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The effect of biological agent and botanical fungicides on maize downy mildew

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Abstract. *Prasetyo J, Ginting C, Akin HM, Suharjo R, Niswati A, Afandi A, Adiwijaya R, Sudiono, Nurdin M. 2021. The effect of biological agent and botanical fungicides on maize downy mildew. Biodiversitas 22: 1652-1657.* This study was conducted to evaluate the effect of the combination of *Trichoderma asperellum* and mycorrhizae with botanical fungicides made from betel leaf extract or turmeric extract against maize downy mildew. The study used a randomized complete block (RCB) design with three replications. The treatments applied were: single applications of *T. asperellum* (Ta); arbuscular mycorrhizal fungi (AMF); turmeric extract (T); betel extract (B); the combination of *T. asperellum* and AMF (TaM); turmeric extract and *T. asperellum* (TTa); turmeric extract and AMF (TM); turmeric extract + *T. asperellum* + AMF (TTaM); betel extract + *T. asperellum* (BTa); betel extract + AMF (BM); betel + *T. asperellum* + AMF (BTaM); and Control (C). The observed variables were disease incidence, incubation period, and shoot dry weight. The data were analyzed using ANOVA. Differences between treatments were tested by the LSD test at 5% significance level. The results showed that the combination of botanical fungicides with biological agents could reduce the incidence of downy mildew, extend the incubation period, and increase the dry weight of corn shoots. The best combination treatment was achieved from the combination of *T. asperellum* with betel extract. There was a synergistic effect between botanical fungicides and biological agents in reducing the disease incidence.

Keywords: Biological agents, botanical fungicides, downy mildew

INTRODUCTION

Corn is one of the most important carbohydrate sources in the world. Corn has an important role in fulfilling national and international market demand for food after rice and wheat (Tanklevska et al. 2020). The market demand for corn from year to year continues to increase along with the increasing population, improvement of community welfare, the development of livestock businesses, and the food industry's advancement. Corn is widely used by the food, beverage, chemical, and pharmaceutical industries (Ranum et al. 2014).

Based on its chemical composition and nutrient content, corn has prospects as a food and industrial raw material (Jabran et al. 2007; Ranum et al. 2014; Shah et al. 2016; Kazerooni et al. 2019). The use of corn as an industrial raw material provides added value for corn. Corn is used as a raw material in the feed and food industry and staple food in several regions in Indonesia (Bantacut et al. 2015). Corn kernels can be processed into corn flour, corn rice, and snacks. Corn can also be processed into cooking oil, margarine, and formula foods. Domestic production of corn in Indonesia has not been able to keep up with demand growth up to 2015 (Bantacut et al. 2015).

According to the Indonesia Central Bureau of Statistics (2016), dry shelled corn production in Lampung, Indonesia experienced fluctuations in production from 2010-2015, i.e., 2,126,571 tons, 1,817,906 tons, 1,760,275 tons, 1,760,278 tons, 1,719,386 tons and 1,502,800 tons, respectively. One of the main factors responsible for decreasing corn production was downy mildew caused by *Peronosclerospora* spp.

Three *Peronosclerospora* species have been reported as the causative agent of downy mildew of corn in Indonesia, namely *P. maydis*, *P. philippinensis*, and *P. sorghi* (Muis et al. 2013; Rustiani et al. 2015; Muis et al. 2016). *P. maydis* (syn. *P. australiensis*) is the causative agent of downy mildew in several corn production areas in Lampung (Suharjo et al. 2020). Production loss in corn infected with this pathogen reaches 80%-100% due to the inability to produce kernels (Soenartiningsih and Talanca 2010).

A metalaxyl fungicide is the primary choice of farmers to control downy mildew until now. However, the use of metalaxyl continuously in the long term has triggered resistance to the pathogen (Gisi and Sierotzki 2008; Burhanuddin 2009). Therefore, it is necessary to find another alternatives method to control the disease, such as the application of biological agents, i.e., *Trichoderma* (Puyam et al. 2016; Sood et al. 2020; Ginting et al. 2020) and mycorrhizal fungi (Akhtar and Siddiqui 2008; Tahat et al. 2010; Cameron et al. 2013; Pérez-de-Luque et al. 2017), and botanical fungicides, like turmeric (Ginting 2006; Rahman et al. 2016; Mamarabadi et al. 2018) and betel (Nalina and Rahim 2006; Rahman et al. 2016). The use of

biological agents and botanical fungicides is also safe for consumers and the environment. Biological agents and botanical fungicides are easily degraded and do not leave chemical residues on agricultural products (Yoon et al. 2013; Nega 2014; Bardin et al. 2015; Zaker 2016). This study was carried out to evaluate the effect of biological agents and botanical fungicides and their combination on downy mildew of corn. Botanical fungicides and biological agents were expected to have a synergistic effect in suppressing downy mildew.

MATERIALS AND METHODS

Plant material and planting media preparation

This study used the P27 variety (Dupont Indonesia) of corn seed. The seeds were planted in 36 plastic polybags (35 cm x 40 cm), each polybag containing 10 seeds. Plant maintenance includes watering and weeding.

The planting media was soil taken from around the Integrated Laboratory, Faculty of Agriculture, University of Lampung. Firstly, planting media (soil) was sterilized by steaming for 2 hours. Ten kg of sterilized soil was put into a polybag.

Preparation of biological agents

Two biocontrol agents were used in this study, namely *T. asperellum* and a consortium of three genera of Arbuscular Mycorrhizal Fungi (AMF), i,e. *Enthropospora* sp, *Gigaspora* sp., and *Glomus* sp. formulated in powder, was bought from Estate Crops Laboratory, Faculty of Agriculture, University of Lampung. The spore density of the AMF was 100 spore/g. The isolate of *T. asperellum* was a collection of the Plant Clinics, University of Lampung. *T. asperellum* was cultured on a sterile petri dish containing 10 mL of Potato Dextrose Agar (PDA). Potato Dextrose Agar (PDA) consists of 200g potato, 20g Dextrose, 20 g Agar, and 1000 mL distilled water. Conidia of *T. asperellum* were harvested by flooding plates containing 7 days old culture with 10 mL of sterile distilled water and grabbed gently using Drigalski spatula. The conidial suspension was transferred into Erlenmeyer 100 mL and diluted to the density of ~10^5 conidia/mL.

Preparation of botanical fungicides

Turmeric rhizome and betel leaves were weighed 500 g each, cleaned with sterile water, cut into small pieces, and air-dried. Subsequently, samples were dried in the oven at 50 °C for 36 hours. After drying, samples were ground using a pestle into a fine powder (600 mess). Botanical fungicide was made by dissolving 10 g of fine powder into 100 mL of sterile distilled water and filtered using filter paper (Whatman no. 42). The suspension was centrifuged for 10 minutes at 300 rpm. The supernatant was collected for further use (Ayu et al. 2013).

Isolation of *Peronosclerospora* sp.

Peronosclerospora used in this study was collected from symptomatic corn plants taken from a cornfield located in Natar, South Lampung. The collection was carried out at 03.00 am. Conidia from infected leaves were harvested using a small brush (no. 2). The brush was moistened before being used by dipping it in 20 mL sterile distilled water in a plastic bowl (400 mL of volume, 11 cm of lid diameter). The brush containing the conidia was immersed in 20 mL of distilled water previously used to moisten the brush. Conidia harvesting was continued until the water in the Petri dish was very cloudy due to the high conidia density. The conidial suspension was transferred into a graduated glass containing 500 mL sterile distilled water (Figure 1). Suspension of conidia was brought to the laboratory, and the conidial density was determined.

Peronosclerospora sp. inoculation and botanical fungicide application

The conidia suspension of *Peronosclerospora* (~10^5 conidia/mL) was mixed with botanical fungicide suspension (1:9) and left for 1 hour. Inoculation was conducted by dropping 1 mL of the mixture of conidia suspension and botanical fungicide to the shoots of corn at 4-5 am, on the same day as conidia collection. As a control treatment, the plants were inoculated with a suspension of conidia (the same volume and the same conidia density) without applying biological control agents and botanical fungicides. Inoculation was carried out twice at the age of 7 and 10 days after planting.

Application of biological agents

Ten mL of *T. asperellum* conidia suspension (~10^6 conidia/mL) was poured into each planting hole. One g of AMF powder was applied to each plant (Manila and Nelson 2014). The spore density of AMF powder was 100 spore/g.

Figure 1. Harvesting method of *Peronosclerospora* sp. conidia

Table 1. The scale of disease severity

Score category	Symptom depiction
0	No symptom
1	<10% of the total area of the leaves were infected
2	10-25% of the total area of the leaves were infected
3	25-50% of the total area of the leaves were infected
4	>50% of the total area of the leaves were infected

Observation and data collection

Observations were done daily for four weeks. The variables observed were disease incidence, disease severity, incubation period, and shoot dry weight. Downy mildew incidence was observed in the morning and was based on chlorosis symptoms in corn leaves. Disease incidence (DI) was calculated by dividing the number of infected plants (n) with the total plants observed (N). The incubation period is the time required for the appearance of disease symptoms calculated from the inoculation of downy mildew pathogen to the formation of symptoms. The disease severity was estimated on the scale based on the symptoms (Table 1.)

The DSI (Disease severity index) was calculated with the following formula: $[\Sigma(n \times v)/(N \times V)] \times 100\%$, whereas [n] is the total of infected leaves with a specific score, [v] is the score category of the symptom, [N] is total leaves observed, and [Z] is the highest score used. At the end of the observation, the corn plant was removed from the planting medium and then cleaned from the attached dirt. The shoot was cut into pieces, put in the envelope, and dried in the oven at 80 °C for 5 days to the constant weight.

RESULTS AND DISCUSSION

Two biological control agents (*T. asperellum* and AMF) and two botanical fungicides (turmeric extract and betel extract) were evaluated for their capability to suppress the development of downy mildew disease in corn. These biological control agents and botanical fungicides were applied in individual or combination applications.

The result showed that all the treatments, either single or in combination application) were able to suppress disease incidence of corn downy mildew, compared to the control treatment. The symptoms in the control treatment were more severe than in the treatment groups (Figure 2).

Individual application of *T. asperellum* (Ta), Arbuscular Mycorrhizal Fungi (AMF) (M), turmeric (T), or betel (B) significantly reduced the incidence of disease compared to control (C). However, the incubation period and disease severity in these treatments were not significantly different from those in control plants. The treatments of AMF (AM), turmeric, or betel extract produced a significantly higher dry shoot weight than control. Meanwhile, the dry shoot weight of the corn plants in *T. asperellum* treatment was not significantly different from that in control plants.

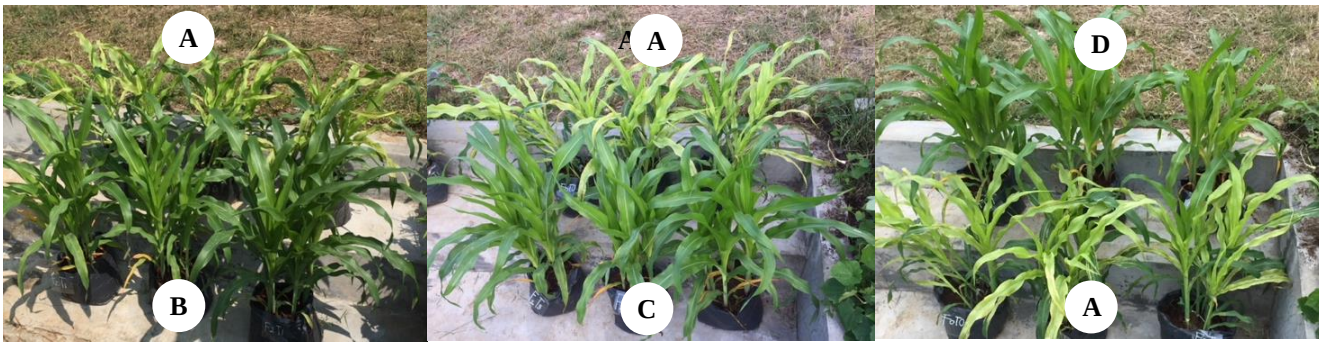

Figure 2. Disease symptoms on the inoculated plants. A. Control, B. Betel + *T. asperellum* (BTa), C. Betel + *T. asperellum* + AMF (BTaM), D. Turmeric + *T. asperellum* (TTa)

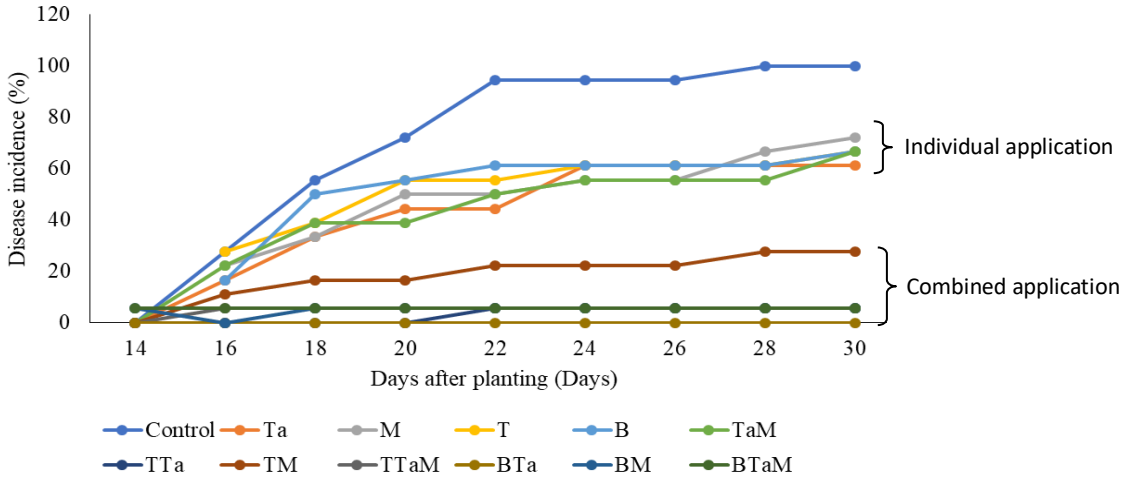

Figure 3. The downy mildew (DM) incidence of maize (%) in various treatments. Ta: *T. asperellum*, M: Arbuscular Mycorrhizal Fungi (AMF), T: turmeric extract, B: betel extract, TaM: *T. asperellum* + AMF, TTa: Turmeric + *T. asperellum*, TM: turmeric + AMF, TTaM: turmeric + *T. asperellum* + AMF, BTa: betel + *T. asperellum*, BM: betel + AMF, BTaM: betel + *T. asperellum* + AMF, C: Control.

Table 3. Average downy mildew incidence, incubation period, and dry shoot weight of corn on treatment combination of *Trichoderma asperellum*, Arbuscular Mycorrhizal Fungi (AMF), and botanical fungicides

Treatment	Downy mildew incidence (%)*	Disease severity index*	The incubation period (days)*	Weight of dry shoot (g)*
Ta	61.11b	66.70a	7.00c	20.73fg
M	66.67b	59.70a	6.00c	41.97bcd
T	66.67b	58.30a	5.33c	45.80bc
B	61.11b	73.60a	6.33c	28.60def
TaM	55.56b	79.20a	5.00c	24.07efg
TTa	5.56d	5.60bc	14.47b	41.30bcd
TM	27.78c	22.20bc	14.00b	33.97cde
TTaM	5.56d	5.60bc	18.70b	54.00b
BTa	**0.00d**	**0.00c**	**25.00a**	**77.20a**
BM	5.56d	5.60bc	19.30ab	36.77cde
BTaM	5.56d	5.60bc	18.30b	50.93bc
C	100.00a	97.20a	5.33c	14.60g

Note: *Numbers in the same column followed by the same letter were not significantly different based on the Least Significantly Difference (LSD) test at 5% of significant level. Ta: *T. asperellum*, M: Arbuscular Mycorrhizal Fungi (AMF), T: turmeric extract, B: betel extract, TaM: *T. asperellum* + AMF, TTa: Turmeric + *T. asperellum*, TM: turmeric + AMF, TTaM: turmeric + *T. asperellum* + AMF, BTa: betel + *T. asperellum*, BM: betel + AMF, BTaM: betel + *T. asperellum* + AMF, C: Control.

The treatment combination of botanical fungicides and biological agents have a synergistic effect in suppressing disease development and increasing plant performance compared to the individual treatment. The treatments' effects were consistent from the beginning to the end of the observation (Figure 3).

Application of *T. asperellum* + AMF (TaM), Tumeric + *T. asperellum* (TTa), Tumeric + AMF (TM), Tumeric + *T. Asperelum* + AMF (TTaM), betel + *T. asperellum* (BTa), betel + AMF (BM), betel + *T. asperellum* + AMF (BTaM) reduced significantly disease incidence than control (C). All the treatments significantly reduce disease severity, longer incubation period, and higher dry shoot weight than control, except treatment of *T. asperellum* + Mycorrhiza (TaM). The disease severity, incubation period, and dry shoot weight of the *T. asperellum* + Mycorrhiza (TaM) treatment group were not significantly different from those of the control (Table 3). The combined application of betel + *T. asperellum* (BTa) produced the best inhibitory activity to suppress disease development of corn downy mildew and the capability to increase dry shoot weight (Table 3).

All combinations of biological agents and botanical fungicides suppressed the disease development of corn downy mildew. *T. asperellum* induced the plant to be resistant to various diseases (Silva et al. 2011; Herrera-Téllez et al. 2019; Ramírez-Olier et al. 2019; Sood et al. 2020). It includes resistance to downy mildew in maize (Ginting et al. 2020). *Trichoderma* produced a variety of compounds that induce plant resistance locally and systemically against plant disease. It also induced plant resistance to survive in unfavorable environmental conditions. Moreover, it can promote plant growth by producing phytohormones (Shafawati and Siddiquee 2013; Mukherjee et al. 2013; Błaszczyk et al. 2014; Singh et al. 2002; Sood et al. 2020). *Trichoderma* spp. produce Indole Acetic Acid (IAA) as growth hormone (Yudha et al. 2016; Contreras-Cornejo et al. 2009) that increase lateral root growth, multiply shoots and increase the biomass of shoots (Contreras-Cornejo et al. 2009; Haryuni 2013)

The Arbuscular Mycorrhizal Fungi (AMF) application affected plant growth and health due to more efficient absorption of nutrients because hyphae are bridging the gap between the root and soil biome (Whipps 2004; Tahat et al. 2010). Mycorrhiza increased the uptake of nitrogen (N), potassium (K), and phosphate (P) (Azcón-Aguilar and Barea 1996; Kabirun 2002; Hasanudin 2003; Musfal 2010), and increases the absorption of Cu and Zn (Liu et al. 2000; Watts-Williams et al. 2015; Diagne et al. 2020). Parapasan and Gusta (2014) reported that the mycorrhizal fungi applied to coffee seeds develop hyphae to infect plant roots. The hyphae increased nutrient absorption to support maximal plant growth (Azcón-Aguilar and Barea 1996; Tahat et al. 2010). Mycorrhiza enhance plant health and activate plant defense mechanism against pathogens (Azcón-Aguilar and Barea 1996; Whipps 2004; Tahat et al. 2010; Kamal et al. 2015; Diagne et al. 2020)

Botanical fungicides kill fungal pathogen due to their antifungal compounds. Sesquiterpene, essential ketone oil, turmeron, and artumeron in turmeric have biological activities as antibacterial, antioxidant, and antihepatotoxic (Mallmann et al. 2017; Amalraj et al. 2017). Turmeric has antifungal activity against several types of plant pathogenic fungi, including *Fusarium udum*, *Colletotrichum falcatum* Went, *Fusarium moniliforme* J. Sheld (Singh et al. 2002), *Xanthomonas axonopodi*s pv. *manihotis* (Kuhn et al. 2006) and *Alternaria solani* (Balbi-Peña et al. 2006). Secondary metabolites in turmeric inhibit the growth of fungal mycelium (Hu et al. 2017).

Betel leaf extract has antifungal and antibacterial activity (Hertiana and Purwati 2002; Nguyen et al. 2020), decreasing the growth and formation of conidial fungi (Nalina and Rahim 2006). Chemical compounds in the essential oils of betel leaf were chavicol, chavibetol (betel phenol), allylprocatechol (hydroxychavikol), allypyro-catechol-mono, diacetate, carvacrol, eugenol, p.cymene,

cineole, caryole, cadeneophyl, esragol, terpenes, sesquiterpene, phenyl propane, tannin, diastase, carotene, thiamine, riboflavin, nicotinic acid, vitamin C, sugar, starch and amino acids (Hertiana and Purwati 2002; Nguyen et al. 2020). Chavikol is a fragrance component in betel and has antibacterial properties with antibacterial activity five times stronger than ordinary phenols. The antifungal activity was contributed by chemical compounds of isoeugenol, limonene, and caryophyllene (Hertiana and Purwati 2002).

Trichoderma asperellum and betel leaf extract (BTa) is the best treatment combination in inhibiting corn downy mildew. BTa treatment increases the dry shoot weight. The BTa treatment can be recommended to control corn downy mildew.

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