Jurnal Pertanian Tropik ISSN NO: 2356- 4725/p- ISSN : 2655-7576
Vol.8.No.2. 2021 (17) 122- 128 DOI: 10.32734/jpt.v8i2, August.6520

Effect of Several Types of Endophytic Fungus Beauveria bassiana isolates in suppressing seed-borne pathogens Colletotrichum spp.

Pengaruh beberapa jenis isolat jamur Endofit *Beauveria bassiana* dalam menekan patogen tular benih *Colletotrichum* spp.

Dini Puspita Yanty[1], Trizelia[2], Darnetty[2], Jumsu Trisno[2]
[1]Mahasiswa Program Doktor, Fakultas Pertanian Universitas Andalas
[2]Fakultas Pertanian universitas Andalas
*Corresponding author : dinipuspitayanty@gmail.com

ABSTRACT

Anthracnose is one of the important seed-borne diseases in chili plants because this disease is able to reduce the quantity and quality of chilies and cause considerable economic losses. The purpose of this study was to obtain the best isolates of various types of endophytic fungi Beauveria bassiana in suppressing the growth of chili seed-borne pathogens caused by the fungus Colletotrichum spp. The design used in this study was a completely randomized design (CRD) consisting of 6 treatments. The treatments consisted of control and 5 types of B. bassiana isolates (PD114, TD312, WS, BbKo, KT2B23). The parameters observed in this study were the percentage of chili seeds that germinated on the blotter test, normal seed germination on the paper-to-paper test, the length of the plumule and the radicle. The results showed that different types of B. bassiana isolates could increase the percentage of chili seeds that grew and also the germination ability of chili seeds. KT2B23 isolate was the best isolate in increasing chili seed germination and also suppressing chili seed-borne pathogens caused by Colletotrichum spp.

Key words : B. bassiana, seed borne pathogen, Colletotrichum spp, chili fruit

ABSTRAK

Antraknosa merupakan salah satu penyakit tular benih yang penting pada tanaman cabai karena penyakit ini mampu menurunkan kuantitas dan kualitas buah cabai serta menimbulkan kerugian ekonomi yang cukup besar. Tujuan penelitian ini adalah untuk mendapatkan isolat terbaik dari berbagai jenis jamur endofit *Beauveria bassiana* dalam menekan pertumbuhan patogen tular benih cabai yang disebabkan oleh jamur *Colletotrichum* spp. Rancangan yang digunakan dalam penelitian ini adalah Rancangan Acak Lengkap (RAL) yang terdiri dari 6 perlakuan. Perlakuan terdiri atas kontrol dan 5 jenis isolat *B. bassiana* (PD114, TD312, WS, BbKo, KT2B23). Parameter yang diamati dalam penelitian adalah persentase benih cabai yang berkecambah pada uji blotter, daya kecambah benih normal pada uji antar kertas, panjang plumula dan radikula. Hasil penelitian menunjukkan bahwa jenis isolat jamur *B. bassiana* yang berbeda dapat meningkatkan persentase benih cabai yang tumbuh dan juga kemampuan daya kecambah benih cabai. Isolat KT2B23 merupakan isolat yang terbaik dalam meningkatkan perkecambahan benih cabai dan juga menekan patogen tular benih cabai yang disebabkan oleh jamur *Colletotrichum* spp.

Kata kunci : B. bassiana, patogen tular benih, Colletotrichum spp, buah cabai

INTRODUCTION

Anthracnose is one of the seed-borne diseases in chili. This disease is able to reduce the quantity and quality of chili and also cause considerable losses. The damage caused by this fungus is on the fruit. Affected fruit becomes rotten, this disease can reduce crop yields by 45-60% (Wiratama et al., 2013). This disease if not properly controlled can reduce yields up to 100% (Duriat et al., 2007).

The pathogen that causes this disease is Colletotrichum spp. Colletotrichum spp. is a type of facultative parasitic fungus from the Order Melanconiales which has the characteristics of conidia arranged in aservulus (asexual structures in parasitic fungi). Fungi from the genus Colletotrichum are included in the class Sodariomycetes (Sudirga, 2016). Colletotrichum spp. has a variety of species. The types of Colletotrichum species are C. capsici, C. acutatum, C. gloeosporioides, C. coccodes and C. dematium (Kambar et al., 2013).

Endophytic fungus Beauveria bassiana has only been reported as insect pest control, but it also has the ability to control plant pathogens (Gothandapani et al., 2014). Batson et al (2000) reported that the treatment of cotton seeds using the fungus Beauveria bassiana had the ability to control the pathogen Rhizoctonia solani in vivo. Azadi et al (2015) reported that the fungus B. Bassiana also had the ability to control the pathogenic R. solani on tomato plants.

Quality testing on seeds is one of the important ways to do in the framework of the certification process. One of the tests carried out is the germination test. Testing the germination of seeds requires optimum conditions on germination media, temperature and humidity. Susanti (2010) reported that there were differences in each type of media suitable for seed germination. ISTA (2014a) also reported that good media for seed germination are paper media (filter paper, blotter paper, and towel paper), sand and organic media.

The purpose of this study was to obtain the best isolates of various types of endophytic fungus Beauveria bassiana in suppressing the growth of chili seed-borne pathogens caused by the fungus Colletotrichum spp.

MATERIALS AND METHOD

This research was carried out at the Laboratory of Biological Control and Phytopathology, Faculty of Agriculture, Andalas University, Padang from January to March 2021.

The tools used were glass petri dishes with a diameter of 9 cm, Bunsen, spatula, erlenmeyer, measuring cup, laminar air flow, autoclave, oven, cork borrer with a size of 0.7 cm, stirring rod, micropipette, analytical balance, electric stove, knife. , beaker, tweezers, dropper, brush, vortex, haemocytometer (Improved Neubeur), binocular stereo microscope, binocular microscope, sprout tub, test tube, scissors, camera, and stationery.

The materials used in this study were local varieties of chili seeds with anthracnose symptoms, Sabouraud Dextrose Agar Yeast (SDAY) media, distilled water, stencil paper, filter paper, 70% alcohol, 3% NaOCl (Sodium Hypochlorite), tween 80 solution, spiritus, paper label, wrapping, plastic, tissue, isolate PD114 (Beauveria bassiana endophytic chili leaves), isolate TD312 (Beauveria bassiana endophyte wheat), isolate WS (Beauveria bassiana endofit walang sangit), isolate BbKo fruit (Beauveria bassiana endophyte coffee fruit), isolate fruit KT2B23 (Beauveria bassiana endophytic cocoa fruit) which is a collection of Prof. Dr. Ir. Trizelia, MSi

This study used a completely randomized design (CRD) with 6 treatments and 16 replications.

The treatments used are as follows:
A= No Treatment (Control), B= PD114 isolate (chilli leaf endophyte)
C= Isolate TD312 (wheat stem endophyte), D = Isolate WS (walang sangit), E= BbKo isolate (coffee fruit),F= Isolate KT2B23 (cocoa fruit)

Observational data were analyzed by means of variance, if the results were significantly different, then continued with the LSD distance test at the 5% level.

This study used a completely randomized design (CRD) with 6 treatments and 8 replications. Each stencil paper contains 50 chili seeds. The treatment used is the same as the blotter test.

Observational data were analyzed by means of variance, if the results were significantly different, then continued with the LSD distance test at the 5% level.

Preparation of chili seeds

Chili seeds were obtained from chili cultivation in chili production centers in West Sumatra. The varieties used are local varieties. The seeds taken were seeds from fruit that physically appeared to be suffering from anthracnose disease. The seeds taken are only seeds in the middle 2/3 of the fruit. The seeds were air-dried for 2 days. The seeds are taken and brought to the laboratory for testing. The tested seeds were selected randomly for each treatment.

The B. bassiana mushroom used is a collection of Prof. Dr. Ir. Trizelia, MSi. Mushrooms propagated on SDAY media. Pure mushroom cultures of 0.7 cm were placed in petri dishes containing new SDAY media, and incubated at room temperature for 21 days until fungal conidia were formed and ready for use.

Figure 1. Culture of B. bassiana on SDAY media (age 21 dsi).

Bassiana mushroom suspension preparation

Conidia isolates of B. bassiana were taken at the age of 21 days by adding 10 ml of sterile distilled water and tween 80 as grading material into a petri dish. All ingredients were mixed and crushed using a medium-sized brush to remove the conidia. The suspension was put into a test tube and homogenized with a vortex. To obtain the required conidia, serial dilutions were carried out to 10-3 and the concentration used was 108 conidia/ml. Conidia density was calculated using a Neubauer Improved haemocymometer. The formula for dilution is:

V1.N1=V2.N2

Information :

V1 = Volume of distilled water in basic solution, V2 = Volume of distilled water after addition, N1 = Initial population of conidia, N2 = Desired inoculum population (108 conidia/ml)

Seed treatment

Before being treated, chili seeds were disinfected using a 3% NaOCl solution for five minutes, then the seeds were washed three times using sterile distilled water. Then the seeds were air-dried in a laminar air flow cabinet. Furthermore, the seeds were immersed in a suspension of conidia B. bassiana according to each treatment. The treated seeds were then air-dried before planting.

Blotter Test

Chili seeds that have been treated and controlled are arranged at the same distance in a glass petri dish as many as 25 seeds/petri which have been previously lined with 3 sheets of filter paper that have been moistened with sterile distilled water. The prepared seeds were incubated for 7 days at room temperature.

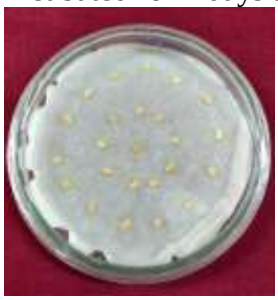

Figure 2. Arrangement of seeds in petri dishes on Blotter test.

Germination test

The germination test of chili seeds was carried out using the Inter-Paper Test (UAK) method. Chili seeds that have been treated and controlled are tested for germination using stencil paper. A total of 2 layers of stencil paper were moistened with distilled water and sown 50 seeds/stencil paper. After that, it was covered with 1 stencil paper that had been moistened with distilled water, then incubated in a germinator (sprout chamber) for 14 days.

Figure 3. Preparation of chili seeds on stencil paper

Jurnal Pertanian Tropik ISSN NO: 2356- 4725/p- ISSN : 2655-7576
Vol.8.No.2. 2021 (17) 122- 128 DOI: 10.32734/jpt.v8i2, August.6520

Observation in the Laboratory Percentage of chili seeds growing

The percentage of chili seeds that grew was calculated on the 7th day after incubation. The percentage of infected chili seeds, calculated using the formula:

$$P = \frac{Chilli\ Growth}{\text{Total of seeds}} \text{ X } 100\%$$

Description: P = Percentage of seeds that grow

To calculate the effectiveness of each treatment on the percentage of seeds attacked by Colletotrichum spp. with the formula:

$$E = \frac{\text{Control} - \text{Treatments}}{\text{Control}} X100\%$$

Description: E= Effectiveness (%)

Percentage of normal seed germination

Observation of normal seed germination was carried out on the 14th day using the formula:

$$P = \frac{\text{Total Seed growth Normally}}{\text{Total seed germination}} \text{ X } 100\%$$

Descripstion : P= Total seed growth Normally

For calculation affectivities each treatments using this formula:

$$E = \frac{\text{Treatments} - \text{Control}}{\text{Control}} X100\%$$

Description: E= Effectiveness (%)

Plumule and radicle length

Plumule and radicle lengths were measured on the 14th day after sowing.

RESULTS AND DISCUSSIONS

Percentage of chili seeds growing

The results of analysis of variance on the percentage of chili seeds that grew after being treated with various isolates of B. bassiana showed that the application of various isolates of B. bassiana was significantly different from the control. The results of the LSD distance test at the 5% level can be seen in Table 1.

Table 1. Percentage of chili seeds that grew after being treated with various isolates of the fungus B. bassiana.

Treatment	results (%)	Effectiveness (%)
PD114	63.00 a	75.00
KT2B21	60.25 a	67.36
TD312	51.25 b	42.36
BbKo	50.50 b	40.27
WS	45.75 b	27.08
Kontrol	36.00 c	0

Numbers followed by the same lowercase letter in the same column are not significantly different according to LSD at the 5% level

In Table 1 it can be seen that the application of various isolates of B. bassiana fungus can increase the percentage of chili seeds that grow and show significantly different results between treatments. Isolates PD114 and KT2B21 were the best isolates in increasing the percentage of chili sprouts that grew, namely 63.00% and 60.25% with effectiveness of 75.00% and 67.36%, respectively. While the chili seeds in the control have the ability to grow that is 36.00% with 0% effectiveness.

Percentage of normal seed germination

The results of analysis of variance on the percentage of normal seed germination showed that the application of various isolates of B. bassiana was significantly different from the control. The results of the LSD distance test at the 5% level can be seen in Table 2.

Table 2. Percentage of normal seed germination after treatment with various isolates of B. bassiana fungus.

Treatment	results (%)	Effectiveness (%)
TD312	71.25 a	42.50
KT2B21	69.00 a	38.00
PD114	61.00 ab	22.00
BbKo	55.00 bc	10.00

WS	54.00 bc	8.00
Kontrol	50.00 c	0

Numbers followed by the same lowercase letter in the same column are not significantly different according to LSD at the 5% level

In Table 2 it can be seen that the application of various isolates of B. bassiana fungus can increase the percentage of normal seed germination in chili seeds. The best percentage of normal seed germination was found in isolates TD312 and KT2B21, namely 71.25% and 69.00% with 42.50% and 38.00% effectiveness, respectively. Meanwhile, the lowest percentage of normal chili seed germination was found in the control, which was 50.00%.

The application of various types of B. bassiana isolates to chili seed-borne pathogens caused by the pathogenic fungus Colletotrichum spp affected the growing chili seeds and seed germination ability. The seeds can live in the chili seed tissue hence they will compete and inhibit seed-borne pathogenic fungi. In addition, B. bassiana is thought to produce secondary metabolites with antifungal properties so that the fungus Colletotrichum spp. on the surface of the seed will be disturbed its development. B. bassiana fungus is capable of producing secondary metabolites that function as antibacterial, antifungal, cytotoxic and insecticide. Ownley et al. (2010) said that the fungus B. bassiana produces many secondary metabolites that are toxic and can inhibit the growth of pathogenic fungal colonies including beauvericin, bassianin, beauverolides, bassianolone, bassianolides and oosporein. The research results of Ownley et al. (2008) that the application of B. bassiana through seed immersion was able to control the pathogens of R. solani on tomato plants and P. myriotylum, X. axonopodis pv. Malvacerum on cotton plants.

a. **Plumule length**

The results of the analysis of variance on the length of the plumule showed that the treatment of various isolates of B. bassiana was significantly different from the control. Further test results can be seen in Table 3.

Table 3. Average length of chili seed plumule after soaking with B. bassiana at different times

Treatment	Plumule Length (cm)	Effectiveness (%)
KT2B21	2.000 a	181.10
PD114	1.500 b	129.57
TD312	1.500 b	147.76
WS	1.125 b	82.98
BbKo	0.625 c	44.03
Kontrol	0.125 d	0

Numbers followed by the same lowercase letter in the same column are not significantly different according to LSD at the 5% level

In Table 3 it can be seen that the application of various types of B. bassiana isolates to chili seeds was able to increase the length of the plumule. The highest plumule length was found in Isolate KT2B21, which was 2,000 cm with an effectiveness of 181.10%.

The application of suspension of B. bassiana not only suppressed Colletotrichum spp., but also increased seed growth on germination. Chili seeds soaked with suspension of B. bassiana can increase the percentage of normal seed germination using the paper-to-paper method compared to the control. It is suspected that B. bassiana is capable of producing high growth metabolites and is not pathogenic in plants. Meanwhile, the best isolate of B. bassiana that was able to increase the maximum germination of normal seeds was isolate KT2B21. Putra (2019) reported that B. bassiana fungus treated on chili seeds could increase germination and growth in the seedling phase. The fungus B. bassiana has been shown to have effective antifungal activity against the fungus F. oxysporum which can cause wilt disease in tomato plants (Parine et al., 2010).

a. Radicle Length

The results of the analysis of variance on radicular length showed that the treatment of various isolates of B. bassiana was significantly different from the control. After the LSD

distance test was carried out at the 5% level, the results can be seen in Table 4.

Table 4. Average radicle length of chili seeds after soaking with B. bassiana mushrooms at different times

Treatment	Radicle Length (cm)	Effectiveness (%)
KT2B21	3.500 a	88.95
TD312	2.875 b	52.76
PD114	2.250 c	33.71
WS	2.125 c	21.77
BbKo	2.125 c	24.89
Kontrol	1.875 c	0

Angka-angka yang diikuti oleh huruf kecil yang sama pada lajur yang sama adalah berbeda tidak nyata menurut LSD pada taraf 5%

Table 4 can be seen that the application of B. bassiana fungus through seed immersion was able to increase the length of the radicle. The highest radicular length was found in Isolate KT2B21, which was 3,500 cm with an effectiveness of 88.95%.

Observation of plumule and radicle length by soaking chili seeds with B. bassiana mushroom suspension gave a higher effect than control. The increase in the length of the plumule and radicle of chili seeds indicated that B. bassiana had the ability to produce a high number of growth-promoting metabolites. Growth-promoting substances such as gibberellins, auxins, and cytokinins are produced by endophytic fungi (Dai et al., 2008). Meanwhile, according to Srivastava (2002) that the concentration of auxin can affect the growth of the plumule and radicle. The results of Putra's research (2019) that the length of the plumule and radicle of chili seed germination were better in the seed soaking treatment with B. bassiana than the control.

CONCLUTION

The results showed that different types of B. bassiana isolates could increase the percentage of chili seeds that grew and also the germination ability of chili seeds. KT2B23 isolate was the best isolate in increasing chili seed germination and also suppressing chili seed-borne pathogens caused by Colletotrichum spp.

REFERENCES

Azadi N, Shirzad A, Mohammadi H (2015a) Study some of biocontrol mechanisms Beauveria bassiana against Rhizoctonia disease in tomato. M.Sc. Thesis. Azarbaijan Shahid Madani University, Tabriz, Iran. available from www.irandoc.ac.ir

Azadi N, Shirzad A, Mohammadi H (2015b) Study of biological control of tomato damping-off disease by some isolates of Beauveria bassiana. First National Conference on Agriculture, Environment and Food Security, University of Jiroft, Jiroft. available from http://www.civilica.com/ Paper-AEFSJ01-AEFSJ01_262.html.

Batson, Jr., W.E., Caceres, J., Benson, M., Cubeta, M.A., Brannen, P.M., Kermy, D.S., Elliott, M.L., Huber, D.M., Hiclanan, M.V., Keinath, A.P., Dubose, V., Ownley, B., Canaday, C., Rothrock, C.S., Schneider, RW., and Sumner, D.R. 2000a, 1999 Biological and Cultural Tests for Control ofPlant Diseases.15, 149- 150.

Duriat, A.S., Gunaeni, N. & Wul&ari, A. 2007. Penyakit Penting Tanaman Cabai & Pengendaliannya. Bandung: Balai Penelitian Tanaman Sayuran. Retrieved from http://www.balitsa.litbang.pertanian.go.id.

Gothandapani, S., Boopalakrishnan, G., Prabhakaran, N., Chethana B.S., Aravindhan, M., Saravanakumar, M., and Ganeshan, G. 2014. Evaluation of Entomopathogenic Fungus Against *Alternaria porri* (Ellis) Causing Purple Blotch Disease of Onion. Phytopathology and Plant Protection 48(2): 135-144.

Kambar, Y., Vivek, M.N., Manasa, M., Kekuda, P.T.R., Nawaz, N.A.S.

(2013).Inhibitory effect of cow urine against *Colletotrichum capsici* isolated from anthracnose of chilli (*Capsicum annuum* L.). Science, Technology and Arts Research Journal 2(4): 91-93.

Ownley, B. H., Griffin, M. R., Klingeman, W. E., Gwinn, K. D., Moulton, J. K and Pereira, R. M. 2008. *Beauveria bassiana*: Endophytic Colonization and Plant Disease Control. USA. Journal of Invertebrate Pathology 98. 267-270.

Parine, N. R., Kumar, D., Khan, P. A. A., and Bobbarala, V. 2010. Antifungal efficacy of secondary metabolites from entomopathogenic fungi Beauveria bassiana. Journal of Pharmacy Research, Vol.3(4), 855-856.

Putra, F. S. 2019. Aplikasi Cendawan Endofit *Beauveria bassiana* (Bals.) Vuill Pada Benih Cabai (*Capsicum annum*) Untuk Mengendalikan *Myzus persicae* (Hemiptera: Aphididae) dan Meningkatkan Pertumbuhan Tanaman. [Skripsi]. Padang. Fakultas Pertanian. Universitas Andalas.

Sudirga, S.K. 2016. "Isolasi Dan Identifikasi Jamur *Colletotrichum* spp. Isolat Pcs Penyebab Penyakit Antraknosa Pada Buah Cabai Besar (*Capsicum Annuum* L.) Di Bali." 30(1): 23–30.

Srivastava, L. M. 2002. Plant Growth and Development, Hormones and Environment. Academic Press, Orlando. 772 hal.

Wiratama, I.D.M.P., I.P. Sudiarta, I.M. Sukewijaya, K. Sumiartha, M.P. Utama. 2013. Kajian Ketahanan Beberapa Galur dan Varietas Cabai Terhadap Serangan Antraknosa di Desa Abang Songan Kecamatan Kintamani Kabupaten Bangli. E-jurnal Agroekoteknologi Tropika. 2 (2):71- 81.

Susanti, M. 2010. Pengaruh media tanam dan perlakuan pra perkecambahan terhadap perkecambahan benih panggal buaya (Zanthoxylum rhetsa (Roxb.) D.C.) [skripsi]. Bogor (ID): Institut Pertanian Bogor.

[ISTA] International Seed Testing Association. 2014a. International Rules for Seed Testing. Switzerland (CH): ISTA. . [ISTA] International Seed Testing Association. 2014c. International Rules for Seed Testing. Switzerland (CH): ISTA.