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Research Article

Identification of Pathogens Causing Bulb Rot Disease on Garlic (*Allium sativum* L.) in Central Java, Indonesia

Lutfi Arifin[1)], Siwi Indarti[1)]* & Arif Wibowo[1)]

[1)]Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada, Jln. Flora No. 1, Bulaksumur, Sleman, Yogyakarta 55281, Indonesia

**Corresponding author. E-mail: siwi.indarti@ugm.ac.id*

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ABSTRACT

Garlic bulb rot disease was found from garlics (*Allium sativum* L.) cultivated from 2017 to 2019 by farmers in Central Java Province, Indonesia. The initial symptoms of the disease were stunted, leaf yellowing, and necrotizing to rotten bulbs. This research was conducted to determine the major causal agent of garlic bulb rot disease in Central Java. A survey was carried out in five regencies across Central Java that were major garlic-producing areas. Nematodes were isolated using water immersion methode and pathogenic fungi were isolated on Potato Dextrose Agar (PDA). Nematode identification was carried out based on the *Ditylenchus dipsaci* morphological and morphometric character. Seven isolates of Fusarium species were obtained from infected garlic. Identification of four chosen isolates were performed by sequencing the TEF-1α gene. The TEF sequence of isolate TB3, KK1, and KK4 showed 99% similarity with several *F. oxysporum,* BT3 sequences showed 98% identity with several *F. solani,* and all were deposited in the NCBI GenBank. Three locations were positively infected by the interaction between *D. dipsaci* and *Fusarium* sp. Based on the results of the morphological identification, parasitic nematode was identified as *D. dipsaci*, while based on the morphological and molecular identification isolates *Fusarium* were identified as *F. oxysporum* and *F. solani*, respectively, as first report causal agents of garlic bulbs rot in Central Java.

Keywords: association; bulb rot disease; *Ditylenchus dipsaci*; *Fusarium* spp. TEF-1α gene

INTRODUCTION

Garlic is an economic crop cultivated in Indonesia. Bulbs rot disease was found in garlic crop in Central Java, Indonesia (Indarti *et al.,* 2018). The infection of bulb to rot disease in the field causes bulbs to be not sellable. Yield loss caused by bulb rots disease can reache 35–40% (L.-J. Zhang *et al*., 2017). The disease infect bulbs and root of garlic plants. Symptoms include leaf yellowing and premature death. The initial symptoms of bulb rot disease are shrunken bulbs that become softer, darker and eventually decay. Bulb and root rot are caused by *Ditylenchus dipsaci* and its occurrence have been reported in Canada (Hajihassani & Tenuta, 2016), Israel (Aftalion & Cohn, 1990), Mexico (French *et al*., 2017), Ohio (Testen *et al.,* 2014), Turkey (Yavuzaslanoğlu *et al.,* 2015), and Indonesia (Indarti *et al.,* 2018). Garlic bulb rot can also be caused by the infection of fungal pathogen, such as from the genus Fusarium (Dugan *et al.,* 2003; Moharam *et al.,* 2013; L.-J. Zhang *et al.,* 2017).

Interaction between pathogens and other organisms associated with plants can increase the severity of a disease. The association between *Meloidogyne incognita* with *Fusarium clamydosporum* can aggravate the disease (Devappa *et al.,* 2009). Several cases of plant-parasitic nematode associations with fungi have been reported, such as the pathogenic relationship between *D. dipsaci* and *Fusarium oxysporum* f. sp. *medicaginis* that can break the resistance of varieties with nematode and *Fusarium* -resistant characteristics in alfalfa (Griffin, 1990). The synergistic interaction between *D. dipsaci* and *Rhizoctonia solani* were also reported in sugar beets (Hillnhütter *et al.,* 2011). Other association between *M. incognita*

and *F. oxysporum* in tomato plants caused complex diseases (Kassie, 2019). Therefore, investigation on the causal agents of bulbs rot disease was done and the role of both nematode and fungi were determined and the possible interaction between both of them in bulb rot disease incidences.

MATERIALS AND METHODS

Collecting Samples from Infected Garlic

Samples of infected garlic were collected from garlic producing area in Central Java (Magelang, Temanggung, Karanganyar, Tegal, and Brebes) during October 2018–April 2019. Bulbs showing rot symptoms were recorded. Furthemore, samples of infected plants were taken to isolate pathogens on PDA plates. Nematode samples were analized from bulbs and infected roots.

Isolation and Identification of Pathogens

Both nematodes and fungi were isolated from infected bulb, mainly from garlic bulbs showing rotting discoloration or wilting. Nematode extraction was done using the water immersion method (S.L. Zhang *et al.,* 2014). Morphological and morphometrical observation of each nematode specimens was done by comparing to related published data for identification to species level. *Fusarium* collected from rotten bulbs were sterilized in 5% NaOCl solution for 30 seconds, rinsed with sterilized water for five times. Pieces excised from lesion margins were transferred to potato dextrose agar (PDA). Cultures were incubated at 25°C in dark conditions for seven days.

Fungal colonies were purified either by using successive transplanting of the colony edges or by using single spore techniques. The purified fungi were identified according to the fungal morphological and microscopical characteristics as described by Leslie and Summerell (2006). DNA extraction was carried out as described by Zhu *et al.* (2014). Genomic DNA from Fusarium isolates were used for PCR amplification of the TEF-1α gene using the primer EF-1 (forward primer; 5'-ATGGGTAAGGA(A/G) GACAAGAC-3) and EF-2 (reverse primer; 5'GGA (G/A)GTACCAGT(G/C)ATCATGTT-3') following conditions described by O'Donnell *et al.* (1998). The products were sent to Apical Scientific Sequencing, 1st BASE DNA Sequencing. The sequences obtained were assembled and edited manually using BioEdit v. 7.0.9 (Hall, 1999), and then analyzed using Basic Local Alignment Search Tool (BLAST) NCBI to clarify the homology from closest species. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura & Nei, 1993). The tree with the highest log likelihood (-1861,45) was shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 21 nucleotide sequences. There were a total of 689 positions in the final dataset. Evolutionary analyses were conducted in MEGA (Molecular Evolutionary Genetics Analysis) software version X (Kumar *et al.,* 2018). *Athelia rolfsii* was used as an outgroup for the analysis. The obtained culture isolates were maintained on PDA plates and kept in refrigerator at 5°C for further study.

Pathogenicity Test

The pathogenic relationship on plant parasitic nematodes as an opening agent for fungal infection was tested (Griffin, 1990; Hillnhütter *et al.,* 2011; Kassie, 2019). In this study *F. oxysporum* was chosen for the pathogenicity test because it was found in the field and have been reported to interact *D. dipsaci*. To study the effect of simultaneous or sequential infestation. on garlic plant was designed under the greenhouse and maintained at 23 ± 4°C. Plants were inoculated with adding 300 *D. dipsaci* per pot and 5×10^7 microconidia of *Fusarium oxysporum*. Inoculation of all pathogens were applied singularly or in combination. Treatments were 1) uninoculated (control), 2) inoculation of *D. dipsaci* three days before inoculation of *F. oxysporum*, 3) inoculation of *F. oxysporum* three days before inoculation of *D. dipsaci*, 4) inoculation of *D. dipsaci* and *F. oxysporum* simultaneously, 5) inoculation of *D. dipsaci* alone, 6) inoculation of *F. oxysporum* alone. Treatments were replicated 5 times, 15 plants were used for every replication. The experiment was terminated when plants reached 8 weeks-old, plant showed mortality, and bulbs and root weights were measured.

Severity of above-ground symptoms were periodically assessed for each plant on a 0–6 scale, according to the percentage of foliage with yellowing or necrosis: (0 = 0%, 1 = 0.1–19.9%, 2 = 20–39.9%, 3 = 40-59.9%, 4 = 60–79.9%, 5 = 80–99.9%, 6 = dead plant). Disease incidence (D.I.) was calculated according to Devappa *et al.* (2009) as:

$$\text{D.I. (\%)} = \left(\frac{\Sigma\,(n \times v)}{N \times V}\right) \times 100$$

Where, (n) = the number of diseased transplants per category, (v) = category number, N = total number of the transplants, (V) = maximum of category. Reisolation was carried out from infected transplants showing disease symptoms. The nematode and the isolated fungi were compared with the original causal agents cultures used.

Statistical Analyses

Plant growth parameters were subjected to analysis of variance with the general linear models procedure of SAS (SAS Institute, Cary, NC). Differences between root and bulb weights of the treated plants and the untreated (control) plants were determined by using Tukey's test at $P < 0.05$ (SAS Institute, 1998).

RESULTS AND DISCUSSION

Sampling and Diagnosis

Five different district location of Central Java were surveyed and plants along with the soil surrounding them were collected. In total, 94 plant samples exhibiting wilt symptoms were collected from 21 fields in five districts (Table 1). Results from three districts implied interaction between *D. dipsaci* and *Fusarium* spp. on garlic bulb showing rot disease. Garlic bulb rot in the field showed specific symptoms (Figure 1). Symptoms could be observed on leaves, bulbs, and roots of plants since the vegetative and through the generative phases. Chlorosis were observed on leaves. Wilting from the top to the bottom of the plant were also observed. Infected plants were usually easily uprooted from the ground. Early symptoms of this disease were wilted plants and defected growth on roots and tubers of plants. As the disease progresses, the plant bulbs would turn brown and eventually rot (Figure 1c).

Yin *et al.* (2015) reported that the initial symptoms of bulbs rot disease were stunted plant growth, chlorosis in apical leaves, plants withering and dying. However, field symptoms caused by nematode showed

Table 1. *Ditylenchus dipsaci* and *Fusarium* spp. occurrence in garlic crop from survey locations

District	Cultivar	Sample	*D. dipsaci*	*Fusarium* spp.
Magelang	Lumbu Kuning	LK 1	+	+
	Lumbu Kuning	LK 2	+	-
	Lumbu Kuning	LK 3	+	-
	Lumbu Kuning	LK 4	+	+
	Tawangmangu Baru	TB 1	+	-
	Tawangmangu Baru	TB 2	+	+
	Tawangmangu Baru	TB 3	+	-
Temanggung	Tawangmangu Baru	TB 1	-	-
	Tawangmangu Baru	TB 2	+	+
	Lumbu Kuning	LK 1	+	-
	Lumbu Kuning	LK 2	-	-
	Lumbu Kuning	LK 3	+	+
	Lumbu Kuning	LK 4	+	+
Brebes	Tawangmangu Baru	TB 1	+	+
	Sangga Sembalun	SS 1	-	-
	Tawangmangu Baru	TB 1	-	-
Tegal	Tawangmangu Baru	TB 1	+	-
	Sangga Sembalun	SS1	+	-
	Lumbu Putih	LP 1	-	-
Karanganyar	Tawangmangu Baru	TB 1	-	-
	Tawangmangu Baru	TB 2	-	-

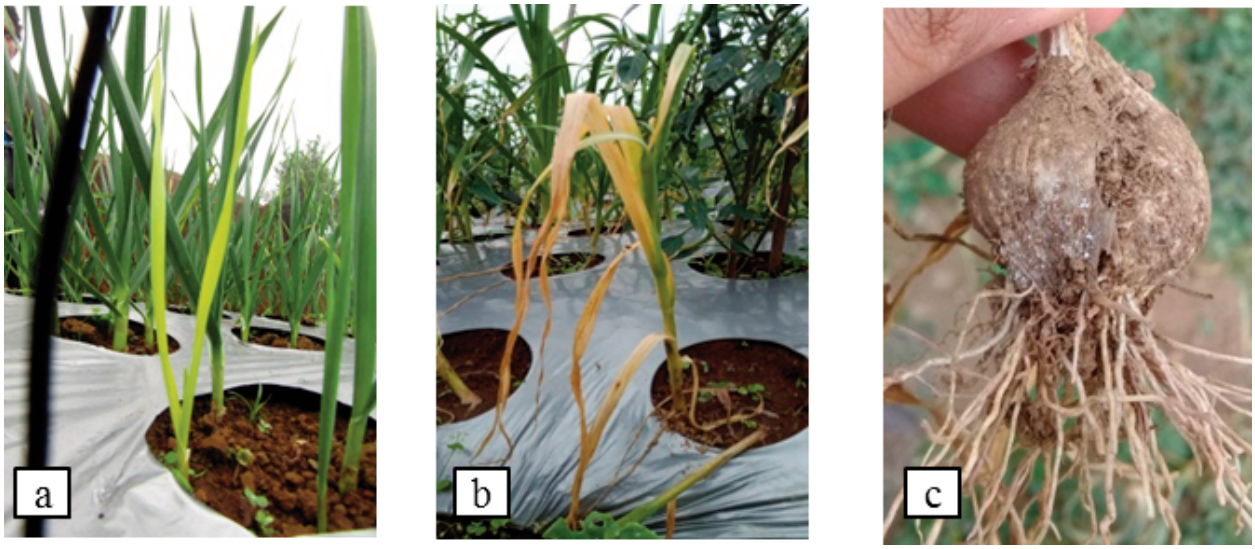

Figure 1. Spesific symptom of bulb rot diseases in location sampling with (a) stunting of plants, (b) leaf distortion and discoloration and (c) rotten garlic bulb

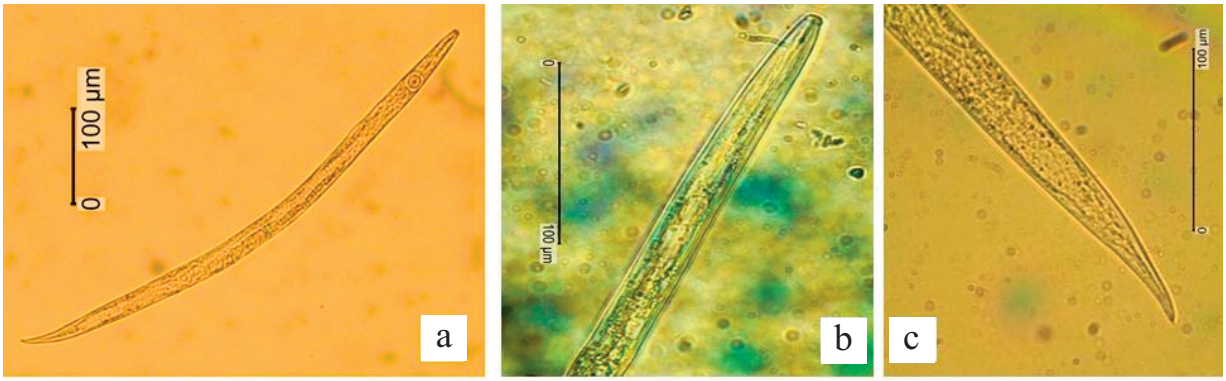

Figure 2. Adult female of *Ditylenchus dipsaci;* (a) full body, (b) anterior, (c) posterior

different or uncertain characteristics. Symptoms could be singular or a combination of several symptoms including dwarf plants or leaf chlorosis or necrosis in bulbs (European and Mediterranean Plant Protection Organization [EPPO], 2017; L.-J. Zhang *et al.,* 2017; Indarti *et al.,* 2018).

Identification of the Causal Patogens

Nematode Identification

Garlic samples tested came from five districts in various garlic cultivars. Detection results showed that from samples of positive garlic bulb rot disease confirmed the presence of *D. dipsaci*. *D. dipsaci* morphological characters had stylets with a length of 7.81–12.42 µm. Knob stylet were rounded and well developed. Median bulb were muscular, with thickening of lumen walls and clearly observable. Basal bulb were not overlapping with the intestine (Figure 2). Tail terminus was pointed. The morphological character of this nematode followed the criteria of *D. dipsaci* (Mai *et al.,* 1968; International Plant Protection Convention [IPPC], 2015; EPPO, 2017).

Baicheva and Budurova (1994) reported that the morphological character of *D. dipsaci* were slim bodies, elongated then tapered body shape, thin and flat nematode lip, clear cuticle annulation, short stylet, short nematode, female nematode had monodelphic vulva, male nematode had a covering that covers bursa until ¾ the tail length, and at the end of the tail was tapered. It had a median bulb shaped starting from its ovoid to fusiform, with an esophagus that does not overlap , with tail length of 4–7 times its body width (IPPC, 2015).

Morphometric measurements for *D. dipsaci* females (n=5) were ± 0.50 (0.35 to 0.62) mm for body length, max body length to body width of ± 30.97 (25.82 to 41.60) µm, body to oesophageal length of ± 5.24 (3.88 to 9.08) µm, and body to tail length of ± 9.13 (7.66 to 12.32) µm. Referring to Indarti *et al.* (2018) the morphometric values complied with the criteria of *D. dipsaci*. Morphological characters resembled as *D. dipsaci*. Yavuzaslanoğlu *et al.* (2015) reported that this nematode infestation caused yield losses to 15% in onions and high intensity attacks could even reach 90% loss. The discovery of *D. dipsaci* in Central Java supports the report of Indarti *et al.* (2018) about the first detection on the discovery of this nematode in Temanggung.

Identification of Pathogenic Fungi

A total of 7 fungal isolates (TK3, TT2, TK4, KT2, KK1, KK4, BT3) were collected from infected garlic crops. Three isolates (KT2, KK1, KK4) were found from Magelang district, 3 isolates (TK3, TT2, TK4) from Temanggung district and 1 isolate (BT3) from Brebes district (Table 1). The colony of all isolates had similar appearance. The colony color of each isolate on the PDA medium showed to initially have white mycelium that then remained white for TT2 and KT2 isolates, turned purple for TK3 and TK4, or pale purple for KK1 and KK4 (Figure 3). Different fungal pigmentation occurs because its growing medium. Wiemann *et al.* (2009) reported that some pigments have a protective role against environmental stresses such as irradiation and oxidation while others contribute to virulence. Therefore all isolates could not clearly be differentiated.

The bottoms surface of the petridish, concentrated color could be found from isolates TK4, KK1 and KK4. Ignjatov *et al.* (2017) suggested that the *Fusarium* sp. colonies are sometimes white but more often slightly purple, where as according to Summerell *et al.* (2003) most of the isolates of *Fusarium* sp. had colonies that were white or accompanied by purple or pink coloration at the center of the colony. This was consistent with findings from Kassie (2019) that reported *F. oxysporum* on PDA media forms a mycelium which is insulated and initially white and gradually turns to purple. The occurrence of color differences over time is more likely due to the accumulation of colors produced during the growth of *F. oxysporum* (Ambar *et al.*, 2019). Based on observations made on each tested isolate, in general the isolates produced abundant air cotton-like mycelium, white in color and sometimes slightly purple.

In general, fusiform were sickle-shaped, mostly with an elongated apical cell and pedicellate basal cell (Figure 4). Macroconidia had thick and smooth walls, with tapered, apical, foot-saped cells at the bottom of the cell. Macroconidium consisted of 3–6 septa attached to a monopialid. BT3 isolates had the longest macroconidium, 40.14 –4.70 µm – 75.75 × 5.00 µm. Kassie (2019) reported that *F. oxysporum* macroconidium is curved, attached to the conidiophoric branches or sporodokium ends, having a sizes of 15.90 × 1.83 µm–46.98 × 4.88 µm with 3–5 septa.

Microconidium observed under the microscope was generally round or oval, oval and sometimes curved if it had septae, with sizes ranging from 2.23 × 2.20 µm –14.87 × 4.83 µm (Figure 5). Isolates BT3 observed

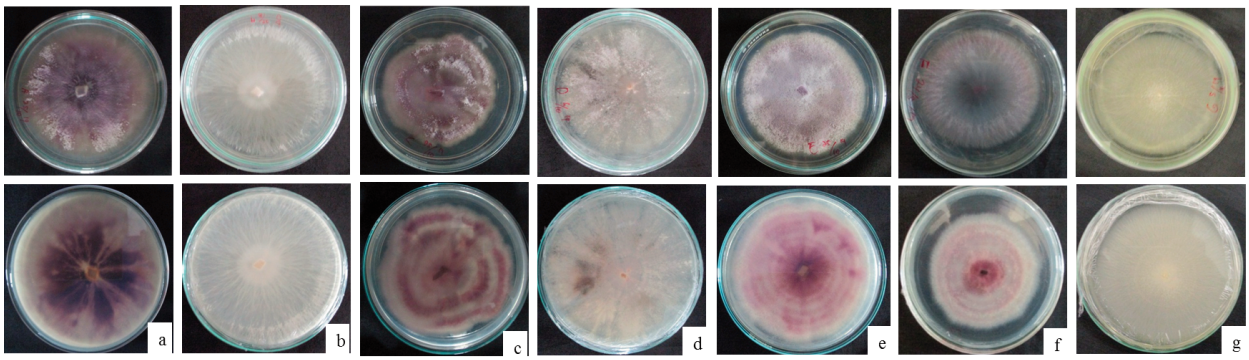

Figure 3. Colour of *Fusarium* spp. colonies on PDA medium from survey locations (a) isolate TK3, (b) isolate TT2, (c) isolate TK4, (d) isolate KT2, (e) isolate KK1, (f) isolate KK4, (g) isolate BT3. The top plate in each pair is the upper surface and the lower plate is the under surface

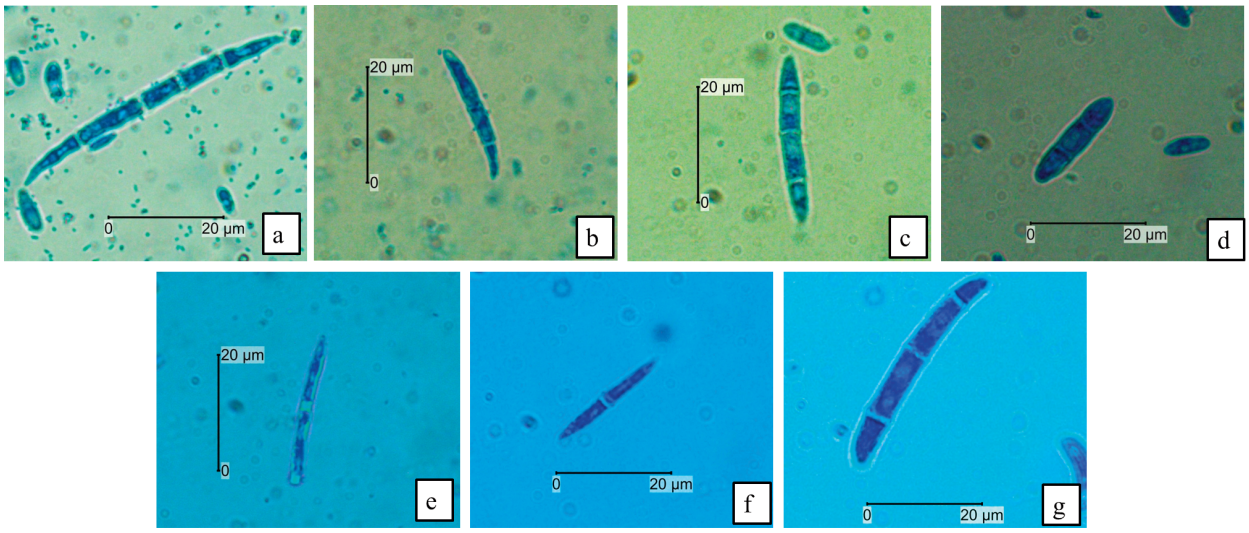

Figure 4. Macroconidia of Fusarium species (a) isolate TK3, (b) isolate TT2, (c) isolate TK4, (d) isolate KT2, (e) isolate KK1, (f) isolate KK4, (g) isolate BT3

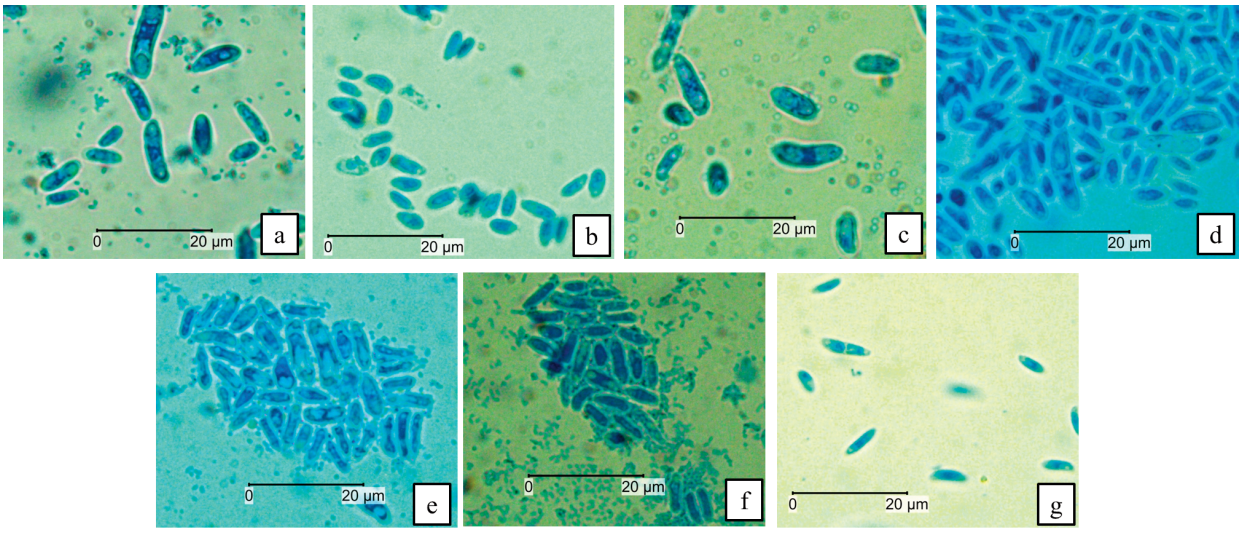

Figure 5. Microconidia of Fusarium species (a) isolate TK3, (b) isolate TT2, (c) isolate TK4, (d) isolate KT2, (e) isolate KK1, (f) isolate KK4, (g) isolate BT3

had microconidium with the longest conidium with sizes ranging from 9.19 × 4.83 µm – 14.87 × 4.83 µm. According to Kassie (2019) the microconidium was attached to the monophialid or conidiophoric branches, oval-ellipses shaped, straight or even curved, measuring between 6.75 × 1.93 µm – 13.56 × 3.4 µm.

Microconidia were always present, especially those that do not act as a result of monophialid or conidiophores with short branches or false heads (Figure 6). *F. oxysporum* had conidiophores (monofialid) with a short stem which is bound at 3-5 microconidium bound (Palmero *et al.*, 2012). This was supported by the statements of Leslie and Summerell (2006) that stated that the length of the *F. oxysporum* phialid is shorter than that of *F. solani*.

Based on the colony's morphology and characteristics of macro and micro conidia, seven fungal isolates were identified as *Fusarium*. After further microscopic observations, six isolates were identified as *F. oxysporum* based on the macroconidia characteristics which were thin walled with 3–5 septate, while based on false head characteristic, one isolate was identified as *F. solani*. Molecular identification are more accurate and can be used as a comparison method between morphological observation methods.

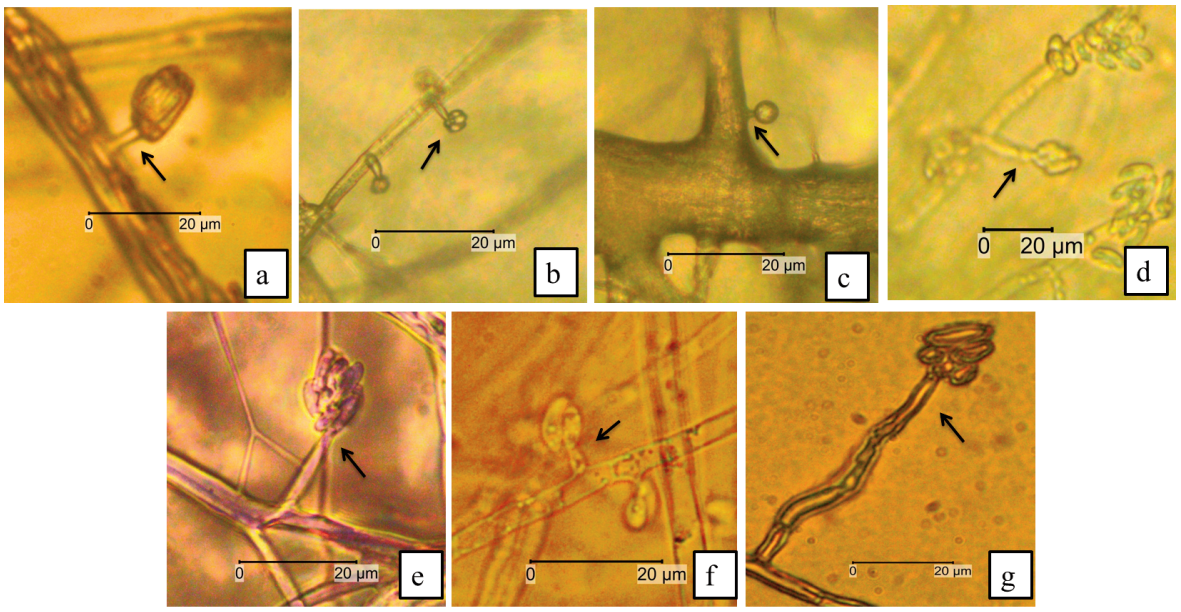

Figure 6. False heads of Fusarium species (a) isolate TK3, (b) isolate TT2, (c) isolate TK4, (d) isolate KT2, (e) isolate KK1, (f) isolate KK4, (g) isolate BT3

Molecular identification was done on 4 isolates based on morphology and sampling site. The four chosen isolates (KK1, KK4, BT3, and KT2) were sequenced for the EF-1α gene. The results using EF1 and EF2 primers, which were specific primers of the *Fusarium* genus, showed DNA bands of 750 bp. O'Donnell *et al.* (1998) produced DNA bands with molecular weights of 700 bp when using this specific primer for PCR method. The TEF sequence of isolate TB3, KK1, and KK4 showed 99% similarity with several *F. oxysporum* sequences. Sequence of BT3 showed 98% identity with several *F. solani* and they were deposited in the NCBI GenBank. Phylogenic trees were arranged based on the results of DNA band sequencing. TEF sequences of Fusarium genus were aligned with the consensus region using CLUSTAL W program and 1000 bootstrap replicates were used on sequence analysis. Three isolates (KK1, KK4, and KT2) were compared to the reference *F. oxysporum* strain (FJ538245.1; JQ965439.1; JQ965441.1; JQ965444.1; KX6097 08.1; MK651510.1; MK266491,1; MH341212.1; MG356947.1). High degrees (94%) of phylogenetic similarity was detected (Figure 7). The results were consisted with morphological result of this study implying that Fusarium isolated from infected garlic bulb in Central Java were *F. oxysporum* and *F. solani*.

Mix Symptom of *Ditylenchus dipsaci* and *Fusarium* spp. in Garlic Crop

Three of the five districts had *D. dipsaci* and *F. oxysporum*. *D. dipsaci* infection showed dwarfed plants or yellow leaves with twisted leaf tips. While infection of both *D. dipsaci* and *F. oxysporum* showed specific symptoms of yellowing of leaves and necrotic to rotting tubers on bulbs. Interaction between pathogens and other organisms associated with plants can increase the severity of a plant disease (Meena *et al.,* 2016).

Association between nematodes and pathogenic fungi may cause far greater infections than only one of the pathogens (Mudawi *et al.,* 2018). Association between plant-parasitic nematodes and pathogenic fungi caused severe damaged on plant tissues. Plant parasitic nematodes create openings for fungal infection (Wiratno *et al.,* 2019). This was indicated by garlic bulb showing rot and collected from Tegal which only found the presence of *D. dipsaci* with dwarf plant symptoms. In contrast, Magelang samples possessed *D. dipsaci* and *Fusarium* sp. and showed severely damaged plants with yellow leaves and bulb rot.

Development of disease symptoms is not only determined by the role of pathogens, but also depends on the complex interrelationships between host plants, pathogens, and prevailing environmental conditions, and for soil-borne pathogens will be largely determined

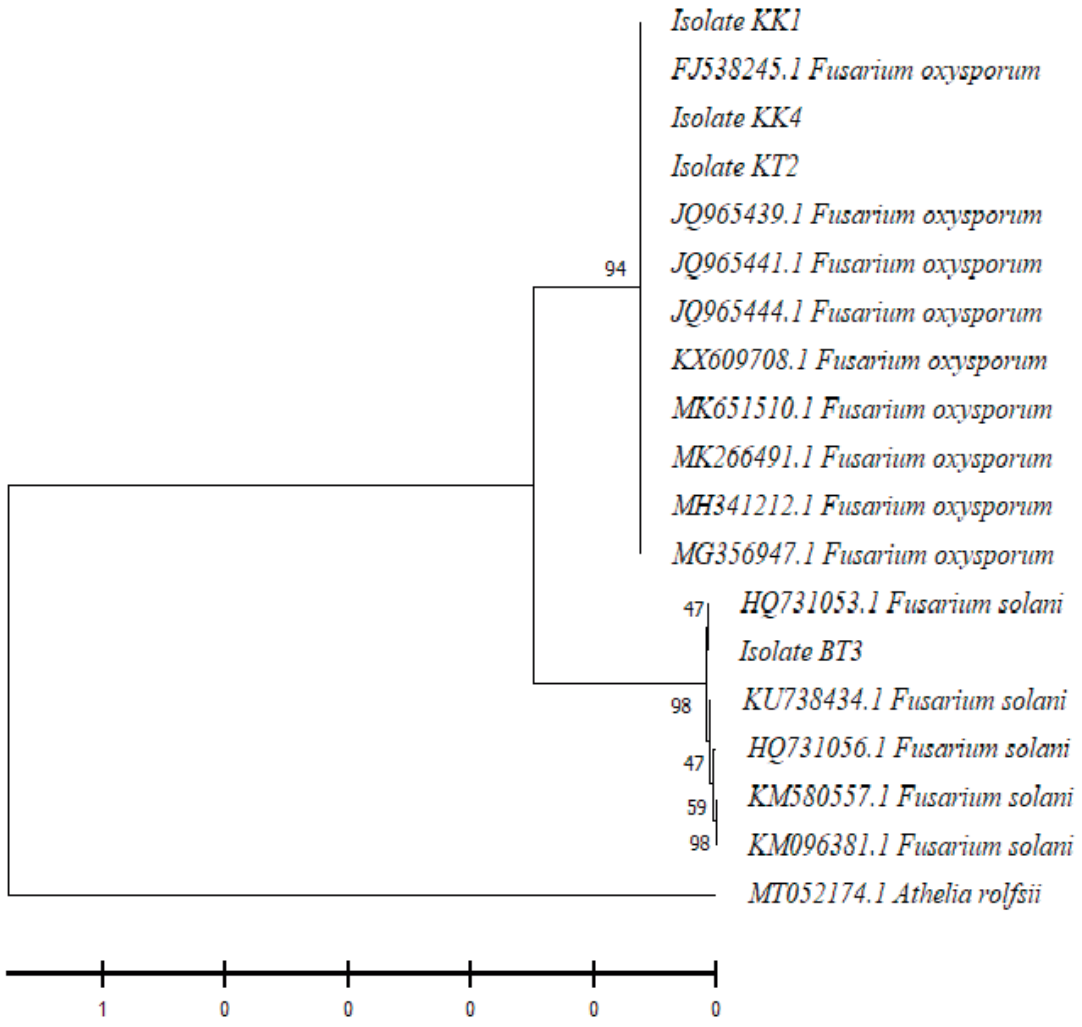

Figure 7. The phylogenic construction of the four isolates of Fusarium isolated from symptomatic bulbs in Central Java based on PCR analysis using primers EF1 and EF2

by their interactions with other microorganisms that occupy the same ecological niches. Endoparasitic nematodes, such as *Ditylenchus*, will penetrate roots through the root tip which is still actively growing and damage caused by nematodes determines the severity of disease by soil-borne pathogens such as *Fusarium* (Back *et al.,* 2002; Williamson & Gleason, 2003). According to Hillnhütter *et al.* (2011), *D. dipsaci* penetration into plant bulbs will result in injuries that occur mechanically and chemically causing easier infection of fungi.

Pathogenicity Tests

The role of *D. dipsaci* and *F. oxysporum* as a cause of bulb rot disease, was tested by inoculating *D. dipsaci* and *F. oxysporum* on garlic plants. Infected plants and disease intensity on leaves caused by *D. dipsaci* and *Fusarium* spp. are shown in Figure 8.

L.-J. Zhang *et al.* (2017) reported that the initial symptoms of bulb rot disease were stunted plant growth, chlorosis on apical leaves, plants withering and dying. However, field symptoms caused by nematode had different or uncertain characteristics. Symptoms could be single symptoms or a combination of several symptoms including dwarf plants or leaf chlorosis or necrosis in bulbs (Indarti *et al.,* 2018).

Single inoculation using *Fusarium* significantly affected the bulb weight, but *D. dipsaci* inoculation alone showed no significant difference compared to the control. Single inoculation treatment of *D. dipsaci* showed that bulb weight was not significantly different from the single inoculation treatment of *F. oxysporum*. However, the bulb weight values indicated that single inoculation of *F. oxysporum* resulted in higher yield. This was presumably because *D. dipsaci*

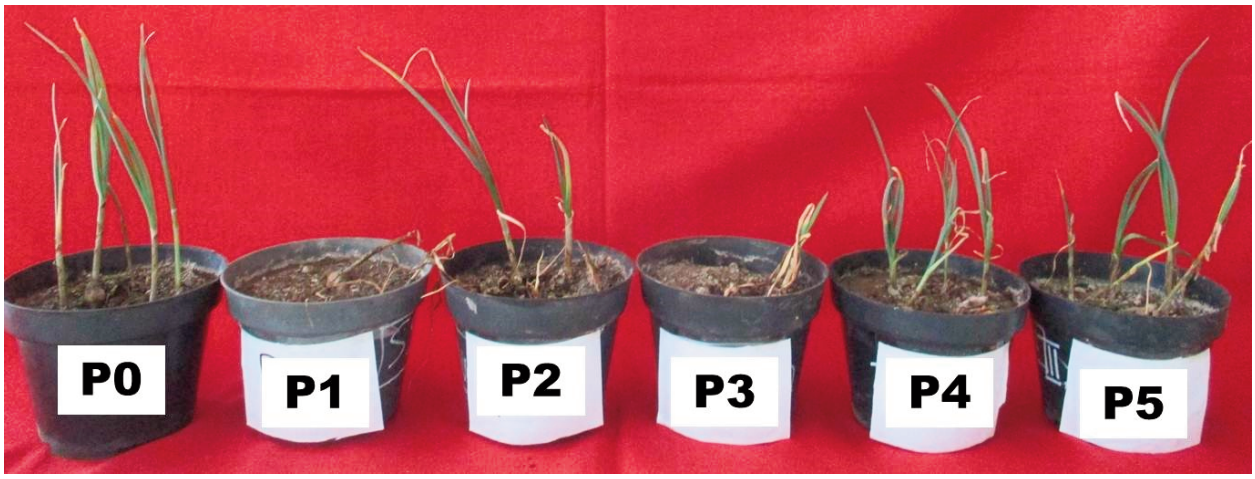

Figure 8. Effect of *Ditylenchus dipsaci* and *Fusarium oxysphorum* inoculation on the garlic plants; P0 = uninoculated (control), P1 = inoculation of *D. dipsaci* three days before inoculation of *F. oxysporum*, P2 = inoculation of *F. oxysporum* three days before inoculation of *D. dipsaci,* P3 = inoculation of *D. dipsaci* and *F. oxysporum* simultaneously, P4 = inoculation of *D. dipsaci* alone, P5 = inoculation of *F. oxysporum* alone

Table 2. Effect of *Ditylenchus dipsaci* and *Fusarium oxysporum* inoculation on disease intensity, root weight, and bulb weigthof garlic plants

Treatments	Disease Intensity (%)	Root weight (g)	Bulb weight (g)
uninoculated (Control)	0.00 c	0.29 a	1.72 a
D. dipsaci followed by *F. oxysporum*	94.44 ab	0.22 ab	1.20 b
F. oxysporum followed by *D. dipsaci*	93.33 ab	0.25 ab	1.45 ab
D. dipsaci with *F. oxysporum*	97.60 a	0.16 b	1.38 ab
D. dipsaci	84.44 b	0.17 b	1.42 ab
F. oxysporum	86.60 b	0.17 b	1.11 b

Note: Mean of five replications (three plants per replication). Means not followed by the same letters were significantly different (P≤ 0.05) according to Tukey's test

could not multiply optimally due to suboptimal temperature at the study site. This was supported by Indarti *et al.* (2018) who stated that the abundance of *D. dipsaci* populations was positively correlated with soil temperature. Higher population abundance of *D. dipsaci* were found in soil with lower temperature.

Disease intensity of infected plants caused by *D. dipsaci* and *Fusarium* spp. are shown in Table 2. The results of this study revealed that *D. dipsaci* and *F. oxysporum* could damage singularly (inoculation *D. dipsaci* alone or *F. oxysporum* alone) or simultaneously. The presence of *D. dipsaci* and *F. oxysporum* has been reported as a cause of garlic rot disease (Testen *et al.,* 2014; French *et al.,* 2017; L.-J. Zhang *et al.,* 2017; Indarti *et al.,* 2018).

The disease intensity due to combination of inoculation treatments showed significant difference compared to the control (Table 2). Disease intensity of single treatments (inoculation of *D. dipsaci* alone or *F. oxysporum* alone) were significantly different from the inoculation of *D. dipsaci* and *F. oxysporum* together. The disease intensity of *D. dipsaci* inoculation followed by all treatments did not show significant differences. Inoculation of *D. dipsaci* and *F. oxysporum* together resulted in the highest disease intensity compared to other treatments. This shows *D. dipsaci* and *F. oxysporum* synergistically increase the intensity of bulb rot disease. Following the report of Pujiastuti *et al.* (2014) who stated that the synergistic interactions of the combined pathogen *Meloidogyne* spp. and *F. oxysporum* in garlic increase the intensity of wilting symptoms of basal rot disease.

D. dipsaci and *F. oxysporum* infections caused damage to plants with varying degrees. Inoculation of garlic plants with only *D. dipsaci* or *F. oxysporum* also caused symptoms of bulb rot disease. These results showed that both *D. dipsaci* and *F. oxysporum* were each able to act as a cause of bulb rot disease, but the damage is more severe if *D. dipsaci* and *F. oxysporum* are double inoculated.

CONCLUSION

Garlic bulb rot in Central Java was caused by *D. dipsaci*, *F. oxysporum,* and *F. solani* complexes. The association between *D. dipsaci* and *F. oxysporum* increased the intensity of bulb rot diseases by up to 98% in garlic plants.

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