

Phytopathogenic fungi as potential biocontrol agents against an invasive weed, *Asystasia gangetica*, at Sakambangan rubber plantation in Garut, West Java, Indonesia

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Abstract. Yulia E, Mahmudah AI, Rahayu A, Nasahi C, Maharani Y, Suganda T, Widayat D. 2022. Phytopathogenic fungi as potential biocontrol agents against an invasive weed, *Asystasia gangetica*, at Sakambangan rubber plantation in Garut, West Java, Indonesia. *Biodiversitas* 23: 4532-4538. Many weeds are invasive species causing both economic and ecological losses. *Asystasia gangetica* is one of invasive species from the Acanthaceae family and has now been established as one of the most important weeds of rubber plantation. Mechanical control of *A. gangetica* is ineffective, while chemical control is harmful to the environment. Thus, it is imperative to explore alternative control methods. This study aimed to carry out an inventory of pathogenic fungi isolated from *A. gangetica* and test their pathogenicity to *A. gangetica* and rubber seedlings. This research was conducted from November 2021 to February 2022. The methods used were field observation and laboratory test conducted at the Sakambangan rubber plantation, Garut District, West Java and the Phytopathology Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran. Pathogenicity test was carried out using water-culture method to grow the tested plants without soil. Fungi isolated from *A. gangetica* identified as *Fusarium* sp., *Pestalotiopsis* sp., *Colletotrichum* sp. and *Cercospora* sp. were tested to be pathogenic to *A. gangetica*, and deemed potential biocontrol agents. However, *Pestalotiopsis* sp. and *Cercospora* sp. were also pathogenic to rubber seedlings which make them ineligible as biocontrol agents for *A. gangetica* in rubber plantations.

Keywords: Acanthaceae, biocontrol, *Colletotrichum*, *Fusarium*, weed

INTRODUCTION

Weeds are important biological factor in crop production that cause yield reduction since they compete with the crops for water, light and nutrients to complete their life cycle (Korav et al. 2018). The presence of weeds affects the production of agricultural crops, one of which is rice (Estiati 2019). A number of weed species are reported as invasive species. The presence of those weeds alter structure and function of ecosystems and cause economic damage to managed landscapes (Adhikari et al. 2022). In many cases, the composition of species in the ecosystem is altered because native plants are unable to compete with invasive species and eventually become extinct. Weeds in rubber plantations compete with the rubber plants for nutrients, sunlight, water and space (Supawan and Hariyadi 2014). Burgos and Ortuoste (2018) mentioned that weeds compete with young rubber plants for moisture and nutrients strongly enough to reduce latex yield.

Asystasia gangetica (L.) T. Anderson is an annual herb that belongs to family Acanthaceae. It can grow in a mat-forming habit and smother neighboring ground plants, thus potentially affecting agriculture or reducing biodiversity

(Westaway et al. 2016). *Asystasia gangetica* is a weed pest of rubber, oil palm and coffee plantations and is on the alert list for environmental weeds of invasive plants that threaten biodiversity and has the potential to seriously degrade native ecosystems (Lucidcentral 2016). In rubber plantations, *A. gangetica* is the most common weed species and the only species with an absolute dominance category (higher than 10%) (Adnan et al. 2020).

Weed control methods can be grouped into mechanical, manual, thermal, cultural, chemical and biological techniques. However, any single method is not adequate to attain sustainable weed management (Burgos and Ortuoste 2018). In rubber plantations, mechanical (plowing and mowing) and chemical (herbicide application) are the primary methods used to manage weeds. However, both techniques have limitations or disadvantages as they are less effective on weeds with rhizomes and roots that are capable of sprouting, such as *A. gangetica*. In addition, they also pose risks to human health and the environment (Myers et al. 2016; Burgos and Ortuoste 2018). Thus, it is imperative to explore efficient and sustainable weed management options.

Biological control or biocontrol uses biological agents, which include parasitoids, predators, nematodes, viruses, insects, bacteria, fungi, and applied singly or in various combinations (Brodeur 2017). In deploying a weed biocontrol method, it should be noted that it is not possible to eradicate weeds, instead weed population can only be reduced to a level with a minimum environmental impact. Moreover, it is cheaper, long lasting, harmless to non-target plants, and has no residual effects (Telkar et al. 2015). Day and Witt (2019) mentioned that biocontrol of weeds has resulted in over 500 biological control agents being intentionally released against nearly 200 weed species including invasive weeds. Many of those biological agents have been highly successful and mostly with no recorded non-target impacts. In weed biocontrol, foliar fungal pathogens have been favored than other types of pathogens because they are generally more specific and readily dispersed by wind or rain splash (Morin 2020). Several pathogens including fungi are reported to be associated with *A. gangetica* (Tan and Tow 1992; Tan and Tow 1994; Braun et al. 2015; De Bruyn et al. 2015; Wyant et al. 2015; Soesanto et al. 2020). The aim of this study was to carry out an inventory of pathogenic fungi that infect *A. gangetica* and test their pathogenicity on *A. gangetica* plants as a biocontrol agent and its impact on rubber seedlings as non-target host.

MATERIALS AND METHODS

Study area

Survey and observation were carried out at a smallholder rubber plantation in Sakambangan Hamlet, Mekarwangi Village, Cibalong Sub-district, Garut District, West Java, Indonesia. The study site was an area of 1.25 ha with elevation of 70-75 meters above the sea level and situated at 7°40'33"S, 107°51'50"E (Figure 1). The plantation has a monoculture system.

Field assessment

The research method used was survey and field observation by determining the incidence and severity of the disease and observing the disease symptoms of *A. gangetica* at the study site. The sampling and determination of plant samples used random purposive sampling, namely sampling with certain considerations (Sugiyono 2017) by randomly selecting sample plants with disease symptoms that were easily accessible. Samples of *A. gangetica* plants with disease symptoms were collected. The plant materials were put into labeled Ziploc® plastic bags and then placed into a cooler box to be transported to the Phytopathology Laboratory, Department of Pests and Plant Diseases, Faculty of Agriculture, Universitas Padjadjaran. Samples were stored in a refrigerator at 4°C until further use.

Disease incidence was assessed at five observation points (2 x 2 m²) defined around the four corners of the almost rectangular field and in the corner. Disease incidence (percentage) was calculated by estimating percentage of *A. gangetica* plants showing disease symptoms at each observation point. Disease severity was scored on five *A. gangetica* plants randomly selected in each observation point using a modified 0-4 Horsfall-Barratt's scale (Table 1) (Bock et al. 2010) and then calculated using the formula:

$$I = \frac{\sum(n \times v)}{N \times V} \times 100\%$$

Where:

I : Disease severity (%)

n : Number of plants scored for each rating

v : Rating value/scale

N : Total number of plants scored

V : Highest scale

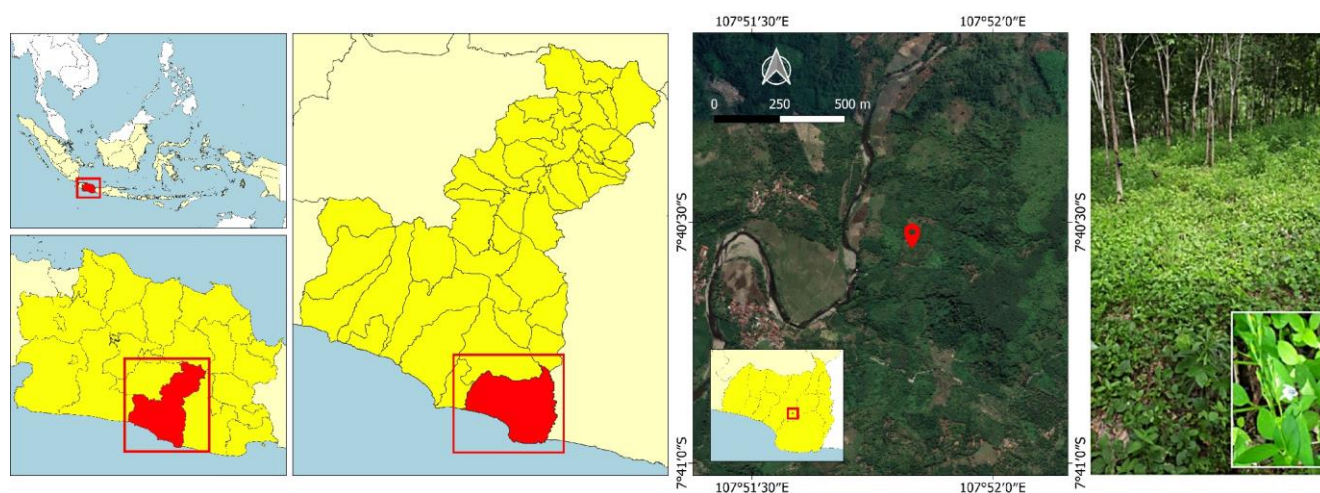


Figure 1. Location of Cibalong Sub-district, Garut District, Indonesia indicating the Walahar Forest (landmark) and the rubber plantation site (7°40'33"S, 107°51'50"E). Right = a very dense *Asystasia gangetica* thicket under the shade of rubber plants

Table 1. Disease severity scale (modified Horsfall-Barratt's scale) with reference to Bock et al. (2010)

Category	Severity	Class
0	Apparently infection-free	Healthy
1	Trace-25% leaves of the total plant area infected	Slight
2	26-50% leaves of the total plant area infected	Moderate
3	51-75% leaves of the total plant area infected	Severe
4	>75% leaves of the total plant area infected (or plant dead)	Very severe

Note: Horsfall-Barratt's scale [interval scales to assess disease severity developed by Horsfall JG and Barratt RW (1945)]

Laboratory work

Pathogen isolation and identification

The medium used for the pathogen isolation and maintenance was Potato Dextrose Agar (PDA). Pathogen isolation was carried out according to Agrios (2005). The symptomatic *A. gangetica* leaves were cut between the healthy and symptomatic parts. The leaf pieces were sterilized using 70% alcohol for 1 min followed by 1% sodium hypochlorite solution for 90 sec, then rinsed three times using sterile distilled water and dried by placing them on sterile filter paper. Next, the leaf pieces were placed on PDA growth medium and incubated at room temperature ($\pm 28^{\circ}\text{C}$) for 3-6 days. The grown mycelium was transferred to a new PDA medium as pure cultures.

Identification of pathogens was carried out macroscopically and microscopically. Macroscopic identification was performed on symptomatic *A. gangetica* specimens or on isolated fungal colonies. Microscopic identification was done by observing the fungus's morphological characteristics, such as hyphae, conidia, or other fungus structures. Identification was based on fungal identification book by Barnett and Hunter (1998).

Pathogenicity test

Pathogenicity tests were carried out on healthy *A. gangetica* plants to test the biocontrol potential and on rubber seedlings to test the non-target hosts. The tested *A. gangetica* plants were whole plants (intact plants) consisting of roots, stems, leaves, and flowers obtained from the field that placed in plastic containers containing 500 mL of water. Meanwhile, rubber seedlings were obtained commercially (± 80 cm height) which were placed in buckets filled with water during the testing. Inoculation was performed on *A. gangetica* leaves as all disease symptoms found in *A. gangetica* leaves in the field. Before inoculation, the tested plants' leaves were wiped using moist tissues and 70% alcohol to remove adhering dirt and sterilize the leaf surface. The leaves were then punctured using a sterile needle to place pathogen inoculum and to facilitate infection. Fungal mycelium (mycelium-agar plug \varnothing 5 mm) of seven-day-old cultures was used as pathogen inoculum while PDA-agar was plug applied in control treatment. Pieces of moist sterile cotton were placed in the inoculation points and the treated leaves were then wrapped using plastic wrap to keep moisture. The number of inoculated leaves was five leaves for each isolate. The

treated *A. gangetica* plants were then placed at room temperature ($\pm 28^{\circ}\text{C}$) in the laboratory but still received sunlight from outside while treated rubber seedlings were placed outdoors with an average temperature of about 28°C during the treatment. Observations were made on the appearance and development of lesions on the inoculated leaves. The diameter of the lesion was also measured at the end of the treatment and other characteristics such as sporulation that formed on the lesion were observed.

RESULTS AND DISCUSSION

Plant disease incidence, severity and symptoms

In addition to the dominance of *A. gangetica*, several weeds from the genera of *Ageratina*, *Clidemia*, *Axonopus*, *Centrosema*, *Cyclosorus* and *Melastoma* were also found in the study area. *Asystasia gangetica* grows well formed a dense ground cover under the shade of rubber plants and was also found along the roadside to the study site. Weed control in research site is usually carried out mechanically using hoes to cut the weeds and break up the soil while chemical control has never been done. The most dominant disease symptoms found in *A. gangetica* were leaf spot and leaf blight. Other symptoms that were relatively less included leaf yellowing and leaf fall. The latter symptoms were often associated or further symptoms of leaf spot or leaf blight. In general, leaf spot symptoms are described as brown colored leaf spot, some are dark brown or blackish brown and some have a yellow halo. The leaf blight symptom is a symptom of dark brown to blackish brown blight with chlorotic halo.

The percentages of disease incidence and severity at the five observation points ranged from 40-75% and 35-70%, respectively (Table 2). A high percentage of disease incidence and severity occurred when spot or blight symptoms were accompanied by yellowing and falling leaves. The disease incidence and severity were categorized as severe at most of the observation points, thus providing an estimation that the pathogens causing disease in *A. gangetica* plants are potential as biocontrol agents. Harding and Raizada (2015) mentioned that fungal pathogens that possess the potency of biocontrol agents must be able to cause relatively high damage to their hosts, including weed hosts.

Table 2. Disease incidence and severity on *Asystasia gangetica*

Site	Disease incidence (%) [†]	Disease severity (%) [†]	
Observation point I	65	55	Severe
Observation point II	70	65	Severe
Observation point III	60	50	Moderate
Observation point IV	75	70	Severe
Observation point V	40	35	Moderate

Note: [†]area of 2 x 2 m²

High disease incidence and severity are presumed because the cropping and environmental conditions support the disease development in *A. gangetica*. The *A. gangetica* growing condition was highly shaded by rubber plants and barely exposed to direct sunlight providing excellent conditions for the growth of the pathogens. Environmental factors such as temperature and humidity greatly affect the life of fungi, whereas pathogenic fungi can grow and develop properly under favorable environmental factors (Suryani et al. 2020). Generally, the optimum, minimum and maximum temperatures for fungal growth are between 22-27°C, 2-5°C and 35-40°C, while the temperature at the research site during sampling was 32°C. *Asystasia gangetica* at the study site grew side by side with various other weeds, which also showed disease symptoms on the leaves. Even in some cases, *A. gangetica* plants were entangled with other weeds, making it highly possible for cross-infections that could have contributed to high disease occurrence. On the other hand, Golan and Pringle (2017) mention that wind is the most commonly considered vector of long-distance fungal dispersal. All of those conditions supported disease incidence at all observation points. Meanwhile, the environmental conditions were assuredly similar among the observation points, so there were no specific factors that could result in differences in fungal infections or the disease incidence and severity at each observation point.

Fungal species associated with disease symptoms in *Asystasia gangetica*

Figure 2 presents the characteristics of fungal species associated with leaf spot and blight symptoms of *A. gangetica*. The pathogens were identified as *Fusarium* sp., *Pestalotiopsis* sp., *Colletotrichum* sp. and *Cercospora* sp. The disease symptom caused by *Fusarium* sp. was a brown to blackish-brown blight with yellow chlorosis (halo) surrounding the blighted area. The symptom was generally found on the edges or tips of the leaves but could also be found in the center. Fungal colony was white in color and yellowish-brown reverse with cottony texture mycelium. Macroconidia were hyaline with typically canoe-shaped, several-celled, slightly curved at the pointed ends while microconidia were one-celled with an oblong shape. Thick-walled chlamydospores were found in the middle or ends of hyphae. Meanwhile, *Pestalotiopsis* sp. caused symptoms of a light brown leaf spot with dark brown and thick margin. The colony was white and slightly brownish-white

reverse with flowery texture composed of dense and thin mycelium while hyphae were hyaline. Conidia were dark, several-celled with hyaline pointed end cells with two or more hyaline apical appendages. Disease symptom caused by *Colletotrichum* sp. was characterized by blackish-brown leaf spots, which were initially round in shape then turned irregular on almost the entire leaf surface. The spots might coalesce to form extensive lesions and the leaves turned yellow or chlorosis, died, and fell. Fungal colony was white and yellowish in the middle at first, but turned grey in color with white and a faint pink color that appeared in the middle. The reverse colony color was white with slightly yellowish-brown center. Conidia were hyaline, one-celled and cylindrical in shape with rounded ends. Leaf spot symptom that was presumably caused by *Cercospora* sp. was blackish-brown spots. Grey color colony with a dull white color in certain parts and cottony texture mycelium. Hyphae were branched, short septate and hyaline, but some tended to brown.

Pathogenicity test results

Pathogenicity test on *A. gangetica* plants showed that the inoculations of *Fusarium* sp., *Pestalotiopsis* sp., *Colletotrichum* sp. and *Cercospora* sp. caused necrotic lesions on the leaves of *A. gangetica* compared to the control treatment without the formation of lesions (Figure 3). Discoloration occasionally found at the inoculation points of the control treatment was a mechanical damage resulting from the injury during inoculation. Based on these results, the four fungal species were confirmed as pathogens of *A. gangetica*.

Lesion diameter was measured at eight days after inoculation or at the end of observation with the diameter ranging from 1.06-1.98 cm. The formed necrotic lesions were generally circular in shape at first because they developed from round mycelium-agar plugs, then developed into larger and irregularly shaped lesions, ranging in color from light brown to dark brown or blackish brown. Lesion symptoms that appear and develop progressively in *A. gangetica* plants in this pathogenicity test are considered to be the result of pathogen infection. The lesions that were initially formed in the inoculation area then developed into larger lesions or blights, gradually causing a yellowish color (chlorosis) on the leaves of *A. gangetica* or even causing the whole leaves to turn yellow and die.

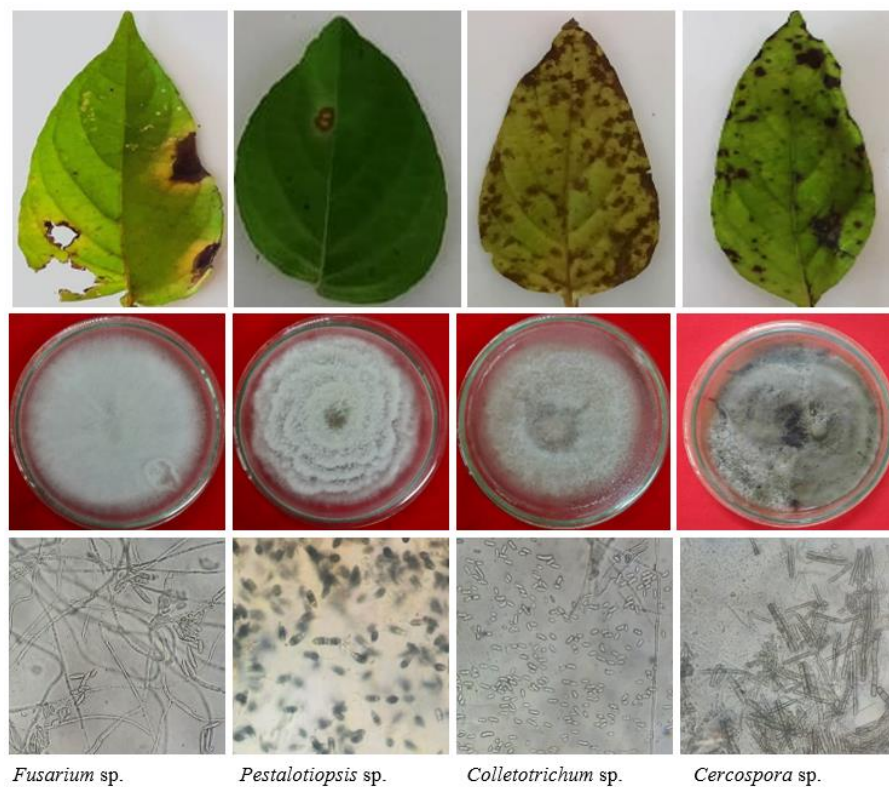


Figure 2. Fungal pathogens associated with leaf disease symptoms of *Asystasia gangetica*

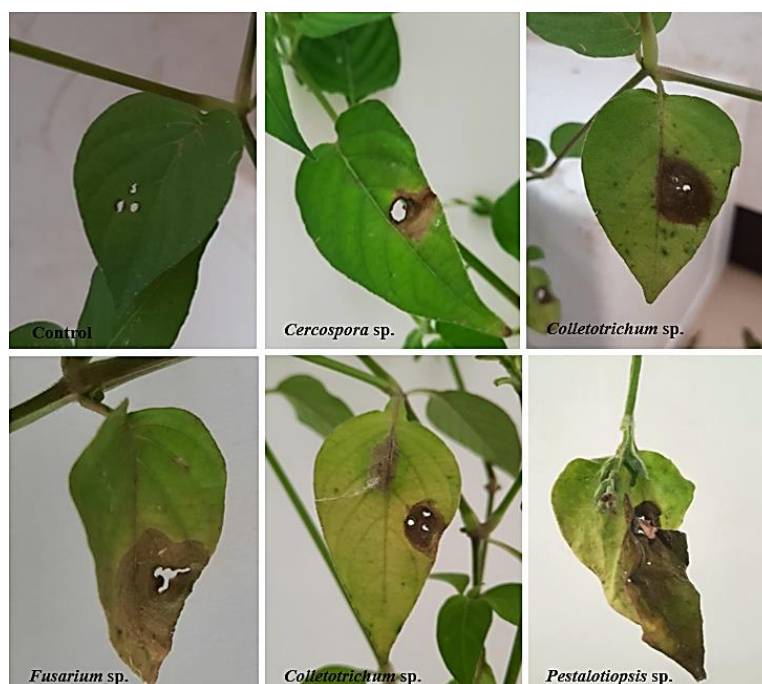


Figure 3. Symptoms development on *Asystasia gangetica* leaves in pathogenicity test

These results indicate the ability of *Fusarium* sp., *Pestalotiopsis* sp., *Colletotrichum* sp. and *Cercospora* sp. to cause damage to *A. gangetica* and also their potential as biocontrol agents of *A. gangetica*. Recently, Soesanto et al. (2020) found that *Fusarium* sp. was a pathogen in several

broadleaf weeds and had potential as a biocontrol agent for those weeds. Previously, *Colletotrichum gloeosporioides* and *C. dematium* were reported by Tan and Tow (1992; 1994) to cause severe infection in *A. gangetica* and *A. nemorum* and also mentioned that the *Asystasia* genus was

susceptible to *Colletotrichum* sp. infection. Meanwhile, *Pestalotiopsis vismiae* was reported to have potential as a biocontrol agent in controlling several weeds (Radi and Hamed 2017), but the role of *Pestalotiopsis* sp. as a biocontrol agent for *A. gangetica* is not known or has not been reported. Likewise, *Cercospora justiciicola* and *Cercospora* cf. *malloti* were reported as pathogens in *A. gangetica* and *A. salicifolia* (Braun et al. 2015; Nguanhom et al. 2015; Cheewangkoon et al. 2021), but their potency as biocontrol agents for *A. gangetica* has not been reported.

All *A. gangetica*-origin pathogenic fungi of *Fusarium* sp., *Pestalotiopsis* sp., *Colletotrichum* sp. and *Cercospora* sp. were used in the pathogenicity test on rubber seedlings. The test results showed there was no formation of lesions or symptoms of infection on the rubber leaves in control treatment. This proves that the control treatment showed good results, clean without any contaminations. Inoculation of *Colletotrichum* sp. showed the same results as the control, which means that the infection is absent or no lesions formed. This result indicates that *Colletotrichum* sp. of *A. gangetica*-origin is not pathogenic to rubber seedlings although the genus *Colletotrichum* has been reported as a pathogen of rubber plants. Liu et al. 2018 reported that anthracnose is the main disease on rubber plants caused by several *Colletotrichum* species, namely *C. fruticola*, *C. siamense*, *C. ledongensis*, *C. Boninense*, and *C. australisinese* with *C. siamense* and *C. australisinese* as major causative agents of the disease.

Inoculation of *Fusarium* sp. on the rubber leaves did not produce obvious symptoms. Although there was discoloration around the inoculation point, there was no visible necrotic symptom development. This discoloration may be due to the toxin produced by *Fusarium* since *Fusarium* produces toxins during plant-pathogen interaction (Perincherry et al. 2019). Thus, from this result it can be assumed that *Fusarium* sp. of *A. gangetica*-origin is not pathogenic to rubber seedlings. Reported fusarium diseases in rubber plants include stem rot caused by *F. oxysporum* which infects roots, stem bases and stems and gummosis disease caused by *F. solani* on rubber stems (Li et al. 2014; Huang et al. 2016). *Fusarium* sp. reported in rubber seedlings infects the stem cause grafting connection and stem bark rots and the death of rubber seedlings (Alimin et al. 2019). This *Fusarium* sp. of *A. gangetica*-origin may be of different species with those *Fusarium* pathogenic to rubber.

Pestalotiopsis sp. infection showed the most obvious lesion symptoms compared to other fungi. The lesions were blackish-grey necrosis that developed from the inoculation points. The genus *Pestalotiopsis* is reported to have a wide range of host plants including rubber plants. There have been reports of outbreaks of disease or severe infections in Indonesia and other countries due to *Pestalotiopsis* sp. or *Pestalotiopsis microspora* in particular, which is a pathogen that causes leaf blight on rubber plants (IRCo 2019). With the characteristics of this wide host range, it can be assumed that *Pestalotiopsis* sp. isolated from *A. gangetica* can also infect rubber plants and vice-versa.

The pathogenicity test of *Cercospora* sp. on rubber seedlings showed symptoms of black spots around the

inoculation point and blackish-grey lesions developed from the initial spots. Thus, the results of this test show that *Cercospora* sp. of *A. gangetica*-origin can infect rubber plant seedlings. There have been no reports of important diseases caused by *Cercospora* sp. on rubber plants. However, the pathogenic fungus *Corynespora cassiicola*, which is originally classified in the *Cercospora* group, is the main pathogen of rubber plants. Symptoms of *C. cassiicola* infection in rubber plants include fish-bone necrosis and spots which ultimately cause the leaves to fall throughout the year (Umoh and Fashoranti 2018). Meanwhile, Sajar et al. (2017) and Sajar (2018) reported that *C. cassiicola* can infect *A. gangetica* with initial symptoms on the leaves in the form of black spots or yellow spots which can then develop into brown or black spots with yellowish chlorotic halos that could result in the death of all leaf tissue. Therefore, *C. cassiicola* is also reported to have potential as a biocontrol agent in the control of *A. gangetica*.

In general, based on the results of pathogenicity tests, there are two fungal pathogens that are pathogenic to *A. gangetica* but do not infect rubber seedlings, namely *Colletotrichum* sp. and *Fusarium* sp. Thus, these two fungal species have the potential as biocontrol agents to control *A. gangetica* in rubber plant cultivation. Meanwhile, despite causing severe symptoms in *A. gangetica*, the use of the pathogenic fungi *Pestalotiopsis* sp. and *Cercospora* sp. of *A. gangetica* cannot be considered because they can also infect rubber plants. Additional future research directions include molecular identification of each fungal isolate, as well as efficacy testing of the fungi on other rubber plantations or on other weed species.

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