International Journal of Agricultural Technology 2018 Vol. 14(7): 1423-1432
Available online http://www.ijat-aatsea.com
ISSN: 2630-0613 (Print) 2630-0192 (Online)

Effective of *Neosatorya* to control *Phomopsis asparagi* causing stem blight of asparagus

Mangkalad, T.[1], Soytong, K.[2], Tangthirasunun, N.[3] and Poeaim, S.[1*]

[1]Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand; [2]Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Ladkrabang, Bangkok, Thailand.

Mangkalad, T., Soytong, K., Tangthirasunun, N. and Poeaim, S. (2018). Effective of *Neosatorya* to control *Phomopsis asparagi* causing stem blight of asparagus. International Journal of Agricultural Technology 14(7): 1423-1432.

Abstract The biocontrol of stem blight of asparagus *Phomosis asparagi* was proved by *Neosartorya* and *Talaromyces*. The symptoms are appearance as oval-shaped lesions with light brown centres and slightly darker margins. Ten isolates of pathogen were isolated by tissue transplanting technique and confirmed by morphology and molecular identification based on internal transcribed spacer (ITS)-nrDNA sequence as *P. asparagi*. Fungal mycelia showed undulate margins and colonies white to gray and reached a diameter of 9 cm petri dish after 10 days. Alpha conidia were oblong or spindle-shaped, with a size of 2–3.5 × 6.5–8.8 µm. The fungal pathogenicity test showed the asparagus was infected by *P. asparagi* within 2 days on moisture chamber at room temperature. *Neosartorya* and *Talaromyces* were proved antifungal acrtivety against *P. asparagi* causing of stem blight of asparagus in dual culture. It revealed that *N. hiratsuka* EU06 was the highest inhibition *P. asparagi* causing the stem blight of asparagus at 59.50%.

Keywords: Stem blight, *Phomosis asparagi*, *Neosartorya* and *Talaromyces*

Introduction

The asparagus (*Asparagus officinalis* L.) became popular for many years and has been used as a vegetable and medicine (Brunning, 2010). In Thailand, asparagus cultivation areas are in western and north east eg. Kanchanaburi, Nakorn Pathom, Suphanburi, Phetchabun and Ratchaburi (Tumtorn, 2018). Including, environment of Thailand able to caultivated asparagus all the year. However, cultivate areas are decrease because it was offended by crown stem and root rot (caused by *Fusarium* sp.), soft rot (caused by *Phytophthora* sp.), rust (caused by *Puccinia asparagi*), purple spot (caused by *Stemphylium* sp.), stem blight (caused by *Phomopsis asparagi*) and anthracnose disease (caused

[*] **Corresponding Author:** Poeaim, S.; **Email**: poeaim@hotmail.com

by *Colletotrichum gloeosporioides*) (Cheah *et al*., 2006; Elena, 2007; Tumtorn, 2018).

The phomopsis stem blight caused by *P. asparagi* is one of the most important plant diseases to loss cultivation crop (Tumtorn, 2018). This disease able spread through trade of contaminated asparagus seeds, crowns and spears (Cheah *et al*., 2006). The stem blight lesion character has light-dark brown oval shape and darker margins and wither in the final stage (Cheah *et al*., 2006; Elena, 2007). For morphology character are greyish white or white colonies, dark brown to back pycnidia, fusiform to eliipsoid conidia (Cheah *et al*., 2006; Elena, 2007; Dinh *et al*., 2018). The conidai has been reported were β-conidia and α-conidia and Uecker and Johnson (1991) show only α-conidia.

In 2015, Nonaka *et al.* were co-culture and evaluated efficacy of *Talaromyces siamensis* FKA-61 with *Phomopsis* sp. FKA62. They founded *T. siamensis* FKA-61 inhibited the growth of *Phomopsis* sp strain FKA-62. Soytong (2015) was reported about *Talaromyces* and *Neosatoya* species could be control *C. coffeanum* causeing antracnose on coffee. Dethoup *et al*. (2017) studied antagonistic activity of *T. tratensis* KUFA 0091 against with *Alternaria padwickii, Bipolaris oryzae, Curvularia lunata* and *F. moniliforme* in rice.

However, information in field of symptoms and biological control have a few. So, in this studied was to efficacy antagonistic activity of the *Talaromyces* and *Neosartorya* to control Phomopsis stem blight disease in asparagus.

Materials and methods

Isolation and identification of fungi

Pathogen

Asparagus stem blight are symtomatic of oval shaped brown and slightly darker margins were collected from different cultivated fields in Nakhon Pathom, Suphanburi, Ratchaburi and Nonthaburi provinces, Thailand (Table 1). The pathogen was isolated and cultured on Potato dextrose agar (PDA) at room temperature. All isolates were separately proved for pathogenicity tes. The morphology was observe under compound microscope (Nikon, Japan). All isolates were kept in PDA slants at 4 ℃ and 40% glycerol suspension at -80 ℃.

The isolates was confirmed which based on DNA sequencing of internal transcribed spacer region (ITS) by using ITS1 (5 ՝TCC GTA GGT GAA CCT TGC GG 3 ՝) and ITS4 (5 ՝TCC TCC GCT TAT TGA TAT GC 3 ՝) primers (White *et al*., 1990) following PCR protocols of Udayanga *et al*. (2012). PCR products were performed for sequencing analysis by Bioneer company, Korea. Finally, Basic Local Alignment Search Tools (BLAST), National Center for

Biotechnology Information (NCBI) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was confirmed *Phomopsis* species.

Antagonist

Pure cultures of antagonistic isolates *N. hiratsuka* EU06, *N. pseudofischeri* EU13, *Neosartorya* sp. EU35, *T. muroii* EU07, *T. muroii* EU18, *T. trachyspermus* EU23, *N. aureola* CHA01-A01, *N. fennelliae* CHA03-A11, *N. spinosa* CHA09-A01 and *T. muroii* CHA03-A03) were obtained from Soytong (2015) and Suksiri and Poeaim. All isolates were cultured on PDA at room temperature and used inn this study.

***Pathogenicity of Phomopsis* sp.**

Pathogenicity test was modified the protocol from Yang *et at*. (2015). Each isolate of *Phomopsis* sp. was cultured on PDA at room temperature for 7 days. The asparagus stem length at 15 cm was cleaned with steriled water and air dried. The surface of asparagus stem was punctured 5 times by sterile needle. The mycelial plugs (5 mm diameter) of pathogen was transferred onto the wounded stem and kept in moisture chamber box for 5 days at room temperature. The PDA plugs without patgogen was used as control. The symptom was observed and the lesions measure diameter and photographed. Finally, the statistical analysis of pathogenicity was done with ANOVA analysis ($p<0.05$).

Dual culture test

P. asparagi RB-19 was grown on PDA for 7 days and incubated at room temperature. The agar pulg of *P. asparagi* RB-19 was placed on one side of PDA plate and the agar plug of pathogen was placed in opposite site, and incubated for 20 days at 25℃ in dark room. Colony diameters were measured. Th was performed withy four replications. The statistical analysis was done with ANOVA analysis ($p<0.05$).

Results

Morphology of P. asparagi

Characteristics of *P. asparagi* were studied (Figure 1 and Table 1) by visually observed under microscope. Lesions were formed on the asparagus stems as oval-shaped, longitudinal scars on stem. Lesions area was light brown

to dark brown and slightly darker margins. The center of lesions was light gray with pycnidia (Figure 1A and 1B). Pycnidia are immersed and dark brown to black and submersed in the stems (Figure 1C and 1D). The pycnidia were formed after 3 weeks and found only α-conidia and not seen β-conidia. The α-conidia was lucent, fusiform to eliipsoid, aseptate and biguttulate α-conidia (Figure 1F). Size of α-conidia showed in Table 1. The mycelia consisted of thin and thick hyphae with various sizes, ranch, smooth and lucent (Figure 1G). The colony character on PDA were raised undulate margins with white to gray (Figure 1H).

The three isolates of NB-02, SP-02 and SP-03 were selected and used to confirm species by sequencing. The isolates proved to be *P. asparagi* (KJ801804, LC203584 and JQ614011) in GenBank which 99% identity.

Pathogenicity

Pathogenicity test of each isolate was evaluatede on asparagus stem after 5 days inoculation. The results were divided into two groups including seven sample asparagus stems without lesion. Three asparagus stems had lesion from inoculated. *P. asparagi* RB-19 showed the large area of lesions of 110.25 mm, and followed by NB-02 and NB-14 which the lesions were 55.63 mm and 44.03 mm, respectively. The inoculated stems was infected and appeared symptom like a burn oval-shaped brown. The center of lesions on stem was light brown. The lesions from pathogenicity test was similar to the symptoms of stem blight collected in the fields. The pathogenicity test is shown in Figure 2.

Table 1. Size of α-conidia of ten *Phomopsis* sp. isolates

Fungal isolates	Location	Size of α-conidia	
		Width (μm)	Length (μm)
NB-02	Nonthaburi	2.2–3.1	6.7–8.4
NB-12	Nonthaburi	2.1–3.2	6.6–8.4
NB-14	Nonthaburi	2.1–3.5	6.9–8.8
NP-01	Nakhon Pathom	2.0–3.2	7.0–8.5
RB-10	Ratchaburi	2.0–3.5	6.7–8.9
RB-19	Ratchaburi	2.0–3.3	7.0–8.3
RB-23	Ratchaburi	2.1–3.4	7.2–9.1
SP-01	Suphan Buri	2.2–3.1	6.8–8.4
SP-02	Suphan Buri	2.0–3.2	6.9–8.3
SP-03	Suphan Buri	2.1–3.2	7.0–8.8

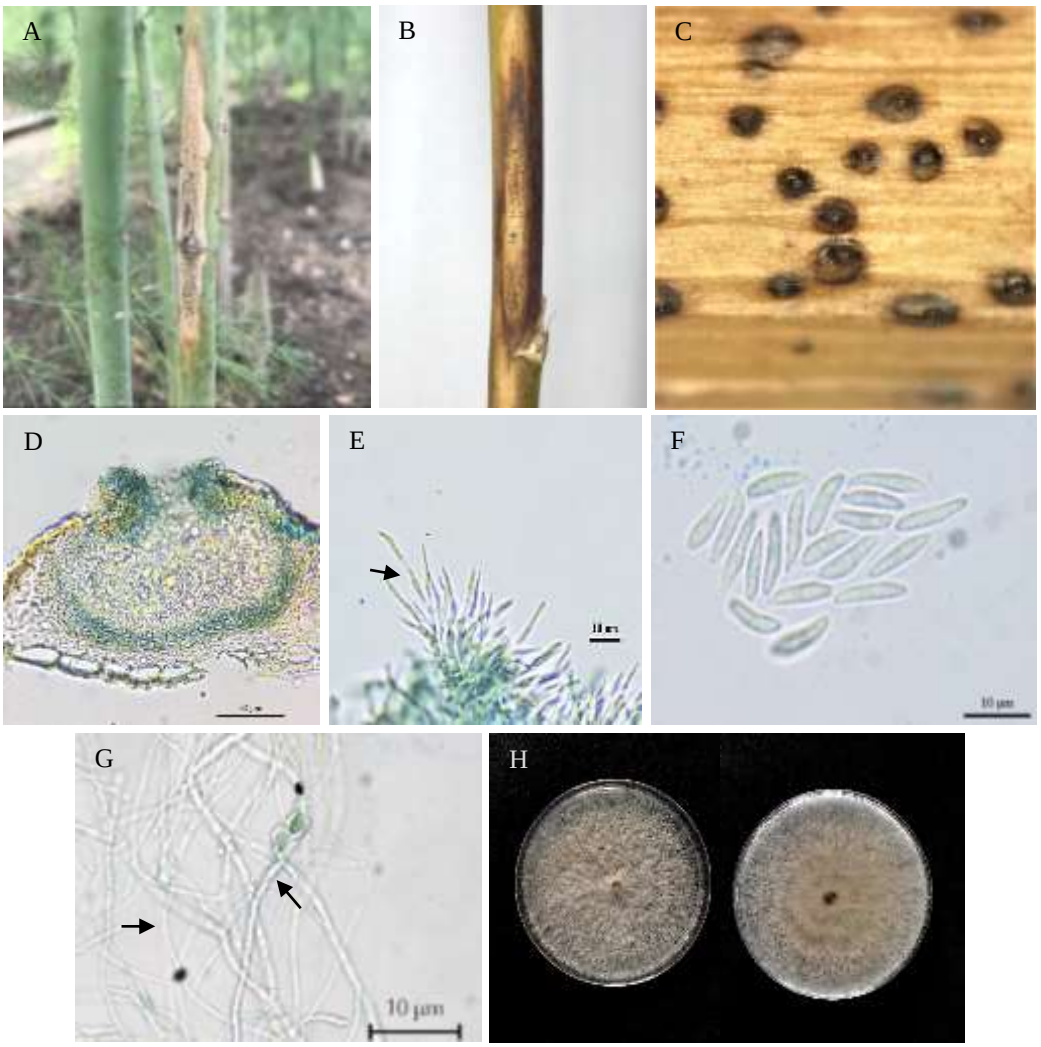

Figure 1. *Phomopsis asparagi* isolate RB-19. (A and B) lesion of stem blight disease on asparagus stem in field. (C and D) Pycnidium of *P. asparagi*. (E) conidiophore (F) α-conidia (G) thin and thick hyphae. (H) *P. asparagi* was cultured on PDA for 10 days at room temperature

Figure 2. Lesion of diseased from asparagus stems during the pathogenicity test that were infected with *P. asparagi* isolates RB-19, NB-02 and NB-14

Figure 3. Dual culture of two fungal strains, *N. hiratsuka* EU06 and *P. asparagi* RB-19

Dual culture test

Dual culture test was done between *P. agparagi* RB-19 and antagonists (*N. hiratsuka* EU06, *N. pseudofischeri* EU13, *N. aureola* CHA01-A01, *N. fennelliae* CHA03-A11, *N. spinosa* CHA09-A01, *Neosartorya* sp. EU35, *T. muroii* EU07, *T. muroii* EU18, *T. trachyspermus* EU23 and *T. muroii* CHA03-A03) which incubated at 25 ℃ for 20 days in dark room. The growth inhibition showed *N. hiratsuka* EU06 was the highest inbibition of 59.50%, followed by *N. pseudofischeri* EU13, *N. aureola* CHA01-A01, *N. spinosa* CHA09-A01, *N. fennelliae* CHA03-A11 and *Neosartorya* sp. EU35 which were 57.55, 57.24, 56.76, 55.96 and 55.26 %, respectively (Figure 3 and Table 2).

Table 2. Colony diameter (mm) of *P. agparagi* RB-19 and antagonists in dual culture test on PDA at 25 ℃ for 20 days

Antagonists	**Colony diameter of *P. agparagi* RB-19 (mm)**		**Growth inhibition (%)**
	Control	**Average**	
N. aureola CHA01-A01	90.00	38.48	57.24[b]
N. fennelliae CHA03-A11	90.00	39.64	55.96[abc]
N. hiratsuka EU06	90.00	36.45	59.50[a]
N. pseudofischeri EU13	90.00	38.21	57.55[b]
N. spinosa CHA09-A01	90.00	38.92	56.76[bc]
Neosartorya sp. EU35	90.00	40.27	55.26[cde]
T. muroii CHA03-A03	90.00	40.37	55.14[de]
T. muroii EU07	90.00	41.35	54.06[e]
T. muroii EU18	90.00	41.58	53.84[e]
T. trachyspermus EU23	90.00	48.46	46.16[f]

[a-f] mean are the different latter in the same colum were significant by Duncan Multiple Range Test (DMRT) at $p < 0.05$

Discussion

The morphology of *P. asparagi* showed the α-conidia size similar to Dinh *et al.* (2018) that found the size of α-conidia as 8.7-9.9 × 2.9-3.5 µm which cultured on PDA medium and Uecker and Johnson (1991) reported the size of α-conidia was 7-8 × 3 µm and not seen β-conidia. Davis (2001) reported not revealed the presence of β-conidia in inoculated stem. However, in this research was not seen beta conidia that may involve temperature. Vidic *et al*. (2013) reported β-conidia formed quickly on medium made of soybean seeds

and mature stems after exposure to low temperature. The species of *P. asparagi* was confirmed by compared with DNA sequences in BLAST. Comparsion with reference sequences in the database was revealed *P. asparagi* accession number include KJ801804, LC203584 and JQ614011 that showed 99% identity. The same characteristic of symptoms was found in infection of *P. asparagi* as reported by Yang *et al*. (2015) whio described that the mycelia can spread into host tissues in 4 days (Elena, 2007).

In the study of dual culture between *P. asparagi* RB-19 and ten antagonists observed that *N. hiratsuka* EU06 inhibited the growth of RB-19 as the highest inhibition of 59.50%. On the other hand, Soytong (2015) studied efficacy of *Neosartorya* and *Talaromyces* against coffee anthracnose who stated that dual culture test on PDA found *T. muroii* EU04 and EU18 can be controlled *C. coffeanum* to 85-90 % but *N. hiratsuka* EU06 can be against the least coffee anthracnose. Efficacy of *Neosartorya* sp. EU35 *and N. pseudofischeri* EU13 can be controlled pathogen more than 50% as same as in this reseach finding. In addition, other research were reported *N. fisheri* can be controlled the growth of other microorganisms (Waing *et al.*, 2015). Nonaka *et al*. (2015) reported *Talaromyces* sp. can be inhibited *Phomopsis* sp. FKA-62 and *T. tratensis* KUFA 0091 against *A. padwickii*, *B. oryzae*, *C. lunata* and *F. moniliforme* (Dethoup *et al*., 2017).

It concluded that **a**sparagus stem blight caused by *P. agparagi* which isolated from diseased samples from Nakhon Pathom, Suphanburi Ratchaburi and Nonthaburi province. Ten isolates of *P. asparagi* were morpgological and molecular identified. *P. asparagi* RB-19 was proved to be highest pathogenic to cause disease in asparagus. *N. hiratsuka* EU06 proved to be antaginized *P. asparagi* RB-19 causing the stem blight of asparagus.

Acknowledgements

This work wrer financially supported by King Mongkut's Institute of Technology Ladkrabang (grant number: A 118-0261-010). Speical thanks are directly expressed to Supattra Poeaim, Mayamor Soytong and Supanan Suksiri for supporting the antagonists.

References

Brunning, P. (2010). Asparagus Liliaceae Asparagacease. The InternationalWine and Food Society Europe and Africa Committee 103.

Cheah, L. H. (2006). Integrated management of new asparagus diseases. New Zealand Institute for Crop and Food Research.

Davis, R. (2001). Asparagus stem blight recorded in Australia. Austrasian Plant Pathology. 30:181-182.

Dethoup, T., Kaewsalong, N., Songkumorn, P. and Jantasorn, A. (2017). Potential application of a marine-derived fungus, *Talaromyces tratensis* KUFA 0091 against rice diseases. Biological Control. 119:1-6.

Dethoup, T. and Manosh, L. (2013). Studies of the efficacy of fungi in Family Trichocomaceae for controlling plant pathogenic fungi. Kasetsart University.

Dinh, T. L., Zaw, M. and Matsumoto, M. (2018). *Diaporthe* species complex occurring on *Asparagus kiusianus* in Japan. Journal of Plant Pathology. 1-7.

Diogo, E. L. F., Santos, J. M. and Phillips, A. J. L. (2010). Phylogeny, morphology and pathogenicity of *Diaporthe* and *Phomopsis* species on almond in Portugal. Fungal Diversity. 44:107-115.

Doyle, J. J. and Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. Focus. 12:13-15.

Elena, K. (2006). First report of *Phomopsis asparagi* causing stem blight of asparagus in Greece. 55:300.

Elena, K. (2007). Asparagus Diseases. The European Journal of Plant Science. 1:76-78.

Johnson, G., Weinberger, K. and Wu, M. H. (2008). The Vegetable Industry in Tropical Asia: Thailand.

Lu, G., Jian, W., Zhang, J., Zhou, Y. and Cao, J. (2008). Suppressive effect of silicon nutrient on *Phomopsis* stem blight development in asparagus. HortScience. 43:811-817.

Nonaka, K., Iwatsuki, M., Horiuchi, S., Shiomi, K., Omura, S. and Masuma, R. (2015). Induced production of BE-31405 by co-culturing of *Talaromyces siamensis* FKA-61 with a variety of fungal strains. The Journal of Antibiotics. 68:573-578.

Punithalingam, E. (1975) Some new species and combinations in Phomopsis. Trans Brit Mycol Soc. 64:427-435.

Sonoda, T., Uragami, A. and Kaji, K. (1997). Evaluation of *Asparagus officinalis* cultivars for resistance to stem blight by using a novel inoculation method. 32:1085-1086.

Soytong, M. (2015). Isolation and identification of genus *Talaromyces* and *Neosartorya* from soil and their efficacy against coffee anthracnose. (Master Thesis). King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

Suksiri, S., Laipasu, P., Soytong, K. and Poeaim, S. (2018). Isolation and identification of *Phytophthora* sp. and *Pythium* sp. from durian orchard in Chumphon province, Thailand. International Journal of Agricultural Technology. 14:389-402.

Tumtorn, P. (2018). Asparagus. Retrieved from http://www.agriman.doae.go.th/home/news/of%20newsyear%202560.html.

Udayanga, D., Liu, X., McKenzie, E. H. C., Chukeatirote, E., Bahkali, A. H. A. and Hyde, K. D. (2011). The genus *Phomopsis*: biology, applications, species concepts and names of common phytopathogens. Fungal Diversity. 50:189-225.

Udayanga, D., Liu, X., Crous, P. W., McKenzie, E. H. C., Chukeatirote, E. and Hyde, K. D. (2012). A multi-locus phylogenetic evaluation of *Diaporthe* (*Phomopsis*). Fungal Diversity. 56:157-171.

Uecker, F. A. and Johnson, D. (1991). Morphology and Taxonomy of Species of *Phomopsis* on Asparagus. Mycologia. 83:192-199.

Waing, K. G. D., Abella, E. A., Kalaw, S. P., Waing, F. P. and Galvez, C. T. (2015). Antagonistic interactions among different species of leaf litter fungi of Central Luzon State University. Plant Pathology and Quarantine. 5:122-130.

White, T. J., Bruns, T., Lee, S. and Taylor, J. (1990). PCR protocols: a guide to methods and applications. San Diego: Academic Press.

Yang, Y. Q., Lan, B., Jian, Y. L., Chang, D. D., Zhang, S. L. and Li, X. M. (2015). Infection Process and Pathogenic Mechanism of *Phomopsis asparagi*, the Asparagus Stem Blight Pathogen. Phytoparasitica. 44:11-18.

(Received: 13 September 2018, accepted: 31 October 2018)