

# The Potensial of *Fusarium* sp. and *Chaetomium* sp. as Biological Control Agents of Five Broad-Leaf Weeds

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## Abstract

Weeds are a major problem in crop cultivation, either in food crops, horticulture, plantations or forests and cause a decrease in the quality and quantity of production. Weed biocontrol, especially by using plant pathogenic fungi, has received attention but is still lacking in application. The purpose of this study was to determine the potential of *Fusarium* sp. and *Chaetomium* sp. as biological control agents against five broad-leaf weeds (*Asystasia gangetica* L., *Ageratum conyzoides* L., *Synendrella nodiflora* (L.) Gaertn., *Wedelia trilobata* (L.) U.S. Hitchc. and *Amaranthus spinosus* L.). The variables observed were the incubation period, disease incidence, disease intensity, as well as weed fresh and dry weight. The results of this study showed that the two pathogenic fungi, *Fusarium* sp. and *Chaetomium* sp., can cause a more intensive disease in *A. conizoides* than *A. spinosus*. *A. gangetica*, *S. nodiflora* and *W. trilobata*; however, the fungi have not been able to inhibit the growth and kill the weeds. Therefore, improvement need to be done by modifying the media to increase the ability of fungi to control weeds.

Keywords: biological control; broad-leaf weeds; Chaetomium sp.; Fusarium sp.

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## **INTRODUCTION**

Weed is a major problem that arises from the beginning of planting preparation until near harvest period in crop cultivation, particularly food crops, horticulture, plantations and forests (Qasem and Foy, 2001). According to Fatonah et al. (2013), the presence of weeds in the middle of cultivated plants can cause substantial losses in the form of a decrease in the quality and quantity of production. This happens because of the high competitiveness of weeds against cultivated plants or staple plants in obtaining nutrients, water, places and sunlight. Losses due to weeds in cultivated plants vary, depending on the type of plant, climate, type of weed and agricultural practices. According to Gharde et al. (2018), in India, the total actual economic loss of about

USD 11 billion was estimated attributable to weeds alone in 10 major crops viz. groundnut (35.8%), soybean (31.4%), green gram (30.8%), pearl millet (27.6%), maize (25.3%), sorghum (25.1%), sesame (23.7%), mustard (21.4%), direct-seeded rice (21.4%), wheat (18.6%) and transplanted rice (13.8%). Weeds exhibited the economic yield losses to the wheat crop, which might range from 24% to 39.95% (Oad et al., 2007). The decline in crop yields due to weeds in Indonesia was estimated to reach 10-20% (Solahudin et al., 2010).

Weeds, based on the morphological characteristics, can be divided into narrow-leaf weeds and broad-leaf weeds. Various species of narrow leaf weeds found in maize and rice include *Cynodon dactilon*, *Digitaria ciliaris*, *Axonopus compressus*, *Eleusine indica*, *Ischaemum* 

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timorense, Panicum repens, Paspalum conygatum, Ottochloa nodosa, Echinochloa crusgalli, Imperata cylindrica and Setaria sp. (Arif et al., 2011; Anwar et al., 2012; Golmohammadi et al., 2018). The most widely found broad leaf weed species are Tridax procumbens (L.), Emilia sonchifolia D.C ex Wight, Ageratum conoyzoides L. and Synedrella nodiflora (L.) Gaertn (Tjitrosoedirjo et al., 2010).

The presence of weeds gives a negative influence on plants because of its adverse nature, namely as an allelopathy, allelomediation and allelopoly (Qasem and Foy, 2001). Allelopathy is a chemical compound produced by plants through washing, root exudation, evaporation and the decay of plant organs and thus inhibiting growth and development, as well as reducing plant production (Mushtaq and Siddiqui, 2017). According to Pereira et al. (2016), weeds are also allelomediation and allelopolistic. Allelomediation is the role of weeds as a place to live for certain types of pests, while allelopoly is the nature of weeds monopolizing water, nutrients,  $CO_2$ ,  $O_2$  and sunlight for plants.

Weed control can be done mechanically, technically and chemically (Marpaung et al., 2013). There are negative effects of mechanical control on weeds, such as the required cost and time that can influence other crop operations. effectiveness that is highly dependent on weather and soil conditions and correct time of application, lower efficacy of intra-row weed control, required skilled labor and high capital cost (Cherati et al., 2011; Karmiłowicz, 2019). The chemical or herbicide control of weeds is more effective than other controlling techniques, but it can have a negative impact on the environment if not wisely performed. However, this raises various problems, including the high cost of supplying herbicides, environmental pollution, decreasing soil organic matter and weeds being tolerant of certain types of herbicides (Kraehmer et al., 2014). Weed control using herbicides applied in the field  $\pm$  only 20% hit the target, while the other 8% fell, accumulated and left residue in the soil. The accumulation causes pollution on agricultural land. With the high level of danger of herbicides, it is necessary to look for the alternative controlling techniques called biological control (Latifa et al., 2015) that are environmentally friendly.

Weed biocontrol, especially by using plant pathogenic fungi, has recently received serious

attention from the researchers in developed countries, because it has minimum negative side effects on the environment (Currie et al., 2020). Weed biological control is advantageous because it is environmentally friendly, reduces the use of pesticides, decreases environmental contamination, avoids health risks for farmers, is permanent, saves energy, does not pollute and is inexpensive compared to other methods and is sustainable (Harding and Raizada, 2015). In addition, biological control of weeds, especially by groups of fungi, has a high specificity (Harding and Raizada, 2015; Radi and Banaei-Moghaddam, 2020). However, weed control by using this pathogenic fungus is still lacking. The research on pathogenic fungi to control broad-leaf weeds has been initially carried out by exploring the fungus on broad-leaf weeds in the field and furthermore, the fungus needs to be tested for virulence on several broad-leaf weeds. This study aims to determine the potential of Fusarium sp. and Chaetomium sp. as biological control agents against five broad-leaf weeds.

### MATERIALS AND METHOD

The research was carried out at the screen house, the Faculty of Agriculture, University of Jenderal Soedirman, Purwokerto, Indonesia, from March to August 2019. A split plot design was used with the main plot of a type of pathogens, including control, Fusarium sp. and Chaetomium sp. and subplots of five broad-leaf weeds, consisting of Asystasia gangetica L. (chinese violet, Acanthaceae), Ageratum conyzoides L. (billygoat-weed, Asteraceae), Synendrella nodiflora (L.) Gaertn. (nodeweed, Asteraceae), Wedelia trilobata (L.) U.S. Hitchc. (Bay Biscayne creeping oxeye, Asteraceae) and Amaranthus spinosus L. (spiny amaranth, Amaranthaceae). Based on these treatments, 15 treatment combinations were obtained and each treatment combination was repeated three times.

The propagation of pathogenic fungi was carried out with potato dextrose broth (PDB). A total of 5 cork of fungal culture isolates ( $\pm$  5 mm, diameter) in a Potato dextrose agar (PDA) were put into 250 ml of PDB and shaker for 7 days at 150 rpm in room temperature. The target weed used in the test was grown in polybags containing soil media and manure (3 : 1) and allowed to grow for 14 days. The application of pathogenic fungi was carried out when the target weed was 14 days

by spraying on the underside of the leaf (Gudesblat et al., 2009). The density of the pathogenic fungus used was  $1 \times 10^7$  conidia mL<sup>-1</sup>.

The variables observed were the incubation period, disease incidence, disease intensity, weed fresh weight and weed dry weight. The incubation period was observed from inoculation until the initial symptoms appeared. The disease incidence was calculated using the formula (Noordzij et al., 2010):

$$DI = \frac{n}{N} \times 100\%$$

Where DI = disease incidence; n = number of diseased plants; and N = number of observed plants. The disease intensity was monitored weekly using the formula:

$$DN = \sum \frac{n \times v}{Z \times N} \times 100\%$$

where DN = disease intensity; n = number of leaves in certain disease symptom categories; v =scale value in each category of disease symptoms; Z = highest scale value of disease symptom category; and N = number of leaves observed. The scale values of disease symptoms were determined as presented in Table 1.

The number of seeds produced by weeds, fresh weights and dry weights were measured at the end of the research activities. The data obtained were analyzed using the F test at  $\alpha$  5%, if there was a real improvement followed by Duncan's Multiple Range Test (DMRT) with  $\alpha$  5%.

Table 1. Score value (Asmaliyah et al., 2016)

Score value	Disease symptom level
0	No disease symptom (healthy plants)
1	Disease symptom $\leq 10\%$
2	Disease symptom $11 < x \le 25\%$
3	Disease symptom $26 < x \le 50\%$
4	Disease symptom $51 < x \le 75\%$
5	Disease symptom > 756%

#### **RESULTS AND DISCUSSION**

The pathogenic fungi caused some symptoms on weeds. Leaf blights, leaf spots, root rot and anthracnose were the common symptoms attributed to fungal pathogens tested on different parts of the weeds. Table 2 indicates that single treatment, with either pathogenic fungi or kind of weeds and the combination treatment between pathogenic fungi and kind of weeds performed significantly difference for the incubation period and differed disease intensity and diseased incidence. The kind of weed as a single treatment influenced all pathosystems and growth components significantly. Meanwhile, the pathogenic fungi and their combination with kind of weeds differed at disease intensity and incidence but they did not differ significantly at growth components. The virulent tests of these pathogens had been done on cultivated plants, namely tomatoes, peanuts and cucumbers. The results of the testing of the two fungi (Fusarium and Chaetomium) were not able to cause disease to the three plants.

Table 2. The results of a variety of influences of pathogenic fungi on five types of broad-leaf weeds

Variable	C (pathogenic fungi)	G (weeds)	CXG
Incubation period	**	**	**
Disease intensity	*	**	*
Disease incidence	*	**	*
Weeds fresh weight	ns	**	ns
Weeds dry weight	ns	**	ns

Note: \* = different; \*\* = significantly different; ns = not significantly different

#### Single treatment of weed pathogenic fungi

The single treatment of *Fusarium* sp. and *Chaetomium* sp. was not different in the incubation period (Table 3). It is suspected that both weed pathogenic fungi have the same virulence in causing symptoms of the disease. This condition corresponds to the intensity of

the disease and the incidence of the disease (Table 3). The ability of both pathogenic fungi shows the same virulence to cause disease symptoms in the same test weed. It is deduced that these two fungi are pathogenic fungi on weeds because the fungi could perform symptoms and caused diseases. According to Casadevall (2007) and Longdon et al. (2015), virulent

pathogens are able to quickly infect their host and produce more inoculums when compared to less virulent pathogens. It is suspected that the fungus *Fusarium* sp. has infectious ability and is supported by the ability of *Fusarium* enzymes to degrade weed cells.

Table 3.	The incubation period, disease incidence, disease intensity, weed fresh weight and weed dry					
weight in the virulence test of wide-leaf weed pathogenic fungi						

	Incubation	Disease	Disease	Weeds fresh	Weeds dry
Treatments	period	incidence	intensity	weight	weight
	(DAI)	(%)	(%)	(g)	(g)
Pathogenic fungi					
Control (C0)	42.00a	0.00b	0.00a	31.28a	5.81a
Fusarium sp. (C1)	12.98b	10.69ab	6.17b	32.02a	4.60a
Chaetomium sp. (C2)	10.93b	13.18a	7.35b	30.42a	4.63a
Kind of weeds					
Asystasia gangetica (G1)	18.89b	10.13ab	2.10b	31.78bc	4.46ab
Ageratum conyzoides (G2)	17.48b	18.48a	14.43c	10.70c	2.62b
Synedrella nodiflora (G3)	16.93b	6.20b	1.93b	38.56ab	6.21ab
Wedelia trilobata (G4)	39.26a	0.48b	0.23a	18.62bc	2.44b
Amaranthus spinosus (G5)	17.20b	4.50b	3.84b	56.56a	9.33a
Combination of the fungi and	d weeds				
C0G1	42.00a	0.00c	0.00a	28.56a	3.52a
C0G2	42.00a	0.00c	0.00a	7.00a	3.15a
COG3	42.00a	0.00c	0.00a	42.00a	6.29a
C0G4	42.00a	0.00c	0.00a	15.53a	2.23a
C0G5	42.00a	0.00c	0.00a	63.33a	13.86a
C1G1	8.00c	12.06bc	2.80cd	38.56a	5.39a
C1G2	5.56c	25.92a	21.10e	10.78a	2.17a
C1G3	4.44c	7.39bc	3.29cd	45.33a	7.61a
C1G4	42.00a	0.00c	0.00a	19.22a	2.40a
C1G5	4.89c	8.09bc	3.68d	46.22a	5.42a
C2G1	6.67c	18.34ab	3.51cd	28.22a	4.47a
C2G2	4.89c	29.51a	22.19e	14.33a	2.54a
C2G3	4.33c	11.20bc	2.51abcd	28.33a	4.73a
C2G4	33.78b	1.44c	0.70abc	21.11a	2.70a
C2G5	5.00c	5.42c	7.84d	60.11a	8.71a

Note: Numbers followed by the same letters in the same column and the same type of treatment are not significantly different based on DMRT  $\alpha$  5%; DAI = day after inoculation

This is in line with the results of study by Michielse and Martijn (2009) that *F. oxysporum* can damage plant tissues because it produces enzymes that degrade compounds contained in cells. Sun et al. (2014) added that *Fusarium* sp. can produce enzymes  $\beta$ -glucosidase, amylase, pectinase, silanase and cellulase (Dwivedi and Enespa, 2015; Basak and Rangan, 2018). The existence of these enzymes causes damage to host plant cells because they can break down pectin, which is a component of cell walls. In addition, the cellulolysis enzymes degrade cell membranes in plant tissue, which can cause damage and disease in host plants. Meanwhile, pathogenic fungus *Chaetomium* sp. as an antagonistic fungus are found in various habitats (Sunayana and Prakash, 2012). This fungus is known to produce lysis enzymes and many other secondary metabolites involved in its virulence mechanism (Zhang et al., 2012). Al-Kharousi et al. (2015) reported that the fungus *Chaetomium* sp. produce cellulase enzymes that degrade cellulosic biomass.

Both weed pathogenic fungi produce enzymes lysis of very complex plant biomass, which mainly contains cellulose. Lignocellulose biomass degradation requires a sophisticated set of enzymes. The complexity of carbohydrate polymers and their cross-linking with lignin require a complex set of enzymes to allow polysaccharide access and release fermentable sugars. Lignocellulose basically consists of plant cell wall components (Mota et al., 2018). In contrast, the two fungi alone had no effect on the components of weed growth, i.e., weeds fresh and dry weights, when compared to control (Table 3). In addition, the presence of a barrier factor or structural resistance on the surface of weed leaves can cause low pathogenic fungal infections (Caffall and Mohnen, 2009).

#### Single treatment of weed types

In Table 3, it appears that the type of weed affected the component of the pathosystem. Wedelia trilobata showed the fastest incubation period compared to other weeds. Wedelia trilobata had the longest incubation period of 39.259 DAI. According to Qi et al. (2014), this is presumably because Wedelia trilobata has the thickest leaf thickness among all weeds so that pathogens require a little longer time to infect the weeds. In addition, Wedelia trilobata is a plant that has allelopathic properties. This causes Wedelia trilobata to become less susceptible to inoculated pathogenic fungi and these weeds can fight independently in their own tissues using secondary metabolites such as phenolic, terpenoids, alkaloids, steroids, polyacetylene and essential oils that drive allelopathic activity (Xianxing et al., 2005). The weed has wider and thinner leaves, so it is infectious on physiological properties, leaf area allocation, bud allocation and growth rate so that the leaves morphology can influence photosynthesis rate, transpiration, nitrogen content in leaf tissue, efficient use of nitrogen and efficient use of water (Wu et al., 2012).

Although *Wedellia trilobata* was shown the most infectious, the development of symptoms of the weeds indicated low disease intensity, when compared with other weeds (Table 3). Ageratum conyzoides actually exhibited a higher disease incidence and disease intensity than other weeds and was significantly different from Synedrella nodiflora, Wedelia trilobata and Amaranthus spinosus. A. conyzoides have amphistomata properties, with stomata anomositis and anisositis, being the first more commonly found (Santos et al., 2016). The large number of stomata causes these weeds to be more easily infected by the pathogenic fungus conidia. According to Sexto

and Howlett (2006), some fungal pathogens enter the host via natural openings (stomata of plants) or even wounds, whereas others secrete toxins and/or enzymes, apply mechanical force, or subvert cellular processes of the host.

This is consistent with the opinion of Gudesblat et al. (2009) and Zeng et al. (2010), that fungal and bacterial pathogens enter and infect the leaves through the stomata, which is shown in a lot of tropical movements towards the stomata. After the infection, microbes can influence stomata behavior in a variety of ways, a fact that is associated with interactions between fungi and plant compounds secreted during plant pathogen interactions. This is supported by disease intensity data (Table 3), which show that the disease intensity in *A. conyzoides* was higher and significantly different compared to all tested weeds.

According to Dalimartha (2002), Ageratum convzoides has thin leaves covered with feathers or hairs (trichomes) on the upper and lower surface of the leaf. The presence of hairs or feathers is one of the factors driving the pathogen infection. The presence of these hairs or feathers allows the pathogen to stick to the hairs or feathers and the pathogen penetrates the lower surface of the leaf, enters the host plant's body tissue and then infects its host plant (Ogbonna and Umunna, 2017). A. conyzoides indicated as more susceptible weed resulted the high disease intensity and the disease incidence. The infection of pathogenic fungi to plants is also affected by the pathogenic virulence and suitable environments for the pathogen development (Chakraborty and Newton, 2011; Velásquez et al., 2018). For A. conyzoides, the wide-leaf size will increase moisture under a high canopy, which allows pathogenic fungi to grow and develop, as well as infect plants (Gudesblat et al., 2009). This is supported that A. conyzoides has the low weight of wet and dry weeds, which are significantly different from other weeds. The decrease in wet and dry weeds of A. conyzoides is not entirely due to the morphological characteristics of the weeds, but also from pathogenic fungal infections. This is in line with more stomata found on A. conyzoides than other weeds (Santos et al., 2016).

## Treatment combination of pathogenic fungi and weeds

The combination between *A. conyzoides* and *Fusarium* sp. and *Chaetomium* sp. exerted

a highly significant effect on all components of the ecosystem compared to the other combinations (Table 3). This is consistent with the single treatment. The ability of weed pathogenic fungi to infect weeds is due to their ability to produce a number of enzymes, specifically cellulose degrading enzymes, which are compounds making up leaf cell walls (Dwivedi and Enespa, 2015; Basak and Rangan, 2018). In addition, the morphological weed leaves also determines the success infection of weed pathogenic fungal by developing the disease symptoms. The infection of fungal microbes to attack plant tissues has many strategies, to optimize growth and to multiply themselves. Bacteria and viruses, as well as some opportunistic fungal parasites, often depend on natural holes or wounds for invasion. In contrast, many true phytopathogenic fungi have developed mechanisms to actively cross barriers to the outer structure of plants, cuticles and cell walls of the epidermis. Fungi generally secrete a number of hvdrolvsis enzymes. including cutaneous. cellulase, pectinase and protease (Uzma et al., 2016).

## CONCLUSIONS

The two pathogenic fungi, *Fusarium* sp. and *Chaetomium* sp., can cause greater disease in *A. conizoides* than *A. spinosus*. *A. gangetica*, *S. nodiflora* and *W. trilobata*. However, the fungi have not been able to inhibit the growth of weeds and kill them and hence, the media need to be modified to improve ability of fungi to control weeds.

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