Control of charcoal rot disease in blackgram caused by *Macrophomina phaseolina*

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Abstract

Methods of controlling charcoal rot disease caused by *Macrophomina phaseolina* on blackgram: Chai Nat 80, were determined at Chai Nat Field Crop Research Center, during October 2012-September 2013. The randomized complete block design was performed with 4 replicates for 6 control methods. The results found that on PDA *Trichoderma harzianum* grew faster than *M. phaseolina*. Likewise, *T. harzianum* also obviously inhibited growing of *M. phaseolina* colony, as compared to control. It was also found that mixing up *T. harzianum*, compost and soil at the ratio of 1:4:10 into the soil for planting blackgram: Chai Nat 80 caused contaminated with the disease incidence 29.0 percent, compared to 58.7 percent of the uncontrolled treatment. In addition using mixed *T. harzianum* also showed the highest yield at 4.38 gram per plant compared to 1.88 gram per plant of the uncontrolled treatment. In addition, mixing up *T. harzianum* into the soil also showed the greatest pods per plant (14.8 pods per plant), seeds per pod (6.4 seeds per pod) and 100 seeds weight (5.76 gram), compared to 8.4 pods per plant, 5.3 seeds per pod and 100 seeds weight at 4.51 gram, respectively.

Keywords: blackgram**,** charcoal rot, *Macrophomina phaseolina*, *Trichoderma harzianum*

1. Introduction

Charcoal rot disease on blackgram caused by *Macrophomina phaseolina* was commonly found in backgram plantations. The outbreak of the fungus severely damages the growth of the blackgram, causing root and stem rot. As the fungus can persist in the soil for a long period of time, the cultivation of the blackgram has even become more difficult. In addition, the fungus can spread to the seed of the blackgram, particularly when the blackgram is transformed into the sprout. This has become a problem that requires an urgent solution. Researchers have conducted several studies to discover the most effective ways to prevent the disease, for instance, blending blackgram seeds with antifungal chemicals. This method, which requires the use of a chemical known as Benomyl, can ensure better germination and decreasing percentage of the disease. (Chauhan et al., 2008, El-Fiki et al., 2004) At the same time, some researchers have applied the use of beneficial microorganisms, such as *Trichoderma,* in controlling the disease aiming to reduce dependence of such chemicals. (McLean et al., 2001) This experimental study aims to seek ways to effectively control charcoal rot disease on blackgram.

2. Materials and Methods

2.1. Study on the use of fungi, using T. harzianum to hinder the growth of M. phaseolina on PDA media.

Nurture *T. harzianum* with *M. phaseolina* on PDA media. Each fungus is required to be nurtured on each particular side of the experiment plate. It is necessary to measure the size of the fungi on every two days until they are plentiful on the plate.

2.2. Preparation of inoculum for M. phaseolina

Nurture *M. phaseolina* on purified millet until it is fully grown. The required inoculum is 2 percent, with inoculum weight per dried soil weight. Prepare the Chai Nat 80 blackgram and purify it with sodium hypochlorite. Grow the purified blackgram plants in the soil with inoculum and *M. phaseolina* prepared in a 12-inch wide clay plot, in which the required proportion is three blackgram plants per one clay pot.

2.3. Study on disease control

The experiment was conducted in RCB with 6 control treatments, 4 replications. The soil was mixed soil with *T. harzianum* before planting. The required proportion of the *T. harzianum* fungus, composed fertilizer and soil at the ratio of 1:4:10. The seed were blended with Benomyl 50% WP before planting, with the required proportion of 3 grams of Benomyl 50%WP per 1 kilogram of seeds. They were then blended with thiophanate methyl 70%WP before planting, with the required proportion of 7.5 grams of thiophanate methyl 70%WP per 1 kilogram of seeds. A spray suspension was prepared with *T. harzianum*, with the condense proportion of 1:200, after seven days of germination. In addition, it is also required to apply the spraying of a spore suspension of *T. harzianum* for every seven days until it reaches the R7 stage. Finally, the seeds were sprayed with Benomyl 50% WP, with the required proportion of 20 g of Benomyl 50% WP per 20 liters of water at 30 days after planting and continue to spray twice on every seven days. Untreated controls were also included.

2.4. Monitoring of other diseases

After the harvest, it is required to apply some random inspection for every repetitive method aiming to seek percentage of possible charcoal rot disease by relying on the agar plate method applied on PDA media.

3. Results and Discussion

The sizes of both *T. harzianum* and *M. phaseolina* increased rapidly. It is quite obvious *T. harzianum* can grow faster than *M. phaseolina*. After the second day, *T. harzianum* had increased growth compared to *M. phaseolina*. Sizes of diameter of *T. harzianum* and *M. phaseolina* were estimated at 2.5 and 1.7 cm, respectively. However, *M. phaseolina* can grow fully in a 9 inch experiment plate after it has been nurtured for four days. (Table 1)

The possibility of charcoal rot disease varies between 29.0-58.7% and the experimental variable is estimated at 21.83%. Blending the soil with *T. harzianum* before planting, with the required proportion of *T. harzianum*, in the ratio of 1:4:10 offers the lowest risk of charcoal rot disease at 29.0%. The mentioned method is slightly different from blending the seeds with Benomyl 50%WP before planting, with the required proportion of 3 grams of Benomyl 50%WP per 1 kilogram of seeds and blending the seeds with thiophanate methyl 70%WP before planting, with the required proportion of 7.5 grams of thiophanate methyl 70%WP per 1 kilogram of seeds. Meanwhile, untreated controls had the highest risk of 58.7% (Table 2)

Blending the soil with *T. harzianum* before planting, with the required proportion of *T. harzianum*, composed fertilizer and soil of 1:4:10 ensures the highest seed weight of 4.38 grams per plant. Spraying a spore suspension of *T. harzianum* in the proportion of 1:200, after seven days of germination, plus the spraying of a spore suspension *of T. harzianum* for every seven days until it reaches the R7 stage, compared to spraying Benomyl 50% WP, gave appropriate weights of 2.76 and 2.24 grams per plant, respectively. In addition, blending the soil with *T. harzianum* before planting provides the highest result of 14.8 pods per plant, 6.4 seeds per pod and 5.76 grams per 100 seeds. Untreated controls had 8.4 pods per plant, 5.3 seeds per pod and 4.51 grams per 100 seeds. (Table 3) In conclusion, blending the soil with *T.*

harzianum with the require proportion of *T. harzianum*, composed fertilizer and soil (1:4:10) can ensure the best result of charcoal rot disease control.

Table 1 Inhibition of *Trichoderma harzianum* to *Macrophomina phaseolina* on PDA at different incubation periods at Chai Nat Field Crops Research Center in 2013.

	colony diameter (cm.)	
Incubation time (days)	*T. harzianum*	*M. phaseolina*
1	1.2	1.0
2	2.5	1.7
3	3.0	1.2
4	4.3	0.2

(Average from 6 replications)

Table 2 Effects of control methods for Charcoal Rot caused by *Macrophomina phaseolina* on seeds of blackgram: Chai Nat 80 at Chai Nat Field Crops Research Center in 2013.

Treatment	% Infected seeds
1. Mixing up with *T. harzianum*	29.0 a
2. Seed treated with benomyl 50% WP	35.9 a
3. Seed treated with thiophanate methyl 70% WP	37.3 a
4. Spraying with *T. harzianum*	40.7 ab
5. Spraying with benomyl 50% WP	39.3 ab
6. Untreated check	58.7 b
CV (%)	21.83

Mean in the same column followed by different letters are significantly different at P<0.05 level by DMRT.

Table 3 Effects of control methods for Charcoal Rot caused by *Macrophomina phaseolina* on yield, 100 seed weight, pods/plant and seeds/pod of blackgram: Chai Nat 80 at Chai Nat Field Crops Research Center in 2013.

Treatment	yield (g/plant)	Pods/plant	Seeds/pod	100 seed weight (g)
1. Mixing up with *T. harzianum*	4.38 a	14.8 a	6.4 a	5.76 a
2. Seed treated with benomyl 50% WP	1.95 c	8.0 b	6.2 ab	4.46 b
3. Seed treated with thiophanate methyl 70% WP	1.91 c	7.8 b	5.7 bc	5.07 ab
4. Spraying with *T. harzianum*	2.76 b	10.5 b	5.9 b	5.31 ab
5. Spraying with benomyl 50% WP	2.24 b	9.3 b	5.3 c	4.99 ab
6. Untreated check	1.88 c	8.4 b	5.3 c	4.51 b
CV (%)	26.9	19.0	8.6	13.1

In the same column, means followed by the same letter are not significantly different at the P<0.05 level by DMRT.

4. Conclusions

Experiments reveal that *T. harzianum* can grow faster than *M. phaseolina*. Blending the soil with *T. harzianum* before planting, with the required proportion of *T. harzianum*, composed fertilizer and soil of 1:4:10 offers the lowest risk of charcoal rot disease of 29.0 percent while applying no control over charcoal rot disease possesses the highest risk of 58.7 percent. Additionally, blending the soil with *T. harzianum* before planting provides the highest result of 14.8 pods per plant, 6.4 seeds per pod and 5.76 grams per 100 seeds. Contrary to applying

no charcoal rot disease control, it unveils the lowest result of 8.4 pods per plant, 5.3 seeds per pod and 4.51 grams per 100 seeds. In conclusion, blending the soil with *T. harzianum* with the require proportion of *T. harzianum*, composed fertilizer and soil (1:4:10) can ensure the best result of charcoal rot disease control.

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