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Application of Three Weed Pathogenic Fungi on Bok choy (*Brassica rapa* subsp. *chinnensis*)

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Abstract. Weed pathogenic fungi play an important role in biological control of weeds. Weeds grow a lot among cultivated plants, the role of weed pathogenic fungi to cultivated plants is not yet known. This research aimed to determine the effect of weed pathogenic fungi on bok choy. The research was carried out in Tambaksogra Village, Sumbang District, Banyumas Regency at an altitude of 100 m above sea level. Randomized block design was used with treatments of weed pathogenic fungi (*Fusarium* sp., *Curvularia* sp., *Chaetomium* sp., *Fusarium* sp. + *Curvularia* sp., *Fusarium* sp. + *Chaetomium* sp., *Curvularia* sp. + *Chaetomium* sp., and *Fusarium* sp. + *Curvularia* sp. + *Chaetomium* sp.) repeated four times. The observed variables included symptoms, incubation period, disease intensity, disease incidence, infection rate, AUDPC, number of leaves, plant height, and plant fresh and dry weight. Results showed that Weed pathogenic fungi of *Curvularia* sp. and *Chaetomium* sp. alone or in combination did not affect bok choy indicated by disease intensity of 0 %, and did not affect plant height, number of leaves, and plant fresh weight. The weed pathogenic fungi can be used as a bioherbicide in bok choy. To control weeds in bok choy plantations, both weed pathogenic fungi are recommended to be used. The further application of weed pathogenic fungi needs to be continued to other cultivated plants besides bokchoy.

Key words: bioherbicides, bok choy, growth and yield, weeds pathogenic fungi.

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INTRODUCTION

Weeds are plants that appear at times, places, and conditions that are not desired by humans in cultivation activities (Clements & Jones, 2021). The presence of weeds in cultivated plants causes a decrease in the quality and quantity of crop yields. Weeds reduce crop yields directly through competition for nutrients and allelopathy, thereby inhibiting plant development (Jabran et al., 2015; Zareen et al., 2022). Weed control is mostly done by mechanical means and the use of synthetic chemical herbicides. Mechanical weed control can only work well in a narrow area, while in a large area it is often inefficient (Woyessa, 2022). Weed control with synthetic chemical herbicides is considered less targeted and has a negative effect on agricultural land (Kraehmer et al., 2014; Tataridas et al., 2022). Therefore, weed control with a combination of various treatments such as the use of synthetic chemical herbicides in low doses, mechanical control, and biological control need to be applied (Kraehmer et al., 2014).

Biological control of weeds is the use of natural enemies (organisms) other than humans to reduce weed populations (Telkar et al., 2015). Biological control is considered safer, more practical, and has very little impact on the environment. One candidate for biological control that is often used is the fungus group, because it can cause high damage to weed and has a very specific host (Chakraborty & Ray, 2021; Mira et al., 2021).

The results of the exploration of weed pathogenic fungi have been identified as *Fusarium* sp., *Chaetomium* sp., and *Curvularia* sp. (Soesanto et al., 2021). The three weed pathogenic fungi have been tested on several weeds (Soesanto et al., 2020, 2021. Pathogenic fungi cause high damage to weeds (Mira et al., 2021) and affect weed growth in the field. Weeds generally grow among cultivated plants, such as bok choy. The application of weed pathogenic fungi to control these weeds will affect cultivated plants (Harding & Raizada, 2015). Meanwhile, the influence of weed pathogenic fungi on growth and production in cultivated plants is not widely known. Based on this, a study was conducted on the application of three weed pathogenic fungi to the growth and production of bok choy. The research was conducted with the aim of knowing the effect of weed pathogenic fungi on the growth and production of bok choy, so that hopefully that it can be used as a bioherbicide in bok choy plantations.

METHODS

The research was conducted in Tambaksogra Village, Sumbang District, Banyumas Regency, Central Java with an altitude of 100 m above sea level. Preparations were carried out at the Plant Disease Laboratory, Faculty of Agriculture, Jenderal Sudirman University.

Preparation of weed pathogenic fungi

Rejuvenation of *Fusarium* sp., *Curvularia* sp., and *Chaetomium* sp. isolates was prepared on PDA in Petri dishes and incubated at room temperature for 7 days (Atallah et al., 2022).

Propagation of weed pathogenic fungi

Propagation of weed pathogenic fungi was carried out on PDB (Potato Dextrose Both) by means of 3 cork drills (0.6 cm diameter) each weed pathogenic fungus inserted into a 250 mL Erlenmeyer flask containing 125 mL of PDB solution. Furthermore, the flask was shaken with a shaker (Daiki Orbital) for 7 days at a speed of 150 rpm at room temperature (Supriyanto et al., 2020). After completion of shaking, the density of the mushroom conidia was calculated using a haemocytometer then diluted to a concentration of 10^6 conidia/mL solution and ready to use (Akıner et al., 2020).

Preparation of bok choy

Bok choy seeds of Gardena F1 variety were sown on trays with a planting medium of a mixture of soil and compost in a ratio of 2: 1 for 14 days. The bok choy seeds that have been sown are transplanted into plots measuring 100 x 100 cm, with a distance between plots of 20 cm and a distance between groups of 30 cm, with a spacing of 20 x 20 cm (Pangli, 2016).

Aplication of weed pathogenic fungi

Bok choy was sprayed with a liquid formula from *Fusarium* sp., *Curvularia* sp., and *Chaetomium* sp., respectively, with a dose of 10-20 mL per plant, in the afternoon with three sprays on the top and bottom of the leaf surface. The spraying interval was every 5 days, starting after the bok choy plants were 10 days after planting and ending 10 days before harvesting.

Experimental design

The design used was a randomized block design, which consisted of eight treatments and 4 replications. The treatments consisted of: control, *Fusarium* sp., *Curvularia* sp., *Chaetomium* sp., *Fusarium* sp. + *Curvularia* sp. (1 : 1), *Fusarium* sp. + *Chaetomium* sp. (1 : 1), *Curvularia* sp. + *Chaetomium* sp. (1 : 1), and *Fusarium* sp. + *Curvularia* sp. + *Chaetomium* sp. (1 : 1 : 1). The concentration of each of these pathogenic fungi was 10^6 conidia/mL of solution.

Observed variables

The disease symptoms were observed directly on bok choy. The incubation period of the disease was calculated by recording the day when the first symptoms appeared on the plant after fungal inoculation (Leclerc et al., 2014), disease intensity due to *Curvularia* sp. and *Chaetomium* sp. calculated by the formula (Bath et al., 2013):

$$DI = \frac{\sum(nxv)}{NxZ} x\ 100\%$$

Description:
DI = Intensity of plant damage
v = Value (score) of plant damage based on leaf area of all affected plants, (Bath et al, 2013): 0 = no damage at all, 1 = area of plant damage >0-10 %, 2 = area of plant damage 11-20%, 3 = area of plant damage 21-40%, 4 = area of plant damage 41-60%, 5 = area of plant damage >61%
n = number of plants that have a value the same v (plant damage)
Z = the highest score (score) (v=5)
N = the number of plants observed

Disease incidence and disease intensity due to Fusarium sp. calculated by formula (Biswas et al., 2020):

$$ID = \frac{A}{N} x\ 100\%$$

Description:
ID = Occurrence/Intensity of disease
A = Number of affected plants
N = Number of plants observed

The infection rate was calculated based on the pattern of disease progression using the Van der Plank formula (1963). The infection rate of *Fusarium* sp. was calculated by formula:

$$r = \frac{2,3}{t} \left(\log \frac{1}{(1-Xt)} - \log \frac{1}{(1-Xo)}\right)$$

Infection rate of *Curvularia* sp. and *Chaetomium* sp. was calculated by formula:

$$r = \frac{2,3}{t}\left(\log \frac{Xt}{(1-Xt)} - \log \frac{Xo}{(1-Xo)}\right)$$

Description:
r = infection rate
Xo = proportion of initial disease observed
t = 0
Xt = proportion of disease at time t
t = time of observation

AUDPC (area under the disease progress curve) was calculated by formula (Simko & Piepho, 2012):

$$AUDPC = \sum_{i-1}^{n-1}\left[\frac{Xi+Xi+1}{2}\right]\times t_{i+1} - t_i$$

Description:
where x_i is an assessment of a disease (percentage, proportion, ordinal score, etc.) at the $_i$th observation, t_i is time (in days, hours, etc.) at the $_i$th observation, and n is the total number of observations.

The number of leaves per plant was calculated starting from a week after the application of three weed pathogenic fungi until they were ready for harvest which was carried out every 7 days. Plant height was measured from the base of the plant to the highest growing point, starting from a week after the application of three weed pathogenic fungi to being ready for harvest which was carried out every 7 days. Plant fresh weight (grams) was weighed using an analytical balance which was carried out after the plants were harvested. The dry weight of the plant (grams) was weighed using an analytical balance which was carried out after the plants were dried in an oven at 60 °C for two days or until the weight was stable.

Data analysis

Data analysis was carried out using the F test. If there was variation between treatments, it was continued with Duncan's Multiple Range Test (DMRT) at an error level of 5%.

RESULTS AND DISCUSSION

The effect of weed pathogenic fungi on bok choy

Based on the observations, the following symptoms were found in bok choy plants:

Disease symptom

Fusarium sp. application could cause symptoms on bok choy plants in the form of wilting. This shows that *Fusarium* sp. have extensive host plants. The initial symptoms caused by *Fusarium* sp. that was at first the bok choy leaves wilt to look like lack of water (Figure 1a) and end with yellowing and dry leaves (Figure 1b).

According to Maurya et al. (2019), *Fusarium* sp. wilt the first time the leaves appear, the plant will experience epinasty, namely the leaves droop down, the lower leaves turn yellow, the leaves and stalks wither, necrosis and dry up, and end in death. Symptoms of wilting in bok choy plants start from the lower leaves and progress to the upper leaves. This shows that *Fusarium* sp. is a fungal pathogen with a wide range of host plants, not only limited to weeds, but also to cultivated plants (Gálvez & Palmero, 2022).

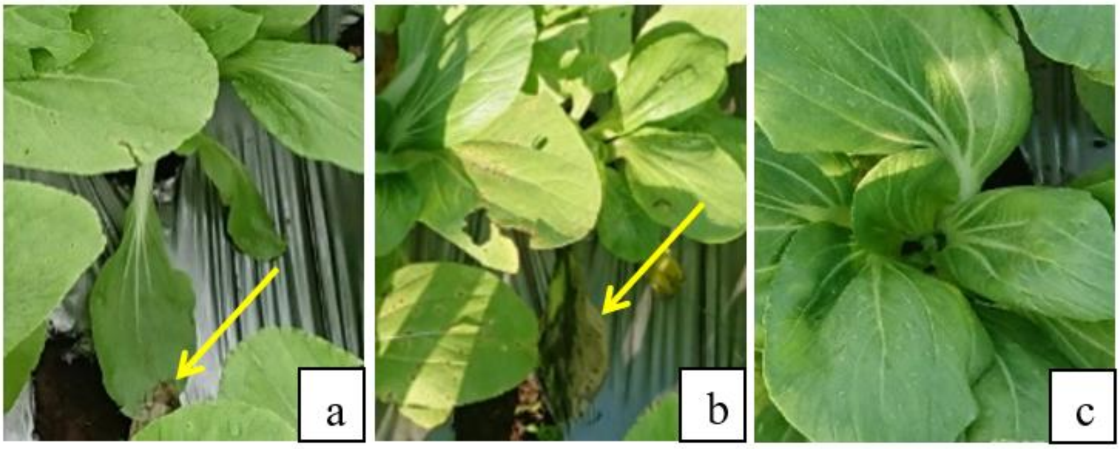

Figure 1. Symptoms of Fusarium sp. on the bok choy plant. Description: (a) Early symptoms of leaf wilting (arrow), (b) Late symptoms of yellowing and dry leaves (arrow), and (c) Healthy leaves

Application of *Curvularia* sp. and *Chaetomium* sp. did not cause symptoms in bok choy plants. This is thought to be caused by several factors including environmental conditions that do not support the growth of pathogens, plants have resistance to *Curvularia* sp. and *Chaetomium* sp. or *Curvularia* sp., and *Chaetomium* sp. are not a fungal pathogen of bok choy plants. In contrast to what was reported by Suganda & Wulandari (2018), it was stated that the fungus *Curvularia* sp. as a new pathogen in mustard plants. This shows that in the same plant genus, pathogenic fungi can cause disease in one species, but in other species, pathogenic fungi may not necessarily cause disease (Li et al., 2020).

Soesanto et al. (2020) reported that *Chaetomium* sp. can cause disease in weeds, but in cultivated plants *Chaetomium* sp. does not cause disease. This can be caused by genetic factors of pathogenic fungi. According to Li et al. (2020), pathogenic fungi have different genetics with different host specifications. Fungal genetics determines the ability of fungi to grow and develop as well as the ability to infect the host (Naranjo-Ortiz & Gabaldón, 2019).

Bok choy disease

Based on Table 1, the incubation period for the treatment of *Fusarium* sp., *Fusarium* sp. + *Curvularia* sp., *Fusarium* sp. + *Chaetomium* sp., and *Fusarium* sp. + *Curvularia* sp. + *Chaetomium* sp. showed a very significant difference compared to the control and other treatments, or each had a speed of 29.8, 19.2, 20.4, and 32.4% compared to the control, which was in line with the incubation period data. The appearance of symptoms in plants is thought to be influenced by host-pathogen interactions and environmental conditions supporting the development of pathogens, so that symptoms can develop properly. This situation affects the ability of the pathogen to carry out the infection process to plants (Shikano & Cory, 2015) which is described by the incubation period. This is in accordance with the opinion of Velàsquez et al. (2018) that the onset of the first symptoms depends on the pathogenicity of the pathogen and supporting environmental factors, such as temperature, humidity, and host resistance. According to Nazarov et al. (2020), the emergence of symptoms is caused by the ability of the pathogen to carry out the infection process in plants. The high and low virulence of the pathogen can affect the incubation period.

In Table 1, the disease intensity showed significantly different results in the treatment of *Fusarium* sp., *Fusarium* sp. + *Curvularia* sp., *Fusarium* sp. + *Chaetomium* sp., and *Fusarium* sp. + *Curvularia* sp. + *Chaetomium* sp. compared to control and other treatments, ie each disease intensity was 7, 8, 9, and 12% compared to control. This means, *Fusarium* sp. has a wide distribution of host plants and is able to infect host plants compared to *Curvularia* sp. + *Chaetomium* sp. In accordance with the opinion Gálvez &

Table 1. Pathosystem component of three weed pathogenic fungi

Treatments	Incubation period (dai)*	Disease intensity (%)	Infection rate (unit/day)	Disease incidence (%)	AUDPC (%-day)
control	25 a	0 b	0 c	0 b	0 c
Fusarium sp.	17.5 bc	2.7 a	0.001371 ab	35 a	10.5 ab
Curvularia sp.	25 a	0 b	0 c	0 b	0 c
Chaetomium sp.	25 a	0 b	0 c	0 b	0 c
Fusarium sp. + *Curvularia* sp.	20.2 b	2.7 a	0.001297 b	40 a	8 b
Fusarium sp. + *Chaetomium* sp.	19.9 b	3.55 a	0.001741 ab	45 a	10.9 ab
Curvularia sp. + *Chaetomium* sp.	25 a	0 b	0 c	0 b	0 c
Fusarium sp. + *Curvularia* sp. + *Chaetomium* sp.	16.9 c	4.4 a	0.002224 a	50 a	15.8 a

Note: Numbers followed by different letters in the same column show a significant difference in the UJGD with an error rate of 5%. *Needs data analysis, incubation period for asymptomatic treatments used until the last observation time (25 days), and dai = days after inoculation.

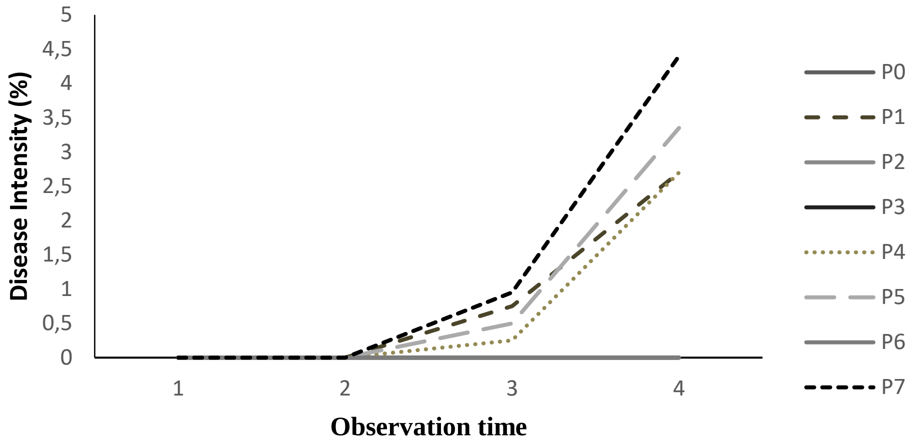

Figure 2. Development of Disease Intensity. Description: P0 = Control, P1 = *Fusarium* sp., P2 = *Curvularia* sp., P3 = *Chaetomium* sp., P4 = *Fusarium* sp. + *Curvularia* sp., P5 = *Fusarium* sp. + *Chaetomium* sp., P6 = *Curvularia* sp. + *Chaetomium* sp., and P7 = *Fusarium* sp. + *Curvularia* sp. + *Chaetomium* sp.

Palmero (2022) that *Fusarium* spp. is a soil-borne pathogenic fungus with a wide distribution and infects many host plants. Meanwhile, the small percentage of plant disease intensity in the treatment of *Curvularia* sp. + *Chaetomium* sp. It is suspected that the plant does not have a close relationship with the pathogen (Li et al., 2020).

The development of disease intensity in bok choy can be seen in Figure 2. The development of disease intensity in controls, *Curvularia* sp., *Chaetomium* sp., and *Curvularia* sp. + *Chaetomium* sp. from the beginning of the observation to the end of the observation it is always at 0 %. The intensity of the disease caused

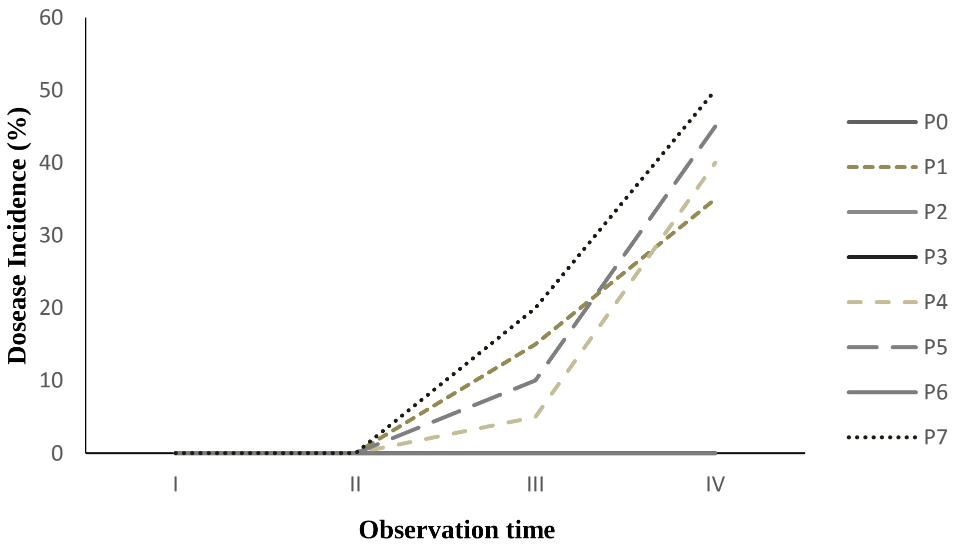

Figure 3. Development of Disease Incidence. Description: P0 = Control, P1 = Fusarium sp., P2 = Curvularia sp., P3 = Chaetomium sp., P4 = Fusarium sp. + Curvularia sp., P5 = Fusarium sp. + Chaetomium sp., P6 = Curvularia sp. + Chaetomium sp., and P7 = Fusarium sp. + Curvularia sp. + Chaetomium sp.

by *Fusarium* sp., *Fusarium* sp. + *Curvularia* sp., *Fusarium* sp. + *Chaetomium* sp., and *Fusarium* sp. + *Curvularia* sp. + *Chaetomium* sp. increased in the third observation with the percentages of 0.75, 0.25, 0.5, and 0.95 %, respectively, and at the end of the observation each became 2.7, 2.7, 3.55, and 4.4 %, respectively. The increased disease intensity is thought to be caused by the number of pathogenic mycelia on plant parts increasing with time so that the damage caused by pathogens is expanding to various parts of the plant. This is in accordance with the opinion of Akhsan et al. (2015), that the longer the time or the length of time available for disease development, the more opportunities for disease to develop, so that the intensity of the disease also increases.

Based on Table 1, the infection rate showed significantly different results in the treatment of *Fusarium* sp. (P1), *Fusarium* sp. + *Curvularia* sp. (P4), *Fusarium* sp. + *Chaetomium* sp. (P5), and *Fusarium* sp. + *Curvularia* sp. + *Chaetomium* sp. (P7) compared to P0 (control) and other treatments, which were 0.001371, 0.001297, 0.001741, and 0.002224 units per day, respectively. This means that the implementation of *Fusarium* sp. + *Curvularia* sp. + *Chaetomium* sp. (P7) had the fastest infection rate compared to other treatments. The value of the infection rate is in line with the intensity of the disease and the incubation period. The higher the infection rate, the higher the intensity of the disease produced and the shorter the incubation period. The higher the infection rate causes the shorter incubation period due to the number of infecting fungal colonies are produced before the time expires. Smith & Casadevall (2022) said that the rate of infection is influenced by the amount of pathogen inoculum and environmental conditions that support the activity of the pathogen and the susceptibility of the host causing the intensity of the disease to be high. Velàsquez et al. (2018) stated that rainfall can affect the host's ability to infect plants. The higher the infection rate, the shorter the period of disease development, so that the epidemic occurs more quickly.

Based on Table 1, the incidence of disease in the treatment of *Fusarium* sp., *Fusarium* sp. + *Curvularia* sp., *Fusarium* sp. + *Chaetomium* sp., and *Fusarium* sp. + *Curvularia* sp. + *Chaetomium* sp. was significantly different from the control and other treatments, which were 35, 40, 45, and 50 % respectively. This means that the implementation of *Fusarium* sp. + *Curvularia* sp. + *Chaetomium* sp. had a better ability to infect host plants than other treatments. The development of disease intensity in bok choy plants can be seen in Figure 3. The development of disease in the treatment of *Fusarium* sp. (P1), *Fusarium* sp. + *Curvularia* sp. (P4), *Fusarium* sp. + *Chaetomium* sp. (P5), and *Fusarium* sp. + *Curvularia* sp. + *Chaetomium* sp. (P7) at the third observation each had a disease incidence of 15, 5, 10, and 20 % and in the fourth observation it increased to 35, 40, 45, and 50 % (Figure 3). The incidence of disease increases with time due to the increase in fungal inoculum that can infect plants. Disease incidence due to treatment made from *Fusarium* sp. proved that *Fusarium* sp. is a fungus that spreads widely and has many host plants (Gálvez & Palmero, 2022). The small increase in disease incidence is due to incompatible host-pathogen relationships and supported by unsuitable environmental conditions in the development of pathogens. According to Smith & Casadevall (2022), disease development will increase in line with increased humidity and lack of sunlight.

Based on Table 1, the AUDPC value in the treatment of *Fusarium* sp. (P1), *Fusarium* sp. + *Curvularia* sp. (P4), *Fusarium* sp. + *Chaetomium* sp. (P5), and *Fusarium* sp. + *Curvularia* sp. + *Chaetomium* sp. (P7) was significantly different from the control (P0) and other treatments, which were 10.5, 8 10.9, and 15.75 %, respectively, compared to the control. The treatment of *Fusarium* sp. + *Curvularia* sp. + *Chaetomium* sp. (P7) was the highest AUDPC value compared to other treatments. The AUDPC value is in line with the disease intensity. The greater the AUDPC value indicates the plant is more susceptible to disease caused by pathogens, and vice versa, the lower the AUDPC value, the more resistant the plant is to pathogens infection. It is known that the treatment of *Fusarium* sp. + *Curvularia* sp. + *Chaetomium* sp. (P7) had the highest AUDPC value and disease intensity compared to others. According to Leitão et al. (2020), the lower the AUDPC value will trigger the effect of plant resistance to disease, and vice versa, the higher the AUDPC value, the lower plant resistance to disease. Simko & Piepho (2012) stated that the lower the AUDPC value, the lower the disease progression.

The effect of three weed pathogenic fungi on bok choy growth and yield

The treatment of three weed pathogenic fungi on disease variables can be seen in Table 2.

Based on Table 2, the treatments of the three

Table 2. Growth and yield component of bok choy

Treatments	Crop height (cm)	Number of leaves	Crop fresh weight (g)	Crop dry weight (g)
control	27.3 a	10.3 a	79.90 a	5.13 abc
Fusarium sp.	28.1 a	11.0 a	74.80 a	4.65 abc
Curvularia sp.	27.8 a	10.5 a	91.30 a	5.60 ab
Chaetomium sp.	27.2 a	10.3 a	92.60 a	5.82 a
Fusarium sp. + *Curvularia* sp.	26.6 a	10.3 a	61.90 a	4.23 bc
Fusarium sp. + *Chaetomium* sp.	26.8 a	9.5 a	52.96 a	3.95 c
Curvularia sp. + *Chaetomium* sp.	27.8 a	10.3 a	81.80 a	4.97 abc
Fusarium sp. + *Curvularia* sp. + *Chaetomium* sp.	26.4 a	9.9 a	54.03 a	3.80 c

Note: Numbers followed by different letters in the same column show a significant difference in DMRT with an error rate of 5%.

weed pathogenic fungi were not significantly different in plant height, number of leaves, and crop fresh weight. The application of three weed pathogenic fungi did not trigger or inhibit plant height, indicating that the fungi applied did not have the ability to disrupt the metabolism of bok choy plants and reduce the ability of the plants to absorb nutrients, so that the plants continued to grow normally. This is because weed pathogenic fungi do not interact with cultivated plants so that they do not cause plant diseases and plant growth normally (Li et al., 2020). Treatment of *Chaetomium* sp. had the heaviest fresh weight of 92.6 g and the treatment of *Fusarium* sp. + *Curvularia* sp. + *Chaetomium* sp. had the lightest fresh weight of 54.03 g and all treatments were not significantly different from the control. This is in line with the pathosystem component. The difference in plant fresh weight was caused by the impact of damage by pathogen attacks, thus affecting the fresh weight of bok choy plants (Shuping & Eloff, 2017).

However, the treatments of the three pathogenic fungi were significantly different on plant dry weight (Table 2). Treatment of *Chaetomium* sp. had the heaviest dry weight of 113.35% of the control and other treatments. The occurrence of significant differences between treatments indicated the effect of the three fungi applied on the dry weight of bok choy plants. The small dry weight in the treatment made from *Fusarium* sp. thought to be caused by disruption of the physiological process of bok choy plants by pathogenic fungi, thereby reducing growth and affecting plant biomass. In accordance with the opinion of Toruño et al. (2016), states that the host plant reacts with the pathogen, allowing changes in plant physiology, such as photosynthesis, respiration, transpiration, and growth.

Efforts to find biological herbicides continue to be carried out considering that the negative effects of herbicides are often found. The results of previous studies have found candidates for biological herbicides, namely weed pathogenic fungi. However, the application of weed pathogenic fungi needs to be tried considering that weeds always grow with cultivated plants, so their effect on cultivated plants needs to be investigated. From the results of this study, new information was obtained that there are weed pathogenic fungi that have no effect on cultivated plants, and which can be developed as candidates for environmentally friendly biological herbicides.

CONCLUSION

Weed pathogenic fungi of *Curvularia* sp. and *Chaetomium* sp. alone or in combination did not affect bok choy indicated by disease intensity of 0 %, and did not affect plant height, number of leaves, and plant fresh weight. The weed pathogenic fungi can be used as a bioherbicide in bok choy. To control weeds in bok choy plantations, both weed pathogenic fungi are recommended to be used. The further application of weed pathogenic fungi needs to be continued to other cultivated plants besides bokchoy.

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