Occurrence of fungal endophytes from *Hevea brasiliensis* leaves in Laguna, Philippines

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Zusammenfassung: Endophytische Pilze aus Blättern von Kautschuk, *Hevea brasiliensis* wurden untersucht und die Isolierungshäufigkeit wurde ausgewertet. *Talaromyces* wurde sowohl von jungen mit 41 % als auch von reifen Blättern mit 22 % am häufigsten isoliert. Es folgte *Nigrospora* mit 20,67 % und 4,67 % von jungen bzw. reifen Blättern. *Penicillium* wurde ebenfalls mit einer hohen Isolationshäufigkeit in jungen Blättern (13,33 %) registriert. Darüber hinaus sind Endophyten an der Immunität der Wirtspflanzen gegen Pathogene und pflanzenfressende Insekten beteiligt. Dies weckt Interesse an der Möglichkeit, dass Endophyten das Überleben der Wirtspflanze verbessern und die Möglichkeit, diese endophytischen Organismen für das Management von Gummibaumkrankheiten auf den Philippinen nutzbar zu machen.

Abstract: Endophytic fungi from foliage of rubber, *Hevea brasiliensis* were examined and isolation frequency was evaluated. It was observed that *Talaromyces* showed the highest percentage of isolation frequency from both young (41 %) and mature (22 %) leaves. This was followed by *Nigrospora* with 20.67 % and 4.67 % from young and mature leaves, respectively. *Penicillium* also recorded with high isolation frequency in young leaf (13.33 %). In addition, endophytes are involved in host plant immunity against pathogens and herbivorous insects. This gain interest for the possibility that endophytes enhance survival of the host plant and the possibility of harnessing these endophytic organisms for the benefit of rubber disease management in the Philippines.

Natural rubber, *Hevea brasiliensis* (WILLD.) MÜLL. ARG., originating from Amazon watershed in South America, is an important crop worldwide. GAZIS & CHAVERI (2010), identified a diversity of fungal endophytes in leaves and stems of wild rubber trees in Peru. Their study showed that not only leaf tissue could harbor a variety of endophytes, but also the sapwood contained an even more diverse assemblage.

Endophytic microbes are an intriguing group of organisms associated with various tissues and organs (STONE & al. 2000). The symbiotic associations between microorganisms and plants are ancient and fundamental, and highly specific between plants and microbes. The use of beneficial fungi has gained significant attention worldwide due to their remarkable antagonistic properties against fungal pathogens (GHORBANPOUR & al. 2018). Endophytes are known to enhance host growth and nutritional acquisition. They enhance plants' ability to tolerate biotic and abiotic stresses and boost the resistance against insects and pests. They produce phytohormones and other bioactive compounds of biotechnological interest (JOSEPH & PRIYA 2011).

Generally, endophytes may play important beneficial roles in the metabolism and physiology of the host plant such as fixing atmospheric nitrogen (DALTON & al. 2004), solubilizing phosphates (FORCHETTI & al. 2007), synthesizing growth hormones (HARDOIM & al. 2008), degrading toxic compounds (SHENG & al. 2008), inhibiting strong fungal activity (BROOKS & al. 1994), and antagonizing bacterial pathogens (VAN BUREN & al. 1993).

Endophytes were the subject of researches over the past decades, due to their many benefits to the host plant. They also serve as source of secondary metabolites for regulation of insect pest and pathogens. GIMENEZ & al. (2007) added that the role of endophytes in plant-pathogen and plant-insect interactions is receiving increasing attention because of its potential use in pest control.

Materials and methods

Site of collection: Healthy looking rubber leaves without any insect damage were collected from Mount Makiling Forest Reserve, College of Forestry and Natural Resources, University of the Philippines Los Baños. The trees chosen based on four criteria: healthy appearance, overall good physiological state, and free from any type of chemical/biological product application. A total of 100 apparently heathy leaves were picked from 10 randomly selected trees in the lower canopy. Collected leaves were blotted dry to remove excess moisture and placed in clean polyethylene, and isolation was done on the same day.

Isolation of endophytic fungi: Collected leaves were washed in flowing tap water. The leaflets were divided into three segments: the apical, middle, and basal segments (Figure 1). Four 6-mm leaf-discs were punched from each segment. Each disc was sterilized following the protocol of GAZIS & CHAVERRI (2010) with modifications. Sterilization was undertaken inside a laminar flow hood to ensure an aseptic process. Each disc was sterilized by sequential washes in 2% sodium hypochlorite for 2 mins, 70% ethanol for 2 mins, rinsed with distilled water twice.

The sterilized leaf-disc samples were placed in a sterilized blotting paper to remove excess moisture, and then placed in petri dish containing PDA. Each plated PDA had 4 leaf-disc samples, sealed by parafilm, and incubated at 26°C, while maintaining dark-light cycle. Growth of endophytic fungi from the leaf tissues were observed daily. Each disc showing growth were recorded and counted. Each fungal growth was placed onto fresh PDA slants and allowed to grow in preparation for identification.

Identification of endophytic fungi: Codes were assigned to the isolated endophytic fungi. All endophytic fungi isolates were sent to Macrogen Korea for DNA sequencing and molecular identification using internal transcribed spacer (ITS) region as universal DNA barcode marker for fungi. The pure culture of endophytic fungi on PDA slants were preserved for future activities.

Determination of endophytes solation frequency per segment: The isolation frequency (IF) of a single endophyte taxon will be calculated using the formula from HATA & al. (2002):

$IF= (Ni/Nt) \times 100$

where: Ni - the number of disc from which the fungus was isolated

Nt – the total number of disc examined

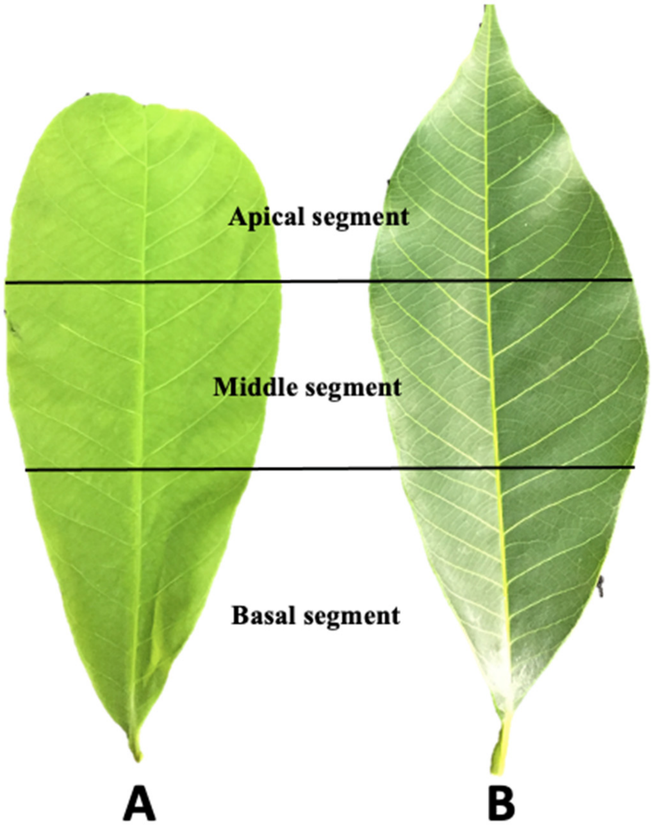

Fig. 1. *A* Young leaf and *B* mature leaf that were divided into three segments: apical, middle, and basal segments.

Results

Endophytic fungi found on apparently healthy rubber leaves were isolated. Isolation frequency and percent occurrence were noted on different leaf segments: the apical, middle, and basal leaf segments. Isolation frequency and percent occurrence of different genera of fungal endophytes on young and mature leaves were compared.

A total of 36 fungal endophytes were isolated from mature and young leaves (Tab. 1).

Tab. 1. Endophytic fungi obtained from rubber leaves. All taxa are *Ascomycota* except *Phanerochaete* and *Phlebia*, which are *Basidiomycota*.

Fungal endophytes	Pathogen code	[1]NCBI Accession no.	[2]Depository accession no.
A. Young leaves			
Talaromyces funiculosus (THOM) SAMSON, YILMAZ, FRISVAD & SEIFERT	BLA-YUO1	LT558961	MCC-MNH-2542
Nigrospora sp.	BLA-YUO2	JF694931	MCC-MNH-2543
Aspergillus tubingensis MOSSERAY	BLA-YUO3	KX664371	MCC-MNH-2544
Phanerochaete chrysosporium BURDS.	BLA-YUO4	AB361644	MCC-MNH-2545
Aspergillus fumigatus FREESENIUS	BLA-YUO5	KU847848	MCC-MNH-2546
Blakeslea trispora THAXTER	BLA-YOU6	HQ248186	MCC-MNH-2547

Tab. 1. Continued.

Aspergillus intermedius BLASER	BLA-YMO7	KX696385	MCC-MNH-2548
Penicillium sp.	BLA-YUO8	KP003825	MCC-MNH-2549
Penicillium sp.	BLA-YMO9	KP003825	MCC-MNH-2550
Penicillium sp.	BLA-YMO10	KP003825	MCC-MNH-2551
Penicillium citrinum THOM	BLA-YLO11	KX958025	MCC-MNH-2552
Penicillium citrinum	BLA-YLO12	KX664347	MCC-MNH-2553
Aspergillus flavus LINK	BLA-YLO13	KXO67886	MCC-MNH-2554
B. Mature leaves			
Talaromyces funiculosus	BLA-MUO1	LT558961	MCC-MNH-2555
Talaromyces funiculosus	BLA-MOU2	LT558961	MCC-MNH-2556
Daldinia eschscholtzii (EHRENB.) REHM	BLA-MOU3	KP050578	MCC-MNH-2557
Nodulisporium sp.	BLA-MLO4	JF314510	MCC-MNH-2558
Aspergillus niger VAN TIEGHEM	BLA-MLO5	KF881765	MCC-MNH-2559
Nigrospora sp.	BLA-MLO6	KT192335	MCC-MNH-2560
Nigrospora oryzae (BERK. & BROOME) PETCH	BLA-MLO7	KT224878	MCC-MNH-2561
Aspergillus tubingensis	BLA-MUO9	KX664316	MCC-MNH-2562
Phomopsis sp.	BLA-MLO10	GU066650	MCC-MNH-2563
Nigrospora sp.	BLA-MLO11	JF694936	MCC-MNH-2564
Diaporthe ceratozamiae CROUS & SHIVAS	BLA-MMO13	KX866903	MCC-MNH-2565
Nodulisporium sp.	BLA-MLO14	JF314510	MCC-MNH-2566
Colletotrichum gloeosporioides PENZ. & SACC.	BLA-MUO15	FJ172224	MCC-MNH-2567
Guignardia sp.	BLA-MUO16	KX611672	MCC-MNH-2568
Aspergillus sp.	BLA-MLO17	KJ528990	MCC-MNH-2569
Acremonium brunnescens LINK	BLA-MLO18	KF993389	MCC-MNH-2570
Nigrospora sp.	BLA-MMO19	KU504314	MCC-MNH-2571
Phlebia acerina PECK.	BLA-MLO20	KU059900	MCC-MNH-2572
Penicillium citrinium	BLA-MLO21	KX958025	MCC-MNH-2573
Aspergillus oryzae (AHLBURG) COHN	BLA-MLO22	KP172534	MCC-MNH-2574
Penicillium citrinium	BLA-MMO23	KM979730	MCC-MNH-2575
Arthrinium phaeospermum (CORDA) ELLIS	BLA-MLO24	JN198505	MCC-MNH-2576
Nigrospora oryzae (BERK. & BROOME) PETCH	BLA-MLO25	HQ608062	MCC-MNH-2577

[1] National Center for Biotechnology Information (NCBI), U.S. National Library of Medicine
[2] Museum of Natural History (MNH)-University of the Philippines-Los Baños (UPLB)

The occurrence of endophytes on segments of both young and mature leaves differ. On mature leaves, most endophytic fungi (30.36 %) occurred on the basal segment. Fungal presence on the middle segment was 19.64 % and 12.5 % on the apical segment. Likewise, in young leaves, highest occurrence of endophytic fungi was obtained in apical segment (19.64 %). The fungi were also present on the basal segment (10.71 %) and on the middle segment (7.14 %). It was revealed further that endophytes were generally prevalent in mature leaves irrespective of the leaf segments. With regard to the leaf age, the result of the study revealed that mature leaves have the most variety in endophytic fungi composition compared to young leaves (Fig. 2).

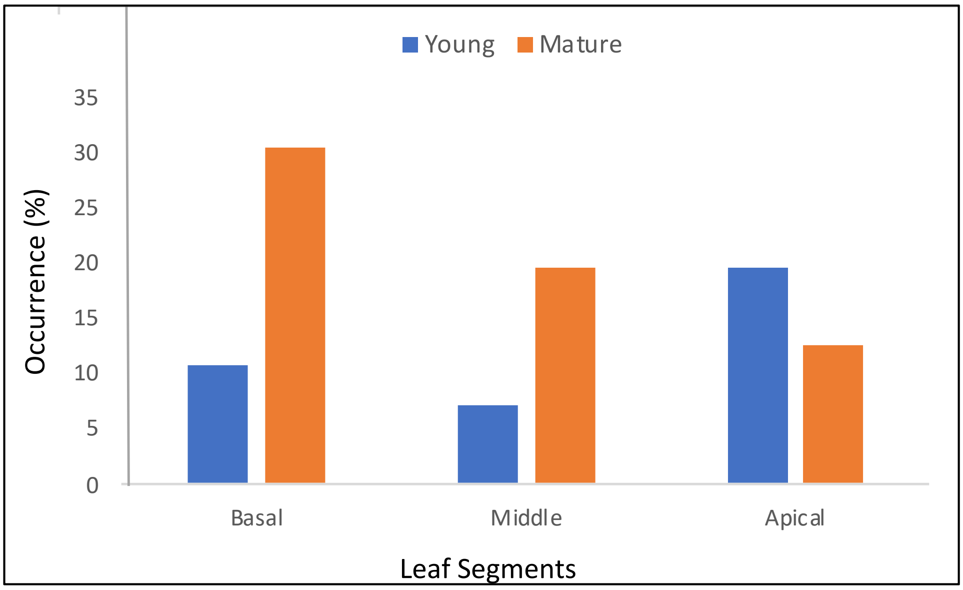

Fig. 2. Occurrence of endophytic fungi on different segments of young and mature leaves.

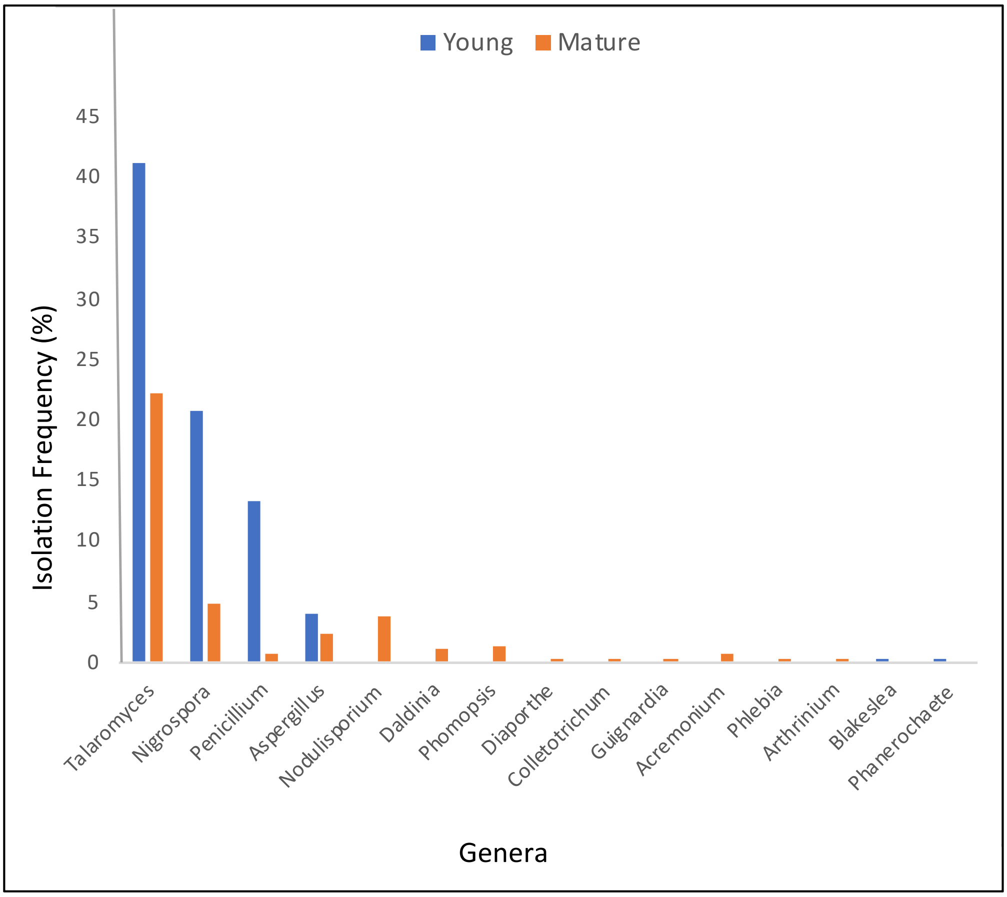

Fig. 3. Isolation frequency (%) of endophytic fungi on young and mature leaves.

Percent fungal isolation frequency in taxa was also evaluated without considering the different leaf segments. It was observed that *Talaromyces* showed the highest percentage of isolation frequency from both young (41 %) and mature (22 %) leaves. This was followed by *Nigrospora* with 20.67 % and 4.67 % from young and mature leaves respectively. *Penicillium* also recorded high isolation frequency in young leaf (13.33 %) (Fig. 3).

Discussion

Fungal endophytes are defined as secondary pathogens by forest pathologists. Plants are considered to be reservoir of untold numbers of organisms called endophytes (BACON & WHITE 2000). Endophytes are microorganisms that inhabit the interstitial spaces of living tissues and do not apparently harm the host plant (AZEVEDO & al. 2000). It seems that these microorganisms can be a source of novel, natural bioactive products that can be used in medicine, industry, and agriculture (BAYMAN & al. 1997).

In the Philippines, most of the studied fungal endophytes were associated with plants of high economic value. Examples of these plants are: pandan (BUNGIHAN & al. 2013), *Musa* spp. (DAGAMAC & al. 2010, REBUTA 2008), sweet potato (HIPOL 2012), nipa palm (CRUZ & CADIENTE 2016), tuba-tuba (SADORAL 2010), pili tree (TORRES & DELA CRUZ 2015), sugarcane (LAPITAN 2018), upland rice (CRISTOBAL 2010), bamboo (DALISAY 1998) and coconut (BIÑAS 2016). In addition, DONAYRE & DALISAY (2016) studied the endophytic fungi associated with the tissues of barnyard grass weed. PUIG & CUMAGUN (2019) explored Mt. Apo in Mindanao Montane Rain Forests ecoregion for endophytes associated with selected rainforest plants. GUERRERO & DALISAY (2018) researched on the occurrence of endophytes in pili fruits. MALVEDA (2019) reported on the endophytic fungi associated with guava fruit.

RAJAGOPAL & SURYANARAYANAN (2000) reported that the frequency of occurrence of foliar endophytes in tropical trees is influenced by environment, and types and chemistry of the host tissues. Endophytes were chemical synthesizers found inside the host plant tissue (RAMESHA & SRINIVAS 2014). They were the less investigated group of microorganisms that produce various bioactive compounds that can be exploited and applied on a wide array of medical, agricultural and industrial areas (STROBEL & al. 2003).

Many studies have demonstrated that leaf age influences the density of endophytes inhabiting leaves of tropical forest trees (ARNOLD & HERRE 2003). Colonization by endophytic fungi is affected by variation in leaf traits during leaf maturation (DUONG & al. 2006). NASCIMENTO & al. (2015) analyzed the community of endophytic fungi of sodom apple, *Calotropis procera* (WILLD.) R. BR. A total of 156 fungal isolates belonging to 19 taxa were obtained. They revealed that the highest number of endophytic isolates was found in older leaves.

Moreover, HILARINO & al. (2011) reported that the composition of endophytic fungi differed among leaf ages. More diverse endophytes can be obtained from mature leaves of orchid trees, *Bauhinia brevipes* L. A similar study was conducted by KUMARESAN (2002) on the endophyte assemblages in young, mature, and senescent leaves of bakauan (*Rhizophora apiculata* Blume). The result revealed that the number of endophytes increased as leaf aged. They added that the increase in density colonization in mature leaves was due to the recurrent re-infection of the leaf over time, perhaps from air-borne inoculum. In addition, mature leaves are more favorable for fungal colonization because of changes in leaf biochemical (FERNANDES & al. 2011). ARNOLD & al. (2003) verified that fungal endophytes in cacao leaves were horizontally transmitted and accumulated over leaf lifetime. Similarly, horizontal mode of transmission of endophytes is high in tropical regions (ARNOLD 2005).

Several studies have been conducted to determine occurrence of the endophytic fungi on different leaf segments. For example, a study was conducted by DONAYRE & DALISAY (2016) on the endophytic fungi associated with *Echinochloa glabrescens* Munro ex Hook F. Fungal endophytes were found highest on both the middle and bottom segments of the lower leaf blade (39.58%) followed by the tip segment (39.17%). Furthermore, the result of this study is comparable to the results of (CANNON & SIMMONS 2002) on the study of the diversity of endophytic fungi obtained from different forest trees species and diversity of endophytes inhabiting the leaves of Japaneses laurel tree [*Neolitsea sericea* (BLUME) KOIDZ.] in broadleaf and conifer (HATA & SONE 2008).

A similar study on the isolation of endophytic fungi from neem tree (*Azadirachta indica*) leaves revealed that frequency occurrence was significantly higher in the basal leaf segment than in the apical and middle segments. The result suggested that the occurrence of foliar endophytes in tropical trees is influenced by the environment and biochemical composition of its host (RAJAGOPAL & SURYANARAYANAN 2000). ARNOLD & HERRE (2003) explained that among the various factors that influence endophytes colonization, the duration of exposure of leaf tissue to viable air inoculum in the field would influence endophyte growth and proliferation.

Further, the study revealed *Talaromyces* was dominantly present in all leaf ages and segments. DUONG & al. (2006) suggested that the wide spread of a particular endophyte may be associated with its ability to inhabit the leaves and colonize. It conformed with the report of SILVA & al. (2018) of high frequency of *Talaromyces* and *Penicillium* when they explored the richness of endophytic fungi in the bromeliad plants. In addition, several reports have described *Talaromyces* as endophytic fungi of diverse plant hosts such as wild ginger, *Amomum siamense* Criab. (BUSSABAN & al. 2001) as well as various medicinal plants in Thailand (THEANTANA & al. 2009).

A similar study was conducted by KUSARI & al. (2013) who isolated more than 90 % endophytic fungi that belonged to genus *Penicillium* on marijuana (*Cannabis sativa* L.) in the Netherlands. Different *Penicillium* species were also obtained from coffee plants (VEGA & al. 2006) and rice (NAIK et al. 2009).

MURALI & al. (2006) reported a species of *Phomopsis* (teleomorph: *Diaporthe*) that dominated the endophytic assemblages of teak, regardless of the location of the host trees. SURYANARAYAN & JOHNSON (2005) on the other hand, reported *Phomopsis* spp. as dominant fungal endophytes when they screened plant species in Southern India.

Furthermore, endophytic fungus *Nigrospora* sp. was found in both young and mature leaves of rubber. *Nigrospora oryzae* is a fast-growing fungus that acts as endophyte, saprophyte, and as weak pathogen, depending upon the host and environmental conditions (KUMAR & al. 2015). Also, EBADA & al. (2016) reported the endophytic fungus *Nigrospora oryzae* isolated from the leaves of Nigerian mistletoe.

Moreover, *Acremonium* species have been recognized as fungal endophytes in grasses. These are obligate seed-borne fungi that form asymptomatic infections in leaves, stems, and inflorescences of grasses (BREEN 1994). Also, the genus *Colletotrichum* is found in many plant host as pathogen, endophyte and occasionally as saprophyte. However, MA & al. (2018) reported a *Colletotrichum* species as endophytic fungi from *Dendrobium* orchid, a new host recorded for Thailand. In Northeastern Brazil, previously reported *Colletotrichum* species causing anthracnose in mango fruits were also recovered as endophytic fungi in mango (VIEIRA & al. 2014). TAO & al. (2013) reported 17 *Colletotrichum* species, including 7 new species recovered from Chinese butterfly orchid (*Bletilla ochracea* SCHLTR). Though numerous researches have been conducted on the genus *Colletotrichum*, understanding it as an endophytic fungus remains limited.

Likewise, MAADON & al. (2018) identified endophytic *Daldinia* species associated with litter of deciduous forest trees in Malaysia. Also, an endophytic *Nodulisporium* sp. has been isolated from balsam tree [*Myroxylon balsamum* (L.) HARMS] in Ecuadorian Amazon (MENDS & al. 2012), broad leaf maiden fern in a rainforest of Central America (HASSAN & al. 2013) and evergreen avocado [*Persea indica* (L.) SPRENG.] in Canary Islands (TOMSHECK & al. 2010).

Endophytic *Guignardia* is an anamorph of *Phyllostica* isolated from leaves of various plants collected from different places in Japan and Thailand. Thus, these fungi can live within various vascular plants, angiosperms, gymnosperms, and pteridophytes (O-KANE & al. 2003). *Guignardia mangiferae* was isolated from the leaves of medicinal plants (BALAKUMARAN & al. 2015). In addition, endophytic *Guignardia citricarpa* Kiely was isolated from leaves of three varieties of mandarin orange (GLIENKE-BLANCO & al. 2002).

Likewise, endophytic *Aspergillus fumigatus* was obtained from the bark of malunggay, *Moringa oleifera* LAM (ARORA & KAUR 2019) and from juniper berry, *Juniperus communis* L. (KUSARI & al. 2009). *Aspergillus clavatus* DESM. was isolated also from *Azadirachta indica* as fungal endophytes (VERMA & al. 2010).

The *Arthrinium* sp. was reported as endophytic fungus isolated from plai (variety of ginger), *Zingiber cassumunar* Roxb. (PANSANIT & PRIPDEEVECH 2018). PRATHYUSHA and and co-workers (2015) reported *Arthrinium phaeospermum* as a rare and infrequent endophyte, mostly isolated from the serpentine plant *Andrographis paniculata* (BURM.f.) NEES. In addition, MARTIN & al. (2015), in their study on the colonization of endophytic fungi in rubber leaves and sapwood, revealed that *Phlebia* and *Phanerochaeta* were among the frequently isolated genera, which have been reported as endophytes of a variety of hosts.

Local studies have recorded different fungal endophytes. GUERRERO & DALISAY (2018) recorded *Aspergillus tubingensis* MOSSERAY, *Guignardia mangiferae* A. J. ROY and *Talaromyces atroroseus* YILAZ, FRISVAD, HOUBRAKEN & SAMSON among the 15 species isolated from all tissues of pili fruit. In addition, DONAYRE & DALISAY (2016) reported that endophytic *Nigrospora oryzae* and *Arthrinium phaeospermum* were among the isolated endophytes from tissues of barnyard grass weed. GENERAL & GUERRERO (2017) recorded six fungal endophytes from mature pili leaves, including *Aspergillus fumigatus*. Similarly, TORRES & DELA CRUZ (2015) recorded 18 fungal endophytic genera, that include *Paecilomyces*, *Colletotrichum*, *Curvularia*, *Fusarium* and *Guignardia* in pili leaves. Moreover, endophytic *Colletotrichum* sp. was isolated from pandan (BUNGIHAN & al. 2013). *Penicillium* sp. was among the isolated fungal endophytes from rainforest plants in Mt. Apo (PUIG & CUMAGUN 2019). CRISTOBAL (2010) isolated endophytic fungi from upland rice. Genera of *Trichoderma* and *Aspergillus* were among the 27 isolated endophytes from rice. SADORAL (2010) recorded genera of

Geotrichum and *Aspergillus* from jatropha plant. Internal mycobiota of healthy banana roots were explored and genera of *Penicillium* and *Aspergillus* were observed with high dominant value of 35.48 % and 19.35 %, respectively (REBUTA 2008).

Among the isolated genera of fungal endophytes, *Colletotrichum* was reported to cause anthracnose in most rubber plantations in Mindanao, Philippines (TANGONAN 2012) and even in other rubber producing countries in Southeast Asia (LIU & al. 2018). The genus *Phomopsis* was also reported as important rubber pathogen. *Phomopsis heveicola*, has been reported to cause root disease of rubber in China (MA & al. 2004) and *P. heveae* has been reported to cause dieback of rubber trees in Brazil, China, India, Indonesia, Malaysia, Sri Lanka and Thailand (UDAYANGA & al. 2011). *Nigrospora* species occur as pathogens, however, there were no existing reports of these fungi affecting rubber locally and abroad. *Nodulisporium* sp. was isolated and confirmed both pathogen and endophyte, however, there was no reported case of this fungus affecting rubber trees. The other isolated endophyte genera, such as *Talaromyces*, *Penicillium*, *Aspergillus*, *Daldinia*, *Guignardia*, *Acremonium*, *Phlebia*, *Arthrinium*, *Blakeslea* and *Phanerochaete* were not pathogenic to rubber.

The results of this study provide information on the endosymbiotic microbiota of rubber leaves. These endophytes may serve as protectant against pathogenic fungi. Endophytes derive their nutrients from plants (WILSON 1995) while plants will benefit due to the endophytes protection against pathogens, enhancement of nutrients uptake, growth promotion (ALVIN & al. 2014), and plant tolerance to stress (LARRAN & al. 2016). Moreover, WIELGUSZ & IRZYKOWSKA (2017) claimed that those plants inhabited by endophytes are healthier compared to those endophyte-free plants.

Conclusion

These findings and observations draw attention to the potential roles of these fungal endophytes in utilization for the benefit of agriculture. Furthermore, endophytic fungi species isolated can possibly be tested as biological control agents against rubber foliar diseases in the Philippines.

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References

ALVIN, A., MILLER, K. I., NEILAN, B. A., 2014: Exploring the potential of endophytes from medicinal plants as sources of antimycobacterial compounds. – Microbiol. Res.**169**(7–8): 483–495.

ARNOLD, A. E., HERRE, E. A., 2003: Canopy cover and leaf age affect colonization by tropical fungal endophytes: ecological pattern and process in *Theobroma cacao* (*Malvaceae*). – Mycologia **95**(3): 88–398.

ARNOLD, A. E., MEJÍA, L. C., KYLLO, D., ROJAS, E.I., MAYNARD, Z., ROBBINS, N., HERRE, E. A., 2003: Fungal endophytes limit pathogen damage in a tropical tree. – Proc. Natl. Acad. Sci. **100**(26): 15649–15654.

ARNOLD, A. E., 2005: Diversity and ecology of fungal endophytes in tropical forests. – In: DESHMUKH, S., (Ed.): Current trends in mycological research, pp. 49–68.– New Delhi, Oxford: IBH Publishing.

ARORA, D. S., KAUR, N., 2019: Antimicrobial potential of fungal endophytes from *Moringa oleifera*. – Appl. Biochem. Biotechnol. **187**(2): 628–648.

AZEVEDO, J. L., MACCHERONI, Jr. W., PEREIRA, J. O., DE ARAÚJO, W. L., 2000: Endophytic microorganisms: a review on insect control and recent advances on tropical plants. – Electronic J. Biotechnol. **3**(1): 15–16.

BACON, C. W., WHITE, J., 2000: Microbial endophytes. – New York: Dekker.

BALAKUMARAN, M. D., RAMACHANDRAN, R., KALAICHELVAN, P. T., 2015: Exploitation of endophytic fungus, *Guignardia mangiferae* for extracellular synthesis of silver nanoparticles and their in vitro biological activities. – Microbiol. Res. **178**: 9–17.

BAYMAN, P., LEBRON, L. L., TREMBLAY, R. L. LODGE, D. J., 1997: Variation in endophytic fungi from roots and leaves of *Lepanthes* (*Orchidaceae*). –New Phytol. **135**(1): 143–149.

BIÑAS, S., 2016: In vitro efficacy of fungal endophytes and medicinal plant extracts against *Fusarium proliferatum* (MATSUSH.) NIRENBERG, causal organism of leaf blight of coconut (*Cocos nucifera* L.) [Undergraduate Thesis]. – College, Laguna, Philippines: University of the Philippines Los Baños.

BREEN, J. P., 1994: *Acremonium* endophyte interactions with enhanced plant resistance to insects. – Annu. Rev. Entomol. **39**(1): 401–423.

BROOKS, D. S., GONZALEZ, C. F., APPEL, D. N., FILER, T. H., 1994: Evaluation of endophytic bacteria as potential biological control agents for Oak Wilt. – Biological Control **4**(4): 373–381.

BUNGIHAN, M. E., NONATO, M. G., DRAEGER, S., DELA CRUZ, T. E. E., 2013: Antimicrobial and antioxidant activities of fungal leaf endophytes associated with *Pandanus amaryllifolius* roxb. – Philippine Sci. Lett. **6**(2): 128–137.

BUSSABAN, B., LUMYONG, S., LUMYONG, P., MCKENZIE, E. H., HYDE, K. D., 2001: Endophytic fungi from *Amomum siamense*. – Canad. J. Microbiol. **47**(10): 943–948.

CANNON, P. F., SIMMONS, C. M., 2002: Diversity and host preference of leaf endophytic fungi in the Iwokrama Forest Reserve, Guyana. – Mycologia **94**(2): 210–220.

CRISTOBAL, D. B., 2010: Isolation and identification of fungal endophytes from upland rice (*Oryza Sativa* L.) and their antagonistic effect on *Rhizoctonia solani* Kuhn, causal organism of sheath blight [Undergraduate Thesis]. – College, Laguna, Philippines: University of the Philippines Los Baños.

CRUZ, J. A., CADIENTE, M. K. M., 2016: Assessment of potential plant growth promoting compounds produced in vitro by endophytic bacteria associated with nipa palm (*Nypa fruticans*). – Philippine J. Crop Sci. **41**(3):74–82.

DAGAMAC, N. H. A., SOGONO, P. G., CABALFIN, R. C. B., ADDUCUL, A. C. Y., 2010: Fungal root endophytes from *Musa* spp. as biological control agents against the plant pathogen *Fusarium oxysporum*. – Acta Manilana **56**: 27–35.

DALISAY, T. U., 1998: Biodiversity of microfungi associated with species of *Bambusa* and *Dendrocalamus*. [PhD Thesis]. – Hongkong: University of Hongkong. http://dx.doi:10.1590/S1517-83822012000400041.

DALTON, D. A., KRAMER, S., AZIOS, N., FUSARO, S., CAHILL, E., KENNEDY, C., 2004: Endophytic nitrogen fixation in dune grasses (*Ammophila arenaria* and *Elymus mollis*) from Oregon. – FEMS Microbiol. Ecol. **49**(3): 469–479.

DONAYRE, D. K. M., DALISAY, T. U., 2016: Identities, characteristics, and assemblages of dematiaceous-endophytic fungi isolated from tissues of barnyard grass weed. – Philippine J. Sci. **145**(2): 153–164.

DUONG, L. M., JEEWON, R., LUMYONG, S., HYDE, K. D., 2006: DGGE coupled with ribosomal DNA gene phylogenies reveal uncharacterized fungal phylotypes. – Fungal Diversity **23**: 121–138.

EBADA, S. S., EZE, P., OKOYE, F. B., ESIMONE, C. O., PROKSCH, P., 2016: The fungal endophyte *Nigrospora oryzae* produces quercetin monoglycosides previously known only from plants. – Chemistry Select **1**(11): 2767–2771.

FERNANDES, G. W., OKI, Y., SANCHEZ-AZOFEIFA, A., FACCION, G., AMARO-ARRUDA, H. C., 2011: Hail impact on leaves and endophytes of the endemic threatened *Coccoloba cereifera* (*Polygonaceae*). – Pl. Ecol. **212**(10): 1687–1697.

FORCHETTI, G., MASCIARELLI, O., ALEMANO, S., ALVAREZ, D., ABDALA, G., 2007: Endophytic bacteria in sunflower (*Helianthus annuus* L.): Isolation, characterization, and production of jasmonates and abscisic acid in culture medium. – Appl. Microbiol. Biotechnol. **76**(5): 1145–1152.

GAZIS, R., CHAVERRI, P., 2010: Diversity of fungal endophytes in leaves and stems of wild rubber trees (*Hevea brasiliensis*) in Peru. – Fungal Ecol. **3**(3): 240–254.

GENERAL, M. A., GUERRERO, J. J. G., 2017: Records of fungal endophytes from *Canarium ovatum* ENGL. (Family *Burseraceae*) leaves. – Philippine J. Sci. **146**(1): 1–5.

GIMENEZ, C., CABRERA, R., REINA, M., GONZALEZ-COLOMA, A., 2007: Fungal endophytes and their role in plant protection. – Current Organic Chemistry **11**(8): 707–720.

GHORBANPOUR, M., OMIDVARI, M., ABBASZADEH-DAHAJI, P., OMIDVAR, R., KARIMAN, K., 2018: Mechanisms underlying the protective effects of beneficial fungi against plant diseases. – Biol. Control **117**: 147–157.

GLIENKE-BLANCO, C., AGUILAR-VILDOSO, C. I., VIEIRA, M. L. C., BARROSO, P. A. V., AZEVEDO, J. L., 2002: Genetic variability in the endophytic fungus *Guignardia citricarpa* isolated from citrus plants. – Genet. Molec. Biol. **25**(2): 251–255.

GUERRERO, J. J. G., DALISAY, T. U., 2018: Fungal Endophytes across tissue layers of *Canarium ovatum* (*Burseraceae*) fruit. – Österr. Z. Pilzk. **27**: 11–21.

HARDOIM, P. R., VAN OVERBEEK, L. S., VAN ELSAS, J. D., 2008: Properties of bacterial endophytes and their proposed role in plant growth. – Trends Microbiol. **16**(10): 463–471.

HATA, K., ATARI, R., SONE, K., 2002: Isolation of endophytic fungi from leaves of *Pasania edulis* and their within-leaf distributions. – Mycoscience **43**(5): 0369–0373.

HATA, K., SONE, K. 2008. Isolation of endophytes from leaves of *Neolitsea sericea* in broadleaf and conifer stands. – Mycoscience **49**(4): 229–232.

HILARINO, M. P. A., OKI, Y., RODRIGUES, L., SANTOS, J.C., CORRÊA JUNIOR, A., FERNANDES, G. W., ROSA, C. A., 2011: Distribution of the endophytic fungi community in leaves of *Bauhinia brevipes* (*Fabaceae*). – Acta Bot. Brasil. **25**(4): 815–821.

HIPOL, R. M., 2012: Molecular identification and phylogenetic affinity of two growth promoting fungal endophytes of sweet potato [*Ipomea batatas* (L.) LAM.] from Baguio City, Philippines. – Electronic J. Biol. **8**(3): 57–61.

JOSEPH, B., PRIYA, R. M., 2011: Bioactive compounds from endophytes and their potential. – Amer. J. Biochem. Molec. Biol. **1**(3): 291–309.

KUMARESAN, V., 2002: Endophytes assemblages in young mature and senescent leaves of *Rhizophora apiculata*: Evidence for the role of endophytes in mangrove litter degradation. – Fungal Diversity **9**: 81–91.

KUMAR, P., KANDAN, A., AKHTAR, J., GAWADE, B. H., GUPTA, A., 2015: *Nigrospora oryzae*: An endophyte potential application in agriculture. – Biotechnology Articles, http://www.biotecharticles.com/Agriculture-Article/Nigrospora-Oryzae-An-Endophyte-ofPotential-Application-inAgriculture-3425.html.

KUSARI, S., LAMSHÖFT, M., SPITELLER, M., 2009: *Aspergillus fumigatus* FRESENIUS, an endophytic fungus from *Juniperus communis* L. Horstmann as a novel source of the anticancer pro-drug deoxypodophyllotoxin. – J. Appl. Microbiol. **107**(3): 1019–1030.

KUSARI, P., KUSARI, S., SPITELLER, M., KAYSER, O., 2013. Endophytic fungi harbored in *Cannabis sativa* L.: diversity and potential as biocontrol agents against host plant-specific phytopathogens.– Fungal Diversity **60**(1):137–151.

LAPITAN, S.C., 2018: Efficacy of fungal endophytes against *Colletotrichum falcatum* WENT. and *Fusarium* sp., causal organisms of red root rot of sugarcane (*Saccharum officinarum* L.) [Undergraduate Thesis]. – College, Laguna, Philippines: University of the Philippines Los Baños.

LARRAN, S., SIMON, M. R., MORENO, M. V., SIURANA, M. S., PERELLÓ, A., 2016: Endophytes from wheat as biocontrol agents against tan spot disease. – Biol. Control **92**: 17–23.

LIU, X., LI, B., CAI, J., ZHENG, X., FENG, Y., HUANG, G., 2018: *Colletotrichum* species causing anthracnose of rubber trees in China. – Scientific Reports **8**(1): 10435.

MA, L., XIANG, M., QI, P., JIANG, Z., 2004: Three new species of *Phomopsis* from Xishuangbanna, China. – Mycosystema **23**(4): 457–460.

MA, X., NONTACHAIYAPOOM, S., JAYAWARDENA, R. S., 2018: Endophytic *Colletotrichum* species from *Dendrobium* spp. in China and Northern Thailand. – MycoKeys 43: 23–57.

MAADON, S. N., WAKID, S. A., ZAINUDIN, I. I., RUSLI, L. S., MOHD, M. S., 2018: Isolation and identification of endophytic fungi from UiTM Reserve Forest, Negeri Sembilan. – Malaysian Sci. **47** (12): 3025–3030.

MALVEDA, R. F., 2019: Efficacy of endophytic fungi against fungal pathogens causing diseases of guava (*Psidium guava* L.) cv Guapple. [Undergraduate Thesis]. – College, Laguna, Philippines: University of the Philippines Los Baños.

MARTIN, R., GAZIS, R., SKALTSAS, D., CHAVERRI, P., HIBBETT, D., 2015: Unexpected diversity of basidiomycetous endophytes in sapwood and leaves of *Hevea*. – Mycologia **107**(2): 284–297.

MENDS, M. T., YU, E., STROBEL, G. A., RIYAZ-UL-HASSAN, S., BOOTH, E., GEARY, B., SEARS, J., TAATJES, C. A., HADI, M. Z., 2012: An endophytic *Nodulisporium* sp. producing volatile organic compounds having bioactivity and fuel potential. – J. Petroleum Environm. Biotechnol. **3**(3): 16.

MURALI, T. S., SURYANARAYANAN, T. S., GEETA, R., 2006: Endophytic *Phomopsis* species: host range and implications for diversity estimates. – Canad. J. Microbiol. **52**(7): 673–680.

NAIK, B. S., SHASHIKALA, J., KRISHNAMURTHY, Y. L., 2009: Study on the diversity of endophytic communities from rice (*Oryza sativa* L.) and their antagonistic activities in vitro. – Microbiol. Res. **164**(3): 90–296.

NASCIMENTO, T. L., OKI, Y., LIMA, D. M. M., ALMEIDA-CORTEZ, J. S., FERNANDES, G. W., SOUZA-MOTTA, C. M., 2015: Biodiversity of endophytic fungi in different leaf ages of *Calotropis procera* and their antimicrobial activity. – Fungal Ecol. **14**: 79–86.

OKANE, I., NAKAGIRI, A., ITO, T., LUMYONG, S., 2003: Extensive host range of an endophytic fungus, *Guignardia endophyllicola* (anamorph: *Phyllosticta capitalensis*). – Mycosci. **44**(5): 353–363.

PANSANIT, A., PRIPDEEVECH, P., 2018: Antibacterial secondary metabolites from an endophytic fungus, *Arthrinium* sp. MFLUCC16-1053 isolated from *Zingiber cassumunar.* – Mycol. **9**(4): 264–272.

PRATHYUSHA, P., RAJITHA SRI, A. B., SATYA PRASAD, K., 2015: Diversity and enzymatic activity of foliar endophytic fungi isolated from medicinal plants of Indian dry deciduous forest. – Pharma Letter **7**(8): 244–251.

PUIG, C. G., CUMAGUN, C. J. R., 2019: Rainforest fungal endophytes for the bio-enhancement of banana toward *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4. – Arch. Phytopathol. Pl. Prot. **52**(7–8): 776–794.

RAJAGOPAL, K., SURYANARAYANAN, T. S., 2000: Isolation of endophytic fungi from leaves of neem (*Azadirachta indica* A. Juss.). – Curr. Sci. **78**: 1375–1377.

RAMESHA, A., SRINIVAS, C., 2014: Antimicrobial activity and phytochemical analysis of crude extracts of endophytic fungi isolated from *Plumeria acuminata* L. and *Plumeria obtusifolia* L. – European J. Experim. Biol. **4**(2): 35–43.

REBUTA, A. J., 2008: Internal microbiota of banana cultivars and their antagonistic activity on *Fusarium oxysporum* f. sp. *cubense* (E. F. SMITH) SYNDER and HANSEN. [Undergraduate Thesis]. – College, Laguna, Philippines: University of the Philippines Los Baños.

SADORAL, J. P., 2010: Survey and identification of leaf spot diseases of *Jatropha curcas* L. in the Philippines and utilization of fungal endophytes and epiphytes as biological control agents against *Colletotrichum* sp. [Master's Thesis]. – College, Laguna, Philippines: University of the Philippines Los Baños.

SHENG, X., CHEN, X., HE, L., 2008: Characteristics of an endophytic pyrene-degrading bacterium of *Enterobacter* sp. 12J1 from *Allium macrostemon* Bunge. – Int. Biodeterioration & Biodegradation **62**(2): 88–95.

SILVA, L. F., FREIRE, K. T., ARAÚJO-MAGALHÃES, G. R., AGAMEZ-MONTALVO, G. S., SOUSA, M. A., COSTA-SILVA, T. A., ... SOUZA-MOTTA, C. M., 2018: *Penicillium* and *Talaromyces* endophytes from *Tillandsia catimbauensis*, a bromeliad endemic in the Brazilian tropical dry forest, and their potential for L-asparaginase production. – World J. Microbiol. Biotechnol. **34**(11): 162.

STONE, J. K., BACON, C. W., WHITE Jr, J. F., 2000: An overview of endophytic microbes: endophytism defined. – Microbial Endophytes **1**: 17–44.

STROBEL, G., DAISY, B., 2003: Bioprospecting for microbial endophytes and their natural products. Microbiol. – Molec. Biol. Rev. **67**(4): 491–502.

SURYANARAYANAN, T. S., JOHNSON, J. A., 2005: Fungal endophytes of tropical plants: a critical review. Microbial diversity: current perspectives and potential applications. – New Delhi, India: IK International 207–224.

TAO, G., LIU, Z. Y., LIU, F., GAO, Y. H., CAI, L., 2013: Endophytic *Colletotrichum* species from *Bletilla ochracea* (Orchidaceae), with descriptions of seven new species. – Fungal Diversity **61**(1): 139–164.

TANGONAN, N. G. (Ed)., 2012: Rubber production and management in the Philippines. – DA-Bureau of Agricultural Research-University of Southern Mindanao, Kabacan, Cotabato.

THEANTANA, T., HYDE, K. D., LUMYONG, S., 2009: Asparaginase production by endophytic fungi from Thai medicinal plants: cytotoxicity properties. – Int. J. Integrative Biol. **7**: 1–8.

TOMSHECK, A. R., STROBEL, G. A., BOOTH, E., GEARY, B., SPAKOWICZ, D., KNIGHTON, B., FLOERCHINGER, C., SEARS, J., LIARZI, O., EZRA, D., 2010: *Hypoxylon* sp., an endophyte of *Persea indica*, producing 1, 8-cineole and other bioactive volatiles with fuel potential. – Microbial Ecol.**60**(4): 903–914.

TORRES, J. M. O., DELA CRUZ, T. E. E., 2015: Antibacterial activities of fungal endophytes associated with the Philippine endemic tree, *Canarium ovatum*. – Mycosphere **6**: 266–273.

UDAYANGA, D., LIU, X., MCKENZIE, E.H., CHUKEATIROTE, E., BAHKALI, A. H., HYDE, K. D., 2011: The genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. – Fungal Diversity **50**: 189.

VAN BUREN, A. M., ANDRE, C., ISHIMARU, C. A., 1993: Biological control of the bacterial ring rot pathogen by endophytic bacteria isolated from potato. – Phytopathol. **83**(2): 140–146.

VEGA, F. E., POSADA, F., PETERSON, S. W., GIANFAGNA, T.J., CHAVES, F., 2006: *Penicillium* species endophytic in coffee plants and ochratoxin A production. – Mycologia **98**(1): 31–42.

VIEIRA, W. A., MICHEREFF, S. J., DE MORAIS, M. A., HYDE, K. D., CÂMARA, M. P., 2014: Endophytic species of *Colletotrichum* associated with mango in northeastern Brazil. – Fungal Diversity **67**(1): 181–202.

VERMA, V. C., KHARWAR, R. N., GANGE, A. C. 2010: Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus *Aspergillus clavatus*. – Nanomedicine **5**(1): 33–40.

WIELGUSZ, K., IRZYKOWSKA, L., 2017: Occurrence of pathogenic and endophytic fungi and their influence on quality of medicinal plants applied in management of neurological diseases and mental disorders. – Herba Polonica **63**(4): 57–69.

WILSON, D., 1995: Fungal endophytes which invade insect galls: insect pathogens, benign saprophytes, or fungal inquilines? Oecologia **103**: 255–260.