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ASSESSMENT OF FUNGI AND VIRUSES IN ARTICHOKE (*Cynara scolymus* L.) IN DA LAT, LAM DONG PROVINCE

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SUMMARY

Artichoke (*Cynara scolymus* L.), a high economic value crop, was brought to Vietnam by the French in the late 19th century. The artichoke was mainly planted in Lam Dong, Lao Cai, Vinh Phuc provinces, etc. At present, the disease situation of Artichoke plants and the lack of disease-free seedlings result in insufficient source of Artichoke for producers. Artichoke plants are mainly vegetative propagation and pathogens easily transferred from mother to daughter plants. Therefore, low propagation rate and fungal infection are two main factors hindering the expansion and development of Artichoke cultivation (in Lam Dong). Therefore, studying and evaluating the situation of fungi and viruses as well as establishing the *in vitro* propagation procedures in order to produce high number of disease-free seedlings are urgent for the current Artichoke shortage. In this study, samples of purple and white Artichoke varieties, which suspected fungal manifestations, were collected to assessment of fungi and viruses in artichoke. In addition, *in vitro* propagation by cultivating apical meristem was applied to produce disease-free seedlings. The recorded results showed that, 19 strains of mold were identified on purple and white Artichoke belonged to nine genera including *Mucor* (*M.* sp., *M. circinelloides, M. fragilis, M. irregularis*, and *M. racemosus*), *Alternaria* (*Alternaria* sp., *A. alterinata, A. gaisen, A. tenuissima*, and *A. tillandsiae*), *Fusarium* (*F. acuminatum* and *F. solani*), *Cylindrobasidium* (*Cylindrobasidium* sp1 and *Cylindrobasidium* sp2), *Actinomucor elegans*, *Curvalaria clavata*, *Plectosphaerella oligotrophica*, *Phoma herbarum*, *Rhizomucor variabillis*; meanwhile, the *Tomato mosaic virus* (ToMV) was isolated only purple Artichoke. Shoot explants obtained from apical meristem culture were completely disease-free and used for micropropagation at the next stage.

Keywords: *Artichoke, disease-free, fungi, Tomato mosaic virus.*

INTRODUCTION

Artichoke (*Cynara scolymus* L.) belonged to the Asteraceae family, which is a high economic value crop, brought to Vietnam by the French in the late 19th century. Artichoke prefers cool weather all year round with a temperature of about 15 - 18°C (altitude of 1,000 - 1,500 m). Therefore, Artichoke is mainly grown in Da Lat (Lam Dong), Sapa, Muong Khuong (Lao Cai), Tam Dao (Vinh Phuc), etc. Initially, Artichoke included A75 variety (cultivated before 1975) and A80 (hybrid variety) planted since 1980. By 1985,

many new Artichoke varieties were researched and breeded, of which the A85 variety was highly appreciated. In addition, Artichoke varieties could be cultivated for 3 main harvesting purposes: flower only, leaf only and both flower and leaf (Lam Dong Agricultural Center, 2017). All plant parts (flower, leaf, stem, root, etc.) are valuable and could be used for many purposes .

The main active ingredient of Artichoke is cynarine (acid 1-3 dicafein quinic – $C_{25}H_{24}O_{12}.H_2O$). In addition, Artichoke also contains other chemical compositions such as inulin, inulinase, tannin, organic salts of metals (potassium, calcium, magnesium, sodium, etc.) as well as antioxidants including quercetin, rutin, anthocyanins, cynarine, luteolin, silymarin (Wang *et al*., 2003; Bundy *et al*., 2004; Wittemer *et al*., 2005). Tannin, flavonic heteroside (cyanoside) and an ether insoluble substance called scolymoside are found in fresh leaves. Particularly, the inflorescence contains protid (3%), lipid (0.1 - 0.3%), sugar (11 - 15.5%, mainly inulin sugar, needed for diabetics), vitamins A, B1, B2, C and manganese, phosphorus, iron minerals (Ministry of Health, 2009).

Due to their benefits for human health, the area of Artichoke plantation has been expanded to meet the growing needs of the farmers, to extract medicinal compounds, as well as to export. However, with the situation of Artichoke disease and the shortage of sources of disease-free seedlings, result in insufficient source of Artichoke for producers. In cultivated Artichoke varieties, about 25 viruses classified into 15 genera, belonging to 10 families of viruses that have been identified so far on samples of *C. scolymus* and *C. cardunculus* (Gallitelli *et al*., 2012). In addition, stunting (*Artichoke patchy chlorotic stunting*, APCS) was the most serious disease on Artichoke, especially in Greece (Kyriakopoulou, 1995). This disease is *Artichoke Italian latent nepovirus* (AILN), belongs Secoviridae family, which is spread by the *Longidorus fasciatus* Roca *et* Lamberti (Kyriakopoulou, 1995). In Vietnam in general and in Lam Dong in particular, *Bemisia argentifolii* and Aphid have been recorded as the two main pests on Artichoke. They affect the growth and development of plants that cause loss of productivity. In addition, leaf spot and wilt disease are common of Artichoke in Lam Dong (Lam Dong Agricultural Center, 2017).

Besides, the cultivated Artichoke varieties have a life span of more than 30 years ago, degraded varieties, low productivity and reduced quality. According to the farmers, only 1.5 - 2 kg of flower was obtained per tree recently, decreased more than 3 times compared to ten years ago. In order to overcome this problem, farmers and agriculture have paid much attention to the restoration of Artichoke in the past, but there have been no significant changes and the efficiency is not high and not yet synchronized. At present, the main source of Artichoke seedlings in Lam Dong is the actual Artichoke seedlings (seedlings imported from foreign countries like France). Vegetative propagation by axillary shoots is often used for propagation. However, the low propagation rate and the fungal infection are the two main factors hindering the expansion and development of Artichoke in the world through plant breeding. In addition, there are many varieties being planted in farmers' gardens. Farmers mainly produce young plantlet spontaneously or buy seed from seed production companies. Moreover, it is not proactive in seed source, quality and uniformity of breeding. Therefore, researching and assessing the situation of fungal diseases as well as establishing the *in vitro* propagation procedures in order to produce high number of disease-free seedlings are urgent for the current Artichoke pharmaceutical crisis.

MATERIALS AND METHODS

Plant samples

Samples of purple (P - 01; P - 02; P - 03; P - 04; P - 05; P - 06) and white (W - 07; W - 08; W - 09) Artichoke varieties, which suspected

fungal manifestations, were collected at Artichoke farmers' gardens in 12 Ward, Xuan Truong and Xuan Tho Commune (Da Lat, Lam Dong) to assess the situation of fungi and virus infections.

Meristem culture

Ex vitro plants (3-month old) collected from farmers' gardens in Da Lat were used as *in vitro* culture materials. The mother plant is pre-treated to collect shoots; after that, the meristem tissue was isolated from the shoots and cultured on MS medium (Murashuige, Skoog 1962) supplemented with 0.2 mg/L BA, 30 g/L sucrose and 8 g/L agar, pH 5.8.

The culture media were sterilized with autoclave at 121°C, 1 atm for 20 min. The explants were cultured *in vitro* under fluorescent light, lighting cycle 12h/day, temperature 25 ± 2°C and humidity 55 - 60%. Shoots were collected to assess the possibility of viral infection.

Fungi isolation

Physiological saline and sample cutting device were prepared. The leaf was washed with NaCl 0.09% twice, then cut the leaf in a rectangular shape with one side shows the sign of disease and one side shows no signs of disease. Samples were placed on PGA (Potato Glucose Agar) and WA (Watter Agar) media containing antibiotics in the incubator at 28°C for 48 hours.

Fungi morphology

After 48 hours incubating, the mycelium system was transferred to grow from isolated specimens into PGA medium and incubated at 28°C for 48 hours. After 48 hours, take out and record the fungal morphology.

Microscopic morphology of fungal and actinomycetes was observed after 24 h, 48 h, 72 hand 96 h under microscope. The mycelium system, structure and shape of spores and petiole were recorded.

Each obtained colony was isolates and examined morphological characteritics by slide (Proctor, 1977). The micro characteristics of each mycelial colony was observed under microscope with 1000× magnification.

Check for viruses

Common viruses on Artichoke were tested on field-collected samples using RT-PCR (Table 1). Nucleic acid extraction by CTAB1 (Weising *et al*., 2005) improved by adding 10% SDS to the extraction buffer and without RNA reduction.

DNA extraction and quality control

The quality and concentration of isolated DNA were checked as described (Weising *et al*., 2005). The optical density at 260 nm represented for DNA concentration and optical density ratio measured at two wavelengths 260 nm and 280 nm for DNA purity. DNA samples (kept at -20ºC) which achieve a purity (1.75 - 1.95) based on the OD260/OD280 ratio can be used for further research.

PCR amplification

A reaction volume (50 µL) including 5 µL My Red HS Taq mix (Bioline), 0.2 µM primer and about 30 ng DNA template were prepared. The amplification is performed on the Eppendorf thermocycler system (Eppendorf, Hamburg, Germany) with the heat program as follows: (1) Initial denaturation at 94°C for 5 min; (2) 36 cycles of 45 s of denaturation 94°C, 45 s of primer annealing at 50°C, and 1 min 30 s of primer extension at 72°C; (3) extends the circuit at 72ºC for 15 min.

Analysis of RT-PCR amplified products

The amplified product is electrophoresis to separate on 2% Agarose gel using TBE buffer for 3 h at 60 V. Then, the sample was stained with ethidium bromide (0.5 µg/mL), photographed under light with a wavelength of 254/312 nm on UVP Gel Studio Plus System (Analitik Jena, Germany).

Table 1. Testing of viruses on Artichoke samples collected in the field.

No.	Type of viruses	Primers 5' – 3'	Amplicon size (bp)	References
1	*Artichoke Italian latent nepovirus* (AILV)	ATTCACTAGTCCCTATTTAG	769	Minutillo *et al*., 2012
2	*Artichoke mottled crinkle tombusvirus* (AMCV)	ATGGCAATGGTAAAGAGAAA	553	Minutillo *et al*., 2012
3	*Artichoke latent potyvirus* (ArLV)	TTGTTCATAAGGGAGCGCGT	499	Minutillo *et al*., 2012
4	*Clover yellow vein virus (*CYVV)	CATTCCAGACAGAGACATCAATGCAG	750	Bariana, 2016
5	*Tomato spotted wilt virus* (TSWV)	AGCTAACCATGGTTAAGCTCACTAAGGAAAGC	760	Sivparad and Gubba, 2008
6	*Cucumber mosaic virus* (CMV)	GTTTATTTACAAGAGCGTACGG	657	Kumar *et al*., 2008
7	*Turnip mosaic virus* (TuMV)	ATTCCTGATACACGCTCCGAGAGCA	986	Sanchez *et al*., 2003
8	*Potato virus X* (PVX)	AAG CCT GAG CAC AAA TTC GC	101	Sanchez *et al*., 2003
9	*Tomato mosaic virus* (ToMV)	GAA AGC GGA CAGAAA CCC GCT G	508	Silva *et al*., 2008

RESULTS AND DISCUSSION

In vitro propagation

Apical meristem tissue with 0.1 – 0.2 mm in size (Fig. 1A, B) derived from *ex vitro* plant was obtained. Then, apical meristem was cultured on shoot regeneration medium in 8 weeks (El-Zeiny *et al*., 2013). Those shoots of purple and white Artichoke were obtained (Fig. 1C, D) and checked for viruses.

Currently, Artichoke was propagated by three main methods: (1) separating seedlings from the mother plant (most common), (2) seedling and (3) plantlet from tissue culture (at least). Besides, some companies have imported Artichoke seeds from foreign countries such as France, USA, etc. However, the propagation is still spontaneous, not synchronized and there is no long-term strategy in controlling seed sources. Therefore, seedlings are of poor quality and carry pathogens (Lam Dong Agricultural Center, 2017).

Besides the low propagation rate, the possibility of fungal infection is the main factor hindering the expansion and development of Artichoke in Lam Dong province as well as in Vietnam. Pests such as *Bemisia argentifolii* and Aphid, are 2 main pests of Artichoke, which affect the growth and development of plants due to reducing the yield. The main previously reported diseases are leaf spot (caused by *Ramularia cynarae*) and wilt disease (caused by *Verticillium dahliae*) which are common of Artichoke in Lam Dong (Lam Dong Agricultural Center, 2017). However, there have been no recorded results on the impact of the origin of harmful microorganisms. The

results of this study will provide more information on the situation of fungal diseases of artichokes in Da Lat (Lam Dong) in order to be more proactive in control measures as well as towards restoring artichoke plantlets by *in vitro* propagation.

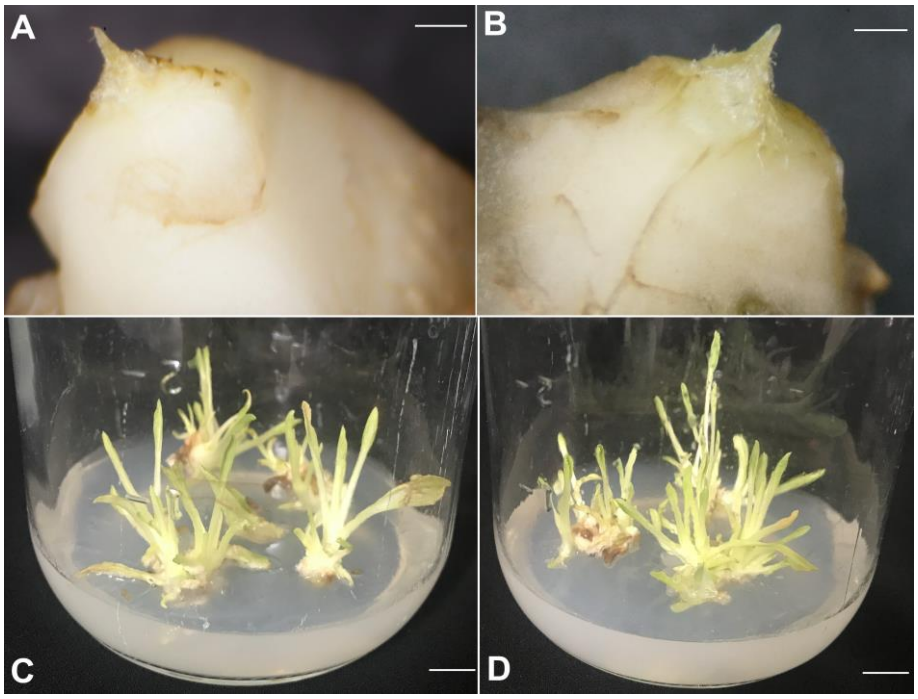

Figure 1. Artichoke's shoots derived from apical meristem culture. **A:** Purple apical meristem; **B:** White apical meristem; **C:** Purple shoots ; **D:** White shoots. *Bars: 0.2 mm (upper panel); 10 mm (lower panel*).

Fungi isolation

Through the process of sample collection in the field, the results have recorded a number of samples showing fungal manifestations for analysis on two varieties of purple and white Artichoke (Fig. 2). Stems, leaves, roots with fungal manifestations of purple and white Artichoke varieties were used to isolate and identify fungi. We isolated 19 strains of mold belonged to 09 genera including *Mucor* (5 species: *M.* sp., *M. circinelloides*, *M. fragilis*, *M. irregularis*, and *M. racemosus*) (Fig. 3), *Alternaria* (5 species *A.* sp., *A. alterinata*, *A. gaisen*, *A. tenuissima*, and *A. tillandsiae*) (Fig. 4), *Fusarium* (2 species *F. acuminatum* and *F. solani*), *Cylindrobasidium* (2 species*: C.* sp1 and *C.* sp2) (Fig. 5), and *Actinomucor elegans*, *Curvalaria clavata*, *Plectosphaerella oligotrophica*, *Phoma herbarum*, *Rhizomucor variabillis* (Fig. 6).

The result showed that fungal infection in artichokes depends on the varieties. *Mucor* sp. and *Fusarium* sp. were obtained from both varieties of Artichokes. *Curvalaria clavata*, *Phoma herbarum* and *Rhizomucor variabillis* were identified from white Artichoke meanwhile, *Actinomucor elegans*, *Plectosphaerella oligotrophica* were isolated from purple Artichoke (Table 2).

Besides the low propagation rate, the risk of fungal infection is the main factor hindering the expansion and development of Artichoke in Lam Dong province as well as in Vietnam. *Bemisia argentifolii* and Aphid are the two main harmful insects of Artichoke. They affect the growth and development of plants; therefore,

can affect the yield of harvests. The main preventive measures are plowing and drying soil thoroughly before cultivating, cleaning gardens, creating ventilation to restrict their habitats, etc. or using chemical methods (Lam Dong Agricultural Center, 2017).

Currently, leaf spot and wilt diseases are common on Artichoke in Lam Dong. Residues of diseased plants far from the farming area are regularly cleared. Plants are grown in high, well-ventilated, well-drained areas as well as adequately fertilized to enhance plant resistance. The disease-free varieties problem should be solved. Therefore, the source of disease-free seedlings with good growth, development and adaptation to local climatic conditions is an urgent need for seeds that will need to be addressed (Lam Dong Agricultural Center, 2017).

Figure 2. Some fungal manifestations on Artichoke plants collected in the field. **A**, **B**, **C**: Purple Artichoke; **D**, **E**, **F**: White Artichoke. *Bar: 2 cm.*

Table 2. Colony characteristics and mold cell morphology isolated on artichoke

No	Strains	Bacterial colony	Cell morphology	Classification
1	P – 01 N1	Mycelium: thin and grows close to the agar surface Colonies diameter: 2 cm (72 h)	Hyphae: branching, no partition; long convex stems without walls, fungal spore-shaped follicles, oval-shaped spores	*Mucor fragilis*
2	P – 01 N2	Mycelium: thin, grows close to the agar surface, light orange-yellow (center) Colonies diameter: 2.3 cm (48	Hyphae: slender, branched, nonspecific septum (r = 29.5 – 37.7 µm), no spores appear	*Alternaria alterinata*

		h) and 3.5 cm (72 h)		
3	P – 01 N3	Mycelium: thick, white, porous and protruding Colonies diameter: 2.5 cm (48 h) and 4.0 cm (72 h)	Hyphae: baffled (l = 53 – 89 µm, r = 10 – 17 µm) Spore stem: long, walled (15 µm) Spores: long, spore tip up (crescent)	*Fusarium solani*
4	P – 01 D1	Mycelium: thin, grows high, light yellow (center), black spores Colonies diameter: 4.0 cm (48 h) and 9.0 cm (72 h)	Follicular spores: cone-shaped, long sporoid, without septum Spore oval (l = 14.22 µm, r = 6.95 µm)	*Mucor fragilis*
5	P – 01 D2	Mycelium: thin, grows high, light yellow in the center Colonies diameter: 2.0 cm (48 h) and 6.0 cm (72 h)	Main hyphae: large (r = 28 – 30 µm), branched to smaller, non-baffled branches Spore: cone-shaped, long, non-septal spleen, oval spores	*Mucor* spp.
6	P – 01 D3	Mycelium: thick, white and grows high, Colonies diameter: 1.5 cm (48 h) and 2.2 cm (72 h: black and gray)	Hyphae: branched, without baffles (r = 7.5 – 8.2 µm). Spores: oval, tapered at both ends (l = 13.2 – 13.5 µm, r = 2.9 – 4.1 µm) Spore stem: short (l ~ 37 µm) without septum	*Alternaria* spp.
7	P – 01 L1	Mycelium: thick, gray-green and grows on the surface of agar Colonies diameter: 2.0 cm (48 h) and 5.5 cm (72 h: black and gray)	Hyphae: thin, branched partitioned walls (l = 119 – 189 µm) Spore stem: branched, walled (l = 53 – 62 µm) Spore pouches: spherical, with dividing walls	*Alternaria gaisen*
8	P – 01 L2	Mycelium: white, smooth, porous, thick and grows above the jelly surface Colonies diameter: 2.0 cm (48 h) and 6.0 cm (72 h)	Hyphae: thin (r = 18 – 22 µm), baffled (l = 125 – 169 µm). Spore: sickle shaped	*Cylindrobasidium* sp.
9	P – 03 N1	Mycelium: thin, white and high Colonies: cotton, porous, pale moss green color	Hyphae: branched, without baffles Spore: oval, long stalk	*Mucor irregularis*
10	P – 03 G1	Mycelium is thin, white and high Colonies: cotton, porous	Hyphae: branched, baffled Spore: oval	*Actinomucor elegans*
11	P – 03 L1	Mycelium: thin and white Colonies: round, growing on the surface of agar	Hyphae: branched, with baffles Spore: slightly oval, short stalk	*Mucor racemosus*
12	P – 03 D1	Mycelium: white, sprouting above the agar surface Colonies: round	Hyphae: branched, without baffles, spherical reproductive organs Spores: spherical, sporangia with protruding spines	*Mucor circinelloides*
13	P – 05 L1	Colonies: white, porous, orange-brown Colonies diameter: 1.5 cm (72 h)	Hyphae: branched, without baffles (r = 4.2 – 12.8 µm) Spore: oval (l = 14.2 – 17.4 µm, r = 4.7 – 6.2 µm), growing in clusters of 2 – 3 spores	*Plectosphaerella oligotrophica*
14	P – 05 L2	Mycelium: white, porous, thin filament center, thick and porous filament	Hyphae: branched, not baffled (r = 5 – 11.6 µm). Spores: long, tapering rods at both	*Cylindrobasidium sp.*

		Colonies diameter: 3.2 cm (72 h)	ends (l = 13.4 – 17.7 µm, r = 5.4 – 7.2 µm), short-stemmed spores, at the position of attaching smaller spores	
15	P – 06 L1	Mycelium: thick, white, grows protruding, black pigment, white filamentous colonies (center), black filamentous (border) Colonies diameter: 1.0 cm (48 h) and 3.5 cm (72 h)	Hyphae: branched, partitioned (l = 109.2 – 146.3 µm, r = 6.3 – 7.8 µm) Spores: oval, tapered at the ends, clustered around the hyphae (l = 32.4 – 33.6 µm, r = 11.5 – 14.9 µm)	*Alternaria tenuissima*
16	P – 06 L2	Mycelium: thick, yellowish-white, protruding, black and gray pigmented (border) and pink (center) Colonies diameter: 1.5 cm (48 h)	Hyphae: thin, branched, baffled (l = 45 – 58 µm), no spores present	*Alternaria tenuissima*
17	P – 06 L3	Mycelium: thick, white, black pigment secretion Colonies diameter: 1.2 cm (48 h) and 2.5 cm (72 h: black gray (center), white sponge (border))	Hyphae: thin, branched, baffled (l = 110 – 115 µm, r = 20 – 29 µm) Spore: cylindrical, slightly rounded at both ends (spore pointed at the tip) (l ~ 226.37 µm, r ~ 72.99 µm) Spore stem: long, bulging at the end of contact with spore, with septum	*Alternaria tillandsiae*
18	W – 07 L1	Mycelium: thin, white, protruding, creating black pigment Colonies diameter: 2.0 cm (48 h) and 5.0 cm (72 h: black gray)	Hyphae: thin, branched, baffled (l = 77.5 – 87.4 µm, r = 7.8 – 11.2 µm) Spore: oval, tip slightly pointed (l = 33 – 42 µm, r = 18.7 – 20.9 µm) Spore stem: long, branched, attached 2-3 spores / stem	*Curvalaria lavate*
19	W – 07 L2	Mycelium: thick, white, grows close to the surface of the medium, producing pink gold pigment Colonies diameter: 3.0 cm (48 h) and 6.0 cm (72 h: yellow (center), pink (next round) and white (border)	Hyphae: thin, branched, baffled (l = 141.8 – 147.07 µm, r = 19.3 – 21.3 µm), tend to be curled into filaments, without the presence of spores	*Fusarium acuminatum*
20	W – 08 L1	Mycelium: white, thick, porous, rising high on the surface of agar Colonies diameter: 1.5 cm (48 h) and 2.5 cm (72 h)	Hyphae: branched, baffled, at the branching site where there is a bulge (such as burning bamboo, l ~ 57.6 µm, r ~ 7.38 µm), no spores appear	*Phoma herbarum*
21	W – 08 N2	Mycelium: thin, white, reaching high, black spores. Colonies: cottony, porous, yellowish (central) Colonies diameter: 2.5 cm (48 h) and 4.0 cm (72 h)	Main hyphae: large in size, smaller in branching and baffled Spore: oval, spherical; peduncle has no baffle	*Rhizomucor variabillis*
22	W – 09 G1	Mycelium: white, flakes close to the surface of the agar Colonies diameter: 4.5 cm (72 h)	Hyphae: branched, baffled Spore: rhomboid, tapered at both ends, 5 -10 spores at a site.	*Mucor circinelloides*

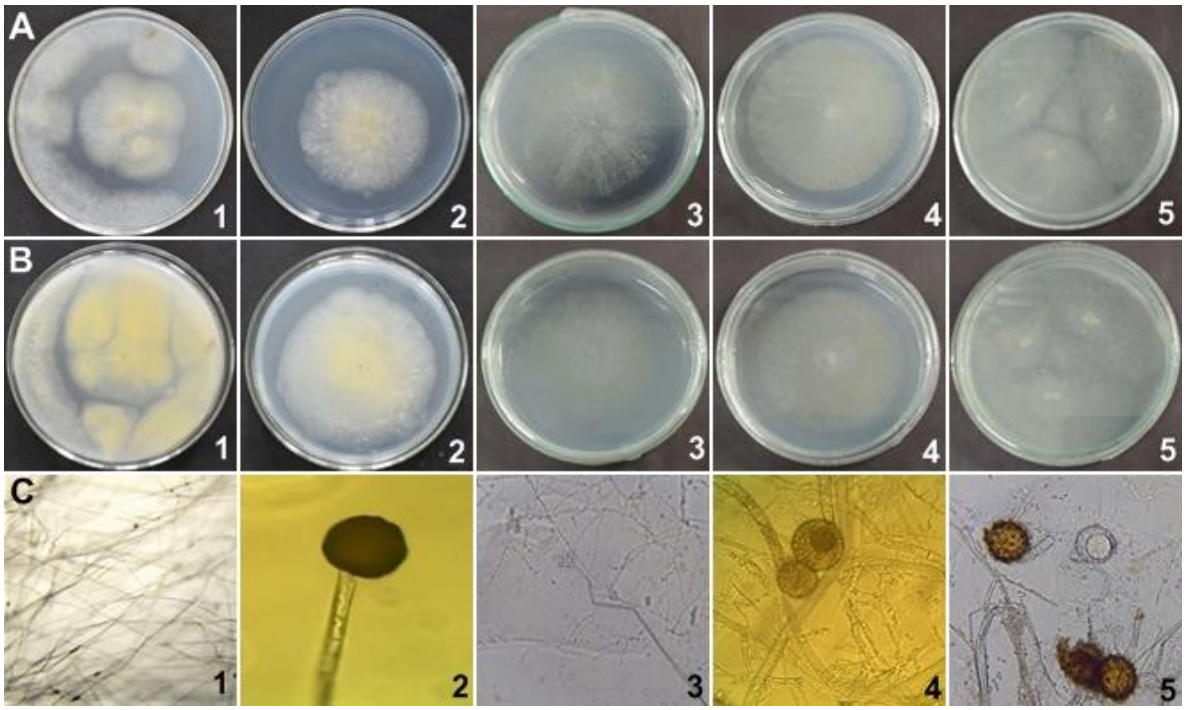

Figure 3. Five *Mucor* species isolated from purple and white Artichoke samples. **A, B:** Colonies (front and back); **C:** Mycelium; **1:** *Mucor* sp.; **2:** *M. circinelloides*; **3:** *M. fragilis*; **4:** *M. irregularis*; **5:** *M. racemosus*.

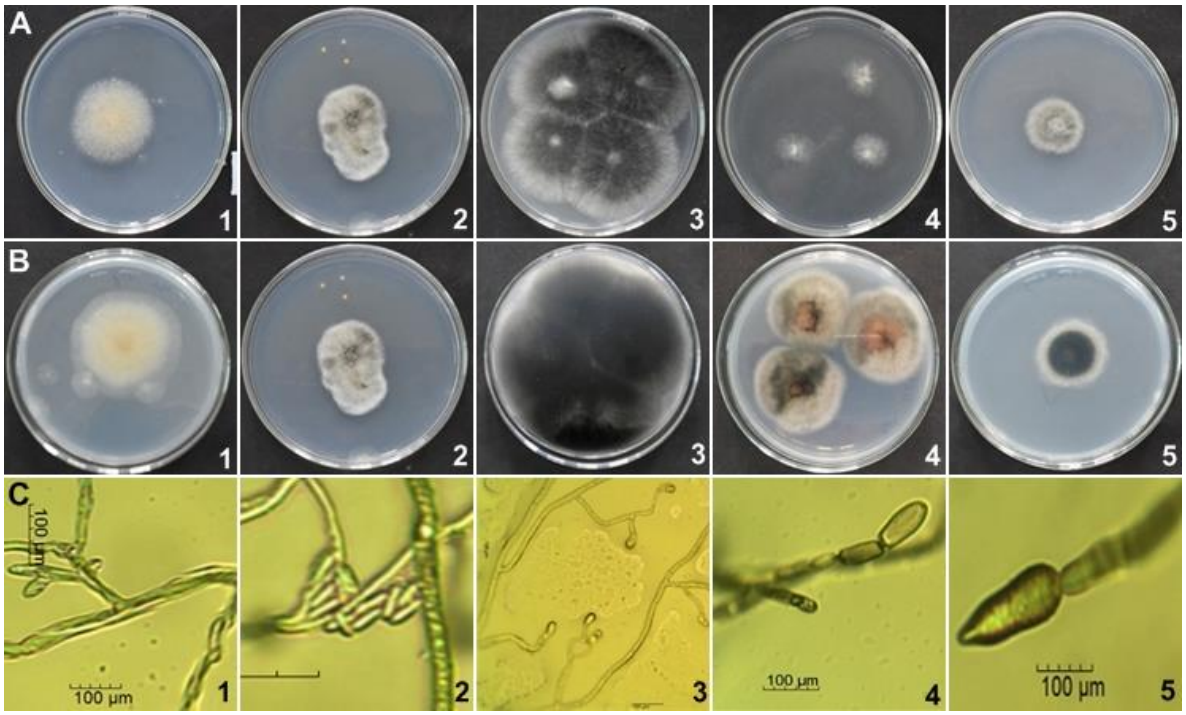

Figure 4. Five *Alternaria* species isolated from purple and white Artichoke samples. **A, B:** Colonies (front and back); **C:** Mycelium; **1:** Alternaria sp.; **2:** *A. alterinata*; **3:** *A. gaisen*; **4:** *A. tenuissima*; **5:** *A. tillandsiae*.

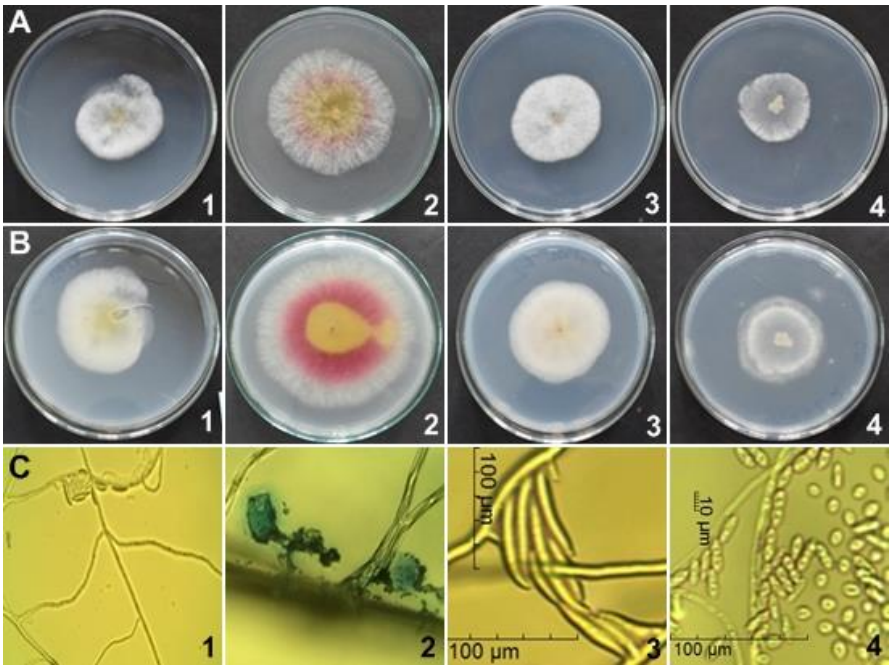

Figure 5. *Fusarium* and *Cylindrobasidium* species isolated from purple and white Artichoke samples. **A, B:** Colonies (front and back), **C:** Mycelium; **1:** *Fusarium acuminatum*; **2:** *F. solani*; **3:** *Cylindrobasidium* sp1; **4:** *Cylindrobasidium* sp2.

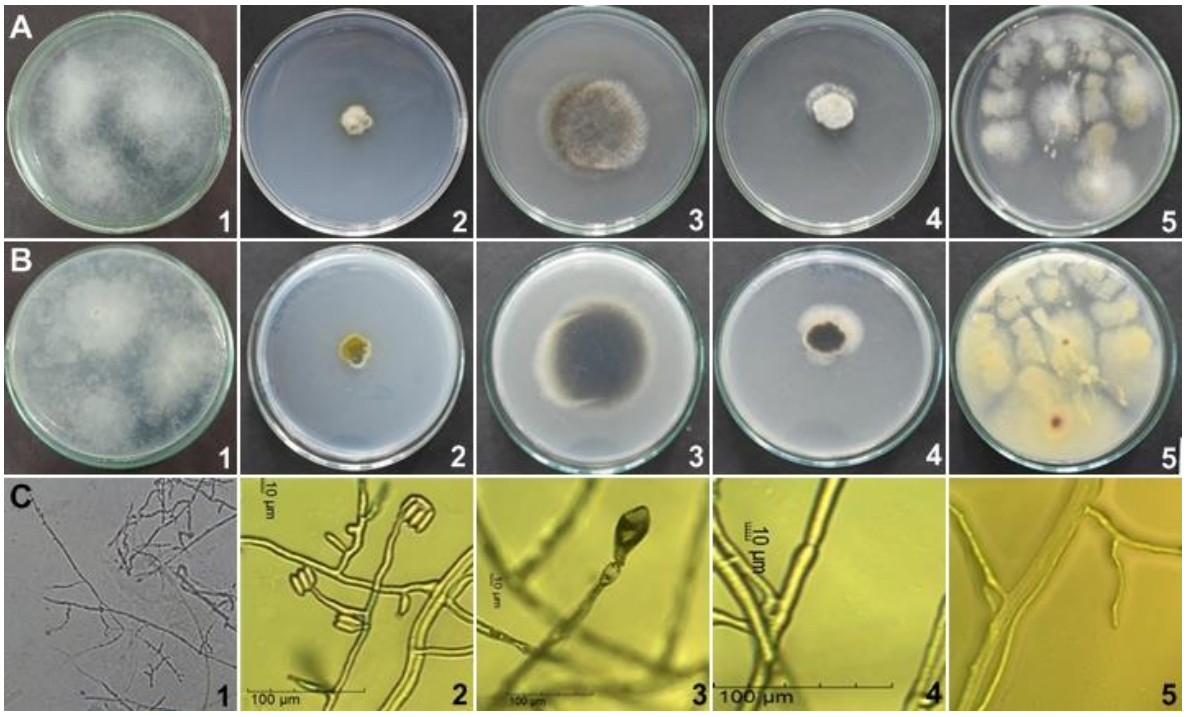

Figure 6. Other fingi species isolated from purple and white Artichoke samples. **A, B:** Colonies (front and back); **C:** Mycelium; **1:** *Actinomucor elegans*; **2:** *Plectosphaerella oligotrophica*; **3:** *Curvalaria clavata*; **4:** *Phoma herbarum*; **5:** *Rhizomucor variabillis.*

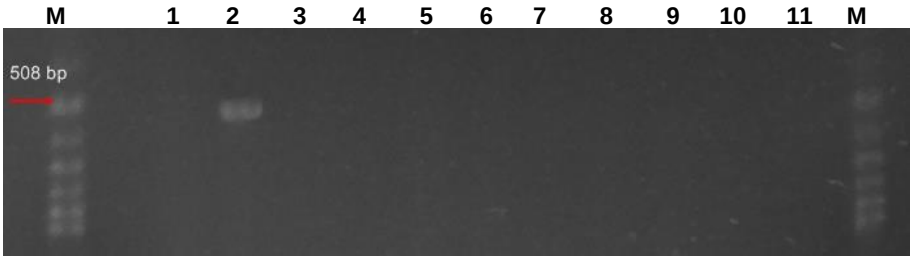

Figure 7. *Tomato mosaic virus* (ToMV) checked by RT-PCR. Purple Artichoke (**1:** P – 01; **2:** P – 02; **3:** P – 03; **4:** P – 04; **5:** P – 05; **6:** P – 06) and white Artichoke (**7:** W – 07; **8:** W – 08; **9:** W – 09), **10:** *in vitro* purple plantlets; **11:** *in vitro* white plantlets.

Check for viruses

Genomic DNA was used as template for RT-PCR to test the presence of 9 viruses (AILV, AMCV, ArLV, CYVV, TSWV, CMV, TuMV, PVX, ToMV) on Artichoke (Table 1). The electrophoresis result showed that only purple artichoke field samples (P **-** 02) were infected with ToMV at a mild level (Fig. 7). All other samples showed free-virus infection of all 9 investigated viruses. The results of this study showed that Artichokes in Lam Dong have not recorded common viruses (except for TMoV in purple varieties). The current low Artichoke production may be due to the fact that current varieties have been cultivated for a long time leading to reduced yields. Therefore, it is necessary to take appropriate breeding measures to improve the current Artichoke yield and quality. Restoration of Artichoke variety via *in vitro* culture (apical meristem culture) can create disease-free and good quality plantlets.

Research on viral diseases in Artichoke has been interesting since about the 1960s. Artichoke virus causes dwarfism, and curly leaves (Morton, 1957) and the latent Artichoke S virus are the two earliest detected viruses. In the next stage, a series of virus causing disease was identified as AILV; ACMV; CMV; TSWV; ToMV; *Cynara* virus, etc. were also isolated from Artichoke disease samples from around the world, respectively (Gallitelli *et al*., 2012). To date, about 25 viruses, classified into 15 genera, belonging to 10 families of viruses have been found on samples of *C. scolymus* and *C. cardunculus* (Gallitelli *et al*., 2012). Stunting (APCS) is the most serious disease in Artichoke (Kyriakopoulou, 1995). Recently, the next generation sequencing technology (NGS) has developed, allowing for complete sequencing of AILN's RNA-1 and RNA-2 molecules (Elbeaino *et al*., 2017).

CONCLUSION

In this study, the results showed that, 19 strains of mold were recorded from 9 samples of white and purple Artichoke varieties. Belonged to 5 *Mucor* species (*Mucor* sp., *M. circinelloides, M. fragilis, M. irregularis*, *M. racemosus*), 5 *Alternaria* species (*Alternaria* sp., *A. alterinata, A. gaisen, A. tenuissima*, *A. tillandsiae*), 2 *Fusarium* species (*F. acuminatum* and *F. solani*), 2 *Cylindrobasidium* species (*Cylindrobasidium* sp1 and *Cylindrobasidium* sp2), *Actinomucor elegans, Curvalaria clavata, Plectosphaerella oligotrophica, Phoma herbarum* and *Rhizomucor variabillis.* In addition, among 9 investigated virus types only ToMV were found in purple Artichoke. The shoots derived from apical meristem culture were free-viruses and used for micropropagation studies at the next stage.

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ĐÁNH GIÁ VỀ NẤM VÀ VIRUS TRÊN CÂY ARTICHOKE (*Cynara scolymus* L.) TẠI ĐÀ LẠT, TỈNH LÂM ĐỒNG

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TÓM TẮT

Artichoke (*Cynara scolymus* L.) là một loại cây có giá trị kinh tế cao được người Pháp đưa vào Việt Nam vào cuối thế kỷ 19. Artichoke được trồng chủ yếu ở các tỉnh Lâm Đồng, Lào Cai, Vĩnh Phúc, v.v. Hiện nay, tình trạng bệnh hại trên cây Artichoke và sự thiếu hụt nguồn cây giống sạch bệnh dẫn đến nguồn nguyên liệu Artichoke vẫn không đủ để cung ứng cho các nhà sản xuất. Nguồn cung cấp giống Artichoke tại Lâm Đồng chủ yếu là cây giống thực sinh và cây có nguồn gốc từ chồi nách. Tuy nhiên, tỷ lệ nhân giống thấp và khả năng lây nhiễm nấm, virus là hai yếu tố chính cản trở việc mở rộng và phát triển của Artichoke. Do vậy, việc nghiên cứu đánh giá tình hình nấm bệnh và phục tráng giống nhằm tạo cây giống sạch bệnh với số lượng lớn là việc làm hết sức cần thiết trước tình trạng thiếu hụt giống Artichoke như hiện nay. Trong nghiên cứu này, những mẫu nghi ngờ có biểu hiện nấm bệnh trên giống Artichoke tím và trắng được thu nhận tại vườn của các hộ nông dân trồng Artichoke tại Đà Lạt và vùng phụ cận được thu nhận nhằm đánh giá tình hình nấm, virus cũng như đưa ra phương pháp nuôi cấy đỉnh sinh trưởng nhằm phục tráng giống sạch bệnh. Kết quả ghi nhận được cho thấy, trên giống Artichoke tím và trắng tại vườn ươm đã phân lập được 19 loại nấm bệnh thuộc các nhóm: 5 loài thuộc chi *Mucor* (*Mucor* sp., *M. circinelloides, M. fragilis, M. irregularis, M. racemosus*), 5 loài thuộc chi *Alternaria* (*Alternaria* sp., *A. alterinata, A. gaisen, A. tenuissima, A. tillandsiae*), 2 loài thuộc chi *Fusarium* (*F. acuminatum* and *F. solani*), 2 loài thuộc chi *Cylindrobasidium* (*Cylindrobasidium* sp1 và *Cylindrobasidium* sp2), và các loài khác như *Actinomucor elegans, Curvalaria clavata, Plectosphaerella oligotrophica, Phoma herbarum, Rhizomucor variabillis*. Ngoài ra, *Tomato mosaic virus* (ToMV) chỉ được phân lập trên giống tím. Mẫu chồi thu nhận từ nuôi cấy đỉnh sinh sinh trưởng là hoàn toàn sạch bệnh và được sử dụng cho các nghiên cứu vi nhân giống ở giai đoạn tiếp theo.

Từ khóa: *Artichoke, nấm bệnh, sạch bệnh, Tomato mosaic virus.*