples from different ecosystems in Ho Chi Minh City were leaf spot, leaf burn, and necrosis. Similar symptoms have also been reported in many previous studies, a diverse fungal species associated with water hyacinth such as *Alternaria* spp. (Shabana et al., 1997), *Curvularia* spp., *Fusarium* spp., *Cercospora* spp. or *Rhizoctonia solani* (Charudattan, 2000; Samuel, 2012) have been previoulsy demonstrated.

In this study, *Curvularia* spp., *Colletotrichum* spp. and *Rhizoctonia* spp. were identified as redundant genera in water hyacinth in Ho Chi Minh City. This result is consistent with previous studies of which *Rhizoctonia solani* was reported as one of the very frequent fungi occured in water hyacinth in numerous countries of South America and some Asian countries (Charudattan, 1990, 2000; Opand et al., 2017). In addition, *Curvularia* spp., *Alternaria* spp. and *Fusarium* spp. were also showed as redundant fungi in water hyacinth in Ethiopia, Mali, Iraq and India (Tegene et al., 2012; Yirefu et al., 2017; Hurria & Hussein, 2018; Govindan & Gunasekaran, 2020).

Rhizoctonia solani is one of the most extensively dispersed fungi in the world, with a wide range of hosts (Walker, 1956) and is considered a parasite virulent species, causing the fastest degradation of water hyacinth (Charudattan, 1990, 2000). In this study, the *Rhizoctonia solani* was reported with the highest disease grade for water hyacinth, and also remained as a pathogenic fungus for all tested plants with high disease severity. Because of its wide host range, *Rhizoctonia solani* has never been seriously considered as a bioherbicide agent although it shows high virulence and destructive capacities. Interestingly, the redundancy of *Colletotrichum* spp. having high fungal pathogenicity and disease index, was found in the water hyacinth in Ho Chi Minh city. This result is also consistent with the findings of Huang et al. (2021), in which *Colletotrichum fructicola* was reported as a causal agent of anthracnose symptoms such as necrotic spots on leaves and stems and stem rot in the water hyacinth in Sichuan, China. According to Dagno et al. (2012), the success of biological control of water hyacinth required several highly virulent fungal pathogens. The combination of *Rhizoctonia solani* and other pathogenic fungi such as *Curvularia* spp*.*, *Colletotrichum* spp with *Neochetina* spp. (Coleoptera: Curculionidae) in stricken areas, lakes, or canals in the city or river promising a biological approach for controlling water hyacinth in Ho chi Minh City, Vietnam.

To the best of our knowledge, this is the first report that *Trichoderma* spp. can cause specific symptoms in water hyacinth. At the first time of inoculation, the lesions were white, then turned to yellow or brown and surrounded by a golden halo, then followed by spreading quickly in the plants. Although this genus could be harmful for water hyacinth, the level of fungal pathogenicity and disease index was not high. Queene et al. (2016) isolated a pathogenic isolates of *Trichoderma* genus in laboratory conditions, but in vivo conditions, these isolated did not shown any symptoms, while the *Alternaria alternate* was found to be the best virulence candidate for control water hyacinth.

5. CONCLUSION

One hundred and six fungal isolates associated with water hyacinth in Ho Chi Minh City belonged to seven fungal genera, in which *Curvularia* spp. and *Colletotrichum* spp. were redundant compared to *Helminthosporium* spp. (4.72%), *Fusarium* spp. (2.83%) and *Alternaria* spp. (1.89%) known as infrequent genera, providing potential application of these fungi to control water hyacinth in Vietnam because these are fungal genera with a wide host range.

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