Alamat : Sekretariat Jurusan Proteksi Tanaman Gedung C. Fakultas Pertanian UHO Kampus Hijau Bumi Tridharma Anduonohu Kendari Telp. (Hp) +62 852 9999 7936
E-mail : panitiapfi2017@gmail.com
Website : http;//uho.ac.id/faperta/pfi2017

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Berdasarkan hasil review dari panitia, maka bersamaini kami sampaikan bahwa abstrak sebagai berikut:

Nama Presenter : Joko Prasetyo, Muhamad Saiful Anwar S., Suskandini Ratih dan Radix Suharjo

Judul Makalah : Preliminary Study on Integrated Management of Maize Downy Mildew Using Zingiberaceae Extracts and Isolates of *Trichoderma* sp.

Layak dan diterima untuk dipresentasikan secara oral pada Seminar Nasional dan Kongres XIV Perhimpunan Fitopatologi Indonesia 2017 pada tanggal 3-5 Oktober di Kendari, olehnya itu untuk keperluan publikasi di Prosiding, dimohon agar Bapak/Ibu segera mengunduh *Attachment* file sebagai berikut :

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Prof. Dr. Ir. Andi Khaeruni R., M.Si

PRELIMINARY STUDY ON INTEGRATED MANAGEMENT OF MAIZE DOWNY MILDEW USING PLANT EXTRACTS AND ISOLATES OF *TRICHODERMA* SPP

Joko Prasetyo, Muhamad Saiful Anwar S, Suskandini Ratih, Radix Suharjo, and Titik N Aeny
Department of plant Protection, Faculty of Agriculture, University of Lampung
Jkdwiprasetyo21@gmail.com; joko.prasetyo@fp.unila.ac.id

Abstract

An experiment was done to investigate the effects of *Trichoderma* spp and plant extracts combination on downy mildew of maize. The experiment was arranged in randomized completely block design with three replicates. The experiment consisted of 13 treatments, i.e. combination of no Trichoderma and no plant extract (T0F0), *Trichoderma* sp. isolate of East Lampung and ginger extract (T1F1), *Trichoderma* sp. isolate of East Lampung and tumeric extract (T1F2), *Trichoderma* sp. isolate of East Lampung and *Alpinia galanga* extract (T1F3), *Trichoderma* sp. isolate of East Lampumg and galangale extract (T1F4), *Trichoderma* sp. isolate of East Lampung and *Curcuma zanthorrhiza* extract (T1F5), *Trichoderma* sp. isolate of East Lampung and *C. aeroginosa* extract (T1F6), *Trichoderma* sp. isolate of Unila Plant Clinics and ginger extract (T2F1), *Trichoderma* sp. isolate of Unila Plant Clinics and tumeric extract (T2F2), *Trichoderma* sp. isolate of Unila Plant Clinics and *A. Galanga* extract (T2F3), *Trichoderma* sp. isolate of Unila Plant Clinics and galangale extract (T2F4), *Trichoderma* sp. isolate of Unila Plant Clinics and *C. zanthorrhiza* extract (T2F5), and *Trichoderma* sp. isolate of Unila Plant Clinics and *C. aeruginosa* extract (T2F6). Investigated variables were disease incidence, incubation periode, plant height, and weight of dry matter. Data obtained were analysed using anova and the difference between means were tested using LSD test at 5% significant level. The results of the experiment showed that all combination of *Trichoderma* isolate and plant extracts tested decreased the incidence of maize downy mildew and increased incubation periode, plant height, and dry matter weight.

Key words: *Trichoderma*, maize, and zyngiberaceae extract.

INTRODUCTION

Maize is an important crop in in the world, including Indonesia. Maize is consumed as food, feed and other miscellaneus usages. The maize demand as food and feed is steadily increasing from year to year. Data in 2013 showed that the consumtion of corn was outspace the production in Indonesia (table 1).

Table 1. Corn Production & Consumption in ASEAN Region - 2013:

COUNTRY	PRODUCTION	CONSUMTION
Indonesia	**18,510,400**	**20,828,000**
Philippinens	**7,372,900**	**7,467,500**
Vietnam	**5,193,400**	**7,142,900**
Thailand	**5,065,000**	**4,716,000**
Myanmar	**1,525,700**	**1,356,400**
Laos	**1,018,200**	**797,300**
Cambodja	**911,100**	**204,500**
Singapore	**-**	**41,000**
Brunei	**-**	**4,500**

Source: ASEAN Food Security Information 2013

In order to increase corn production many efforts have been conducted, among them is the use of hybrid seeds. However, the price of hybrid corn seeds is very higher compared to local seeds. Many farmers still use local seeds in growing corn or growing corn seeds from the harvest of hybrid seeds, without protection. It could promotes the explosive of downy mildew disease, one of the most important disease in maize production.

Downy mildew is serious disease in maize production area. The disease severity varies among maize producing countries, depending upon the varieties grown. Downy mildew can cause total lost in susceptible variety (fig.1). In Indonesia, the loss for downy mildew also varies from maize producing provinces, in 1996, the disease caused 100% of yield loss in Lampung Province (Subandi *et al.* , 1996).

Fig.1 Heavy symptom of maize downy mildew on susceptble variety followed by total loss in Central Lampung.

Recently downy mildew is manage integratedly using resistant varieties and fungicides. Farmers combine resistant varieties with mathalaxyl applied as seed dresser. But, it is known that metalaxyl causes negative impact in the environment. Chemical compound, including methalaxyl, have been used to control plant diseases, but abuse in their employment. Methalaxyl causes negative side effects at least via two ways. Methalaxyl threaten the life of benifit mycroorganism such as mycorrhizal fungi in the rhizosphere of maize (Schecnk, 1982). Other weakness of methalaxyl is that the fungicide caused the fungal pathogens to be resistant against the fungicide (Erwin, 1983; Bains and Dhaliwal, 1994;Tjamos *et al.*, 1992). The fungicide has been used more than 50 years. The results of many researches stated that the use of methalaxyl in the long time could induce the presence of resistant fugal variants. When the metalaxyl resistant variants present in the field, the effectivity of metalaxyl against the pathogens steadily decreases from time to time. Katan and Bashi (1981) and Bains and Dhalliwal (1994) stated that the long use of metalaxyl could induce the presence of resistant fungal variant against the disease. Recently, Some reports stated that the effectivity of metalaxyl to control downy mildew decreased. According to Isakeit and Juster (2005) metalaxyl was not effective anymore against downy mildew caused by *Peronosclerospora sorghi.* It is aledged that some explosives of downy mildew are caused by the presence of resistant fungal variants of *P. sorghi*, *P. maydis*, and *P. phillipinensis*. The variants can adapt with metalaxyl well. In the host, the fungi have evolved to be new races with higher virulence (Perumal *et al.,* 2008). Thus many efforts have to be done to get a new alternative control free from the presence fungal resistant variants problem.

Biological control is becoming the promissing solution in integrated control. The use of microorganisms that protect plant is risk-free to the environment. *Trichoderma* is one of the popular microorganism used in plant disease management (Benites *et al.*, 2004). There were many reports on the potential of *Trichoderma* to manage plant diseases caused by ascomycetous, deuteromycetous, basidiomycetous, which are mainly soil-borne but also air-borne pathogens (Monte, 2001). Only very limited study has been done on the seed-borne pathogens such as *Peronosclerospora* spp using *Trichoderma*.

It is known that *Trichoderma* spp. role many fungtions in plant. *Trichoderma* spp. are antagonists against many pathogenic fungi, role as biocontrol agents. The mechanisms of biocontrol consisted of mycoparasitism, toxin production, competitor for space and resource. *Trichoderma* spp. take part in plant growth as plant growth promoting fungi. They also

known as inducer of plant defense system (Fig.2). Some researchers showed tha the use of *Trichoderma* systemically induce plant defense system. Djonovic *et Al.* (2007) reported the identification, purification, and characterization of an elicitor secreted by *T. virens*, a small protein designated Sm1 (small protein 1), that induced systemic resistance in maize. Fungi such as *Trichoderma* spp. can stimulate plant growth by suppressing plant diseases (Van Wees *et al*., 2008). *Trichoderma* spp. can form endophytic associations and interact with other microbes in the rhizosphere, thereby influencing disease protection, plant growth, and yield. Prasetyo (2009) showed that application of *Tricoderma* sp. isolat 14 could decrease downy midew incidence corn on cultivar Pacific 105. The mechanisms of this phenomenon may occur via induce systemic resistance or growth promoting done by the *Trichoderma* isolate.

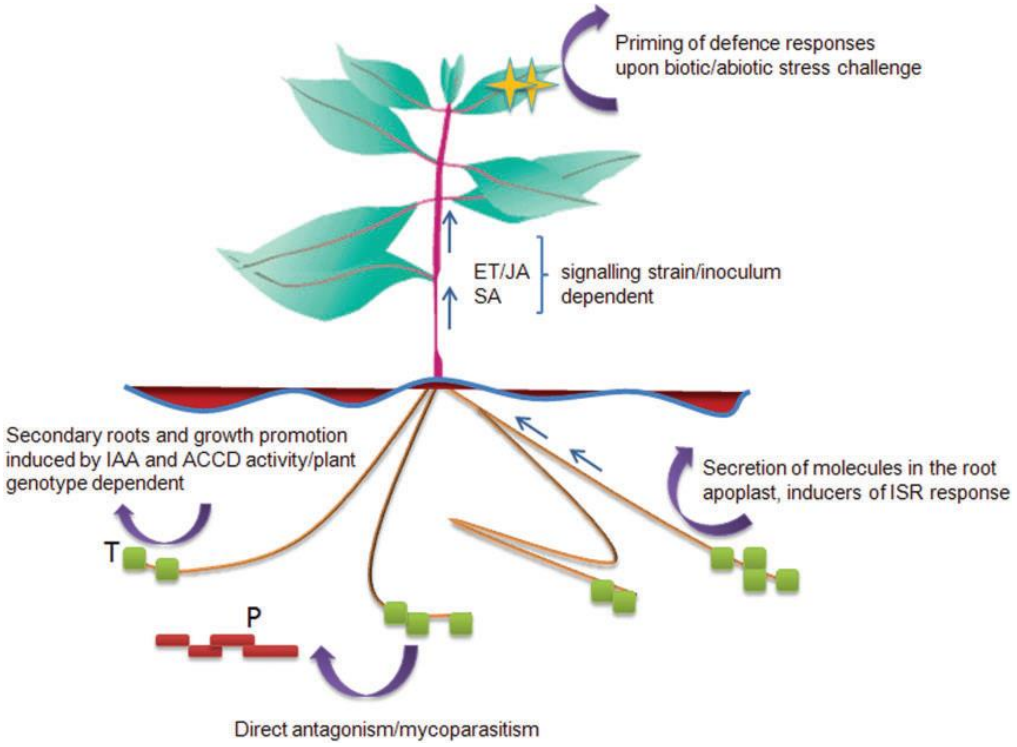

Fig.2 Schematic representation of *Trichoderma*–plant interaction (Hermosa *et al.* 2012). T, *Trichoderma*; P, pathogen; IAA, indole-3-acetic acid; ACCD, ACC deaminase; ET, ethylene; JA, jasmonic acid; SA, salicylic acid; ISR, induced systemic resistance

Plant extracts have been reported effective against plant pathogens and termed as botanical fungicides. In the context of agricultural disease management, the plant extracts are best suited for use in fresh. Plants have ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarin as

active ingredients against plant pathogens (Cowan, 1999). Allicin effectively controlled seed-borne *Alternaria* spp. in carrot, Phytophthora leaf blight of tomato and tuber blight of potato as well as *Magnaporthe* on rice and downy mildew of *Arabidopsis thaliana* (Slusarenko, 2008). Sekarsari (2012) reported that citronella extract decreased the incidence of maize downy mildew. The main componen of citronella are citronellal and geraniol as active ingredients against microbes. Many plants from the family of *Cingiberaceae* also produce active ingredients against fungi, their potential have not been tested to downy mildew of maize.

This study is an effort to integrate the use of *Trichoderma* and plant extracts from the family of *Cingeberaceac* to manage maize downy mildew. It is expected that the application of *Trichoderma* in rhizozphere will induce growth and resistance against downy mildew. The application of plant extract on the shoot will protect maize growing point directly from downy mildew pathogen.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of plant pests and diseases Faculty of Agriculture University of Lampung from December, 2016 to February, 2017. The experiment was aimed to evaluate the combine effects of *Trichoderma* isolates and plant extracts to manage maize downy mildew. Treatments of the experiments were arranged in randomized complete block design with three replicates. The treatments were combination between *Trichoderma* isolate from East Lampung (T1) and *Trichoderma* isolate from Unila Plant Clinics (T2) and *Cingiberaceae* extracts (F). The experiment consisted of 13 treatments, i.e. combination of no Trichoderma and no plant extract (T0F0), *Trichoderma* sp. isolate of East Lampung and ginger extract (T1F1), *Trichoderma* sp. isolate of East Lampung and tumeric extract (T1F2), *Trichoderma* sp. isolate of East Lampung and *Alpinia galanga* extract (T1F3), *Trichoderma* sp. isolate of East Lampumg and galangale extract (T1F4), *Trichoderma* sp. isolate of East Lampung and *Curcuma zanthorrhiza* extract (T1F5), *Trichoderma* sp. isolate of East Lampung and *C. aeroginosa* extract (T1F6), *Trichoderma* sp. isolate of Unila Plant Clinics and ginger extract (T2F1), *Trichoderma* sp. isolate of Unila Plant Clinics and tumeric extract (T2F2), *Trichoderma* sp. isolate of Unila Plant Clinics and *A. Galanga* extract (T2F3), *Trichoderma* sp. isolate of Unila Plant Clinics and galangale extract (T2F4), *Trichoderma* sp. isolate of Unila Plant Clinics and *C. zanthorrhiza* extract (T2F5), and *Trichoderma* sp. isolate of Unila Plant Clinics and *C. aeruginosa* extract (T2F6).

Investigated variables were disease incidence, incubation periode, plant height, and weight of dry matter. Data obtained were analysed using anova and the difference between means were tested using LSD test at 5% significant level.

Preparation of *Trichoderma* isolates.- *Trichoderma* isolates were obtained from collection of Unila Plant Clinics, namely isolate of East Lampung and isolate of Unila Plant Clinics. Seven days old cultures of *Trichoderma* in potato sucrose agar were harvested their spores and suspended in streile water to get 2,4 x 10^6 spore suspension.

Preparation of plant extracts.- Zingiberaceae tubers as much as 200 g were cut to be small pieces, ovened at 50^{o}C for 36 ours. Each of the dried tuber was grinded and sieved to get smooth powder. Alliquat was made by diluting 10 g of the powder in 100 ml of sterile aquadest, and filtered with cloth (Sekarsari *et al.*, 2012). The filtrat was centrifuged 3000 rpm for 10 minits and the pelet was discarded.

Preparation of Downy Mildew Inoculum.- Conidia of *Peronosclerospora* sp. was obtained from the field in Tegineneng, Pesawaran Regency. The inoculum was taken at 1.00 am in the morning. The diseased leaves at lower surface were sprayed with water, rubbed with spatula, and collected in beaker glass. The suspension was homogenized with *rotary mixer*. Conidia density was measured using haemacytometer (Sekarsari *et al.*, 2012).

Application of Trichoderma and Inoculation of *Peronosclerospora* sp.- Maize seeds (F2 of P27) were grown in polybag containing 5 kg of sterilized soil and goat manure (2:1). Each experimental unit consisted of three polybags. In each polybag two plants were grown separately. Suspension of *Trichoderma* (2,4 x 10^6) 10 ml was applied around the maize plant base at 5 days after planting. Twelve days after planting, at 2.00-3.00 am, three drops of conidia suspension (4.8 x 10^5) was inoculated on the growing point of maize plant.

Observation.- Observation was conducted every day along four weeks after planting. Variables investigated were disease incidence, incubation periode, plant height, and plant dry weight. Disease incidence of downy mildew was ditermined with the following furmula:

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

N

Where DI = Disease Incidence
n = Number of diseased plant
N = number of all plant investigated

RESULTS AND DISSCUSSIONS

Disease Incidence

The results of the experiment showed that all combination of *Trichoderma* isolates and *Zingiberacea* plant extracts influanced maize downy mildew (Table 1). All of combination between *Trichoderma* isolates and plant extracts significantly decreased the incidence of maize downy mildew. T1F2, T1F3, T1F6, T2F1, T2F3, T2F5 were more effective compared to other treatments; and T1F1, T2F4 were more effective compared to T1F5, T2F6 in decreasing downy mildew incidence. Other result showed that F2 and F6 were more synergic when combined with T1 than with T2. The potential of all combinations to decrease downy mildew incidence were consistant since the early to the end of the observation (Fig.2)

Trichoderma combined with plant extracts could decrease the disease incidence of maize downy mildew for some reasons. Plant extracts used in this experiment were obtained fron the family of *Zyngiberaceae*. First, Plant in this family produces substances those have ability to inactive microbes such as plant pathogens. Turmeric (*Curcuma longa* Linn.) and Ginger (*Zingiber officinale* Rosc.) contain active ingredients those inactivates *Phytopthora infestans*, *Fusarium solani*, and *Pyricularia oryzae* (Bandara *et al.*, 1989). Suprapto and Khalimi (2009) reported that *A. Galanga* could control stem rot of vanilla. Second, *Trichoderma* spp. have been reported applied in soil could induce systemic resistance in plants (Hermosa *et al.*, 2012). Prasetyo (2009) showed that application of *Tricoderma* sp. isolat 14 could decrease downy midew incidence of corn on cultivar Pacific 105. Appication *Trichoderma hamatum* Uom 13 on pear millet increased the resistance of the plant against downy mildew (Siddaiah *et al.*, 2017)

Table 1. The influance of *Trichoderma* isolates and plant extracts combination on some variables

Treatments	Variables investigated			
	Disease Incidence (%)	Incubation periode (Days)	Plant height (cm)	Dry Weight (g)
T0F0	94.44 d	12 .28 a	57.41 a	9.14 a
T1F1	44.45 b	22.00 b	97.49 c	14.18 b
T1F2	33.33 a	23.83 c	96.36 c	18.27 c
T1F3	33.33 a	24.39 c	93.14 bc	18.86 c
T1F4	33.34 ab	23.11 bc	94.40 c	13.40 ab
T1F5	55.56 c	19.44 b	87.32 b	14.07 b
T1F6	16.67 a	26.89 c	92.34 b	14.12 b
T2F1	27.78 a	25.50 c	94.71 c	14.84 bc
T2F2	50.00 bc	21.28 b	91.48 b	21.20 d
T2F3	33.33 a	25.11 c	100.17 d	22.26 d
T2F4	38.89 b	22.39 b	98.51 c	18.99 cd
T2F5	33.33 a	24.44 c	99.10 cd	18.72 c
T2F6	61.11 c	19.39 b	88.97 b	20.03 d

Note: Number in the same column followed by the same letter is not significantly different accordingto LSD test α=5%. T0F0 (combination of no Trichoderma and no plant extract), T1F1 (*Trichoderma* sp. isolate of East Lampung and ginger extract), T1F2 (*Trichoderma* sp. isolate of East Lampung and tumeric extract), T1F3 (*Trichoderma* sp. isolate of East Lampung and *Alpinia galanga* extract), T1F4 (*Trichoderma* sp. isolate of East Lampumg and galangale extract), T1F5 *Trichoderma* sp. isolate of East Lampung and *Curcuma zanthorrhiza* extract), T1F6 (*Trichoderma* sp. isolate of East Lampung and *C. aeroginosa* extract), T2F1 (*Trichoderma* sp. isolate of Unila Plant Clinics and ginger extract), T2F2 (*Trichoderma* sp. isolate of Unila Plant Clinics and tumeric extract), T2F3 *Trichoderma* sp. isolate of Unila Plant Clinics and *A. Galanga* extract), T2F4 *Trichoderma* sp. isolate of Unila Plant Clinics and galangale extract), T2F5 *Trichoderma* sp. isolate of Unila Plant Clinics and *C. zanthorrhiza* extract), and T2F6 *Trichoderma* sp. isolate of Unila Plant Clinics and *C. aeruginosa* extract).

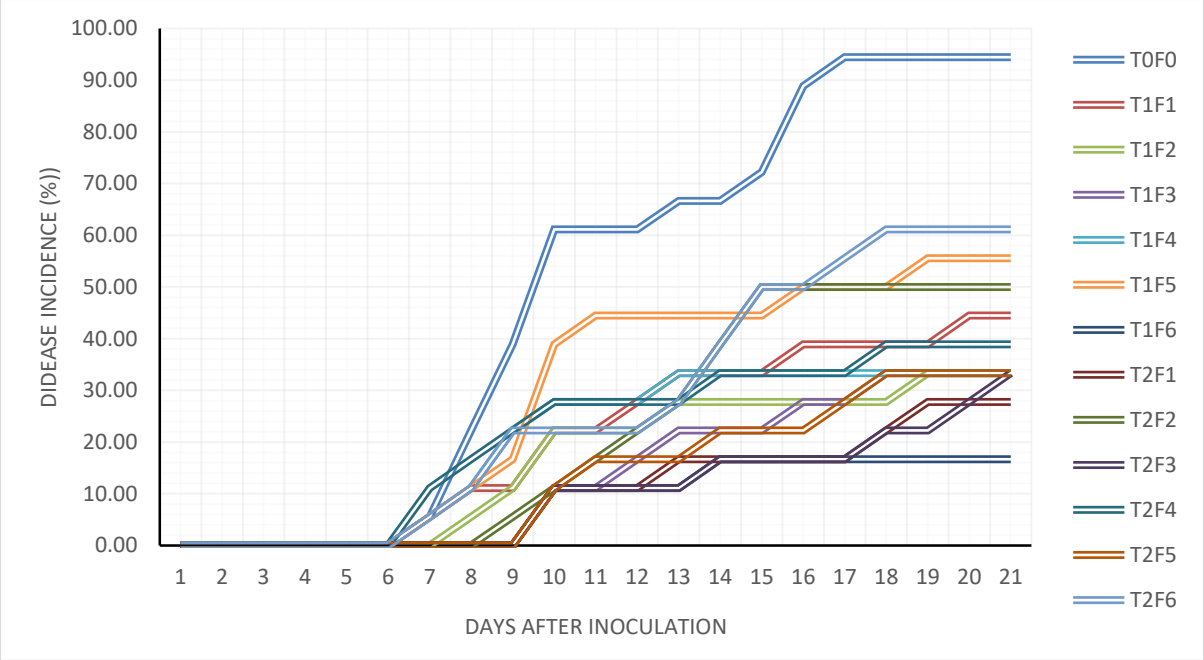

Fig.2 Progress of maize downy mildew under various treatments.

Incubation Periode of Downy Mildew

All of combination between *Trichoderma* isolates and plant extracts significantly increased the incubation periode of maize downy mildew. T1F2, T1F3, T1F6, T2F1, T2F3, and T2F5 were more effective compared to T1F1, T1F5, T2F2, T2F4 in increasing incubation periode. T1 combined with F1 and T1 combined with F5 was less effective compared to T2 combined with F1 and T2 combined with F5, respectively, on the other side T1 combined with F2 was more effective compared to T2 combined F2 in increasing incubation periode.

Combinations of *Trichoderma* isolates and plant extracts could increase th incubation periode at least for two reasons. Plant extracts directly affected by weakening the pathgen inoculum. It retarded the infection procces. *Trichoderma* could induce resistance in maize plant. It has been reported that application of *Trichoderma* induce the plant to produce chitinase and glucanase those able to breakdown the pathogen cell wall. *T. hamatum* treated millet seedlings responded to downy mildew infection with high lignification and callose deposition (Siddaiah iet al., 2017). Analysis of defense enzymes showed that *T. hamatum* treatment significantly enhanced the activities of glucanase, peroxidase, phenylalanine ammonia-lyase, and polyphenol oxidase in comparison to untreated control (Siddaiah *et al.*, 2017)

Plant height and Dry Weight of Plant

All treatments investigated significantly increased plant height compared to control. Treatment of T2F3 was the most effective to increase plant height and followed by T1F1, T1F2, T1F4, T2F1, T2F4, and T2F5. T1F5, T1F6, and T2F2 were less effective compared to T1F1, T1F2, T1F4, T2F1, T2F4, and T2F5 in increasing plant height. T2 combined with F3 was more effective compared to T1 combined with F3; on the contrary T2 combined with F2 was less effective than T1 combined with F2 in increasing plant height.

All the treatments investigated significantly increased dry weight maize plant compared to control. T2F2, T2F3, and T2F6 were more effective compared to T1F2, T1F3, and T2F5; and T1F1, T1F5, and T1F6 were less effective compared to T1F1, T1F2, T1F4, T2F1, T2F4, and T2F5 in increasing plant weight. T1 combined with F2 and T1 combined with F3 were less effective compared to T2 combined with F2 and T2 combined with F3
Combination of *Trichoderma* and plant extracts could increase plant growth via two mechanism. First, plant extracts could directly inactivate pathogen inoculum and kept maize plant to grow well. Second, *Trichoderma* spp have beneficial effects on plant growth. Further study demonstrated that *Trichoderma* increase root developtment and crop yield the proliferation of secondary roots, and seedling fresh weight and foliar area (Harman, 2000). Maize rhizosphere colonization by T. virens also induces higher photosynthetic rates and systemic increases in the uptake of CO2 in leaves (Vargas et al., 2009).

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