

# Isolation and Capability of Dark Septate Endophyte Against Mancozeb Fungicide

## (Isolasi dan Kemampuan Cendawan *Dark Septate Endophyte* terhadap Fungisida Berbahan Aktif Mankozeb)

Jean Nihana Manalu<sup>1\*</sup>, Bonny Poernomo Wahyu Soekarno<sup>2</sup>, Efi Toding Tondok<sup>2</sup>, Surono Surono<sup>3</sup>

(Diterima Juli 2019/Disetujui November 2019)

### ABSTRACT

Mancozeb is a group member of dithiocarbamate fungicides that most widely applied in the field. The use of mancozeb causes residues that have negative effects on agricultural production system and on agricultural environment. This research aimed to obtain dark septate endophyte (DSE) fungal isolates that are tolerant to mancozeb fungicide. The study was consisted of three steps, i.e., the isolation of DSE fungi from chili roots, the effect of DSE fungal isolates on chili seeds, and the growth ability of DSE isolates on medium supplemented with mancozeb. Thirteen DSE fungal isolates were isolated from chilli pepper roots and grown in the field assumed to be contaminated by pesticides. The isolates of DSE fungi caused the growth of chili seeds were normal and abnormal. Seven DSE isolates were able to grow well at PDA medium supplemented with mancozeb at concentrations of 100, 200, 400, and 800 ppm with different levels of relative growth inhibition. Mancozeb on PDA medium influenced the mycelial growth and colony color of DSE fungal isolates. The biomass of DSE fungal isolates were reduced when they were grown at malt extract broth (MEB) medium supplemented various levels of mancozeb.

**Keywords:** dithiocarbamate, relative growth inhibition, tolerant

### ABSTRAK

Mankozeb adalah kelompok fungisida ditiokarbamat yang paling banyak digunakan di lapangan. Penggunaan mankozeb yang tidak sesuai dapat menyebabkan dampak negatif pada sistem produksi dan lingkungan pertanian. Penelitian ini bertujuan untuk memperoleh isolat cendawan *Dark Septate Endophyte* (DSE) yang toleran terhadap fungisida berbahan aktif mankozeb. Penelitian terdiri atas tiga tahap, yaitu isolasi cendawan DSE dari akar tanaman cabai, pertumbuhan isolat cendawan DSE pada tanaman cabai, dan kemampuan tumbuh cendawan DSE pada media yang mengandung mankozeb. Berdasarkan hasil penelitian diperoleh 13 isolat cendawan DSE dari akar tanaman sawit yang terkontaminasi oleh penggunaan pestisida. Isolat cendawan DSE yang diperoleh menyebabkan pertumbuhan benih cabai normal dan tidak normal. Tujuh isolat cendawan DSE mampu tumbuh dengan baik pada media PDA yang telah diberi penambahan konsentrasi mankozeb, yaitu 100, 200, 400, dan 800 ppm dengan hambatan pertumbuhan relatif yang berbeda. Penambahan mankozeb pada media PDA memengaruhi pertumbuhan miselium dan warna koloni isolat cendawan DSE. Pertumbuhan cendawan DSE pada media cair *Malt Extract* (ME) yang ditambahkan konsentrasi mankozeb menyebabkan penurunan biomassa cendawan DSE.

**Kata kunci:** ditiokarbamat, hambatan pertumbuhan relatif, toleran

### INTRODUCTION

Mancozeb is one of the contact fungicides of the dithiocarbamate group that has been used for more than 50 years in agriculture (Szolar 2007; Fatma *et al.* 2017). This fungicide consists of 20% manganese with 2.5% zinc salts and it is widely used to control fungal diseases such as leaf spots, downy mildew, and

blossom end-rot on vegetables and fruit plants (Worthing 1991; Cernohlavkova 2008; Brody *et al.* 2013). The residues of mancozeb can produce the negative impacts on non-target organisms, resulting in changes in nutrient replacement, microbial community structure, and soil quality (Monkiedje *et al.* 2002; Demanou *et al.* 2006; Cycon *et al.* 2010). Organisms that are exposed to the use of mancozeb can damage nerve structure, disruption of survival, and decreased reproduction (Brody *et al.* 2013; Carniel *et al.* 2019).

The negative impact of the use of mancozeb on the environment is caused by the toxic effects of degradation products of mancozeb, namely ethylenethiourea (ETU). ETU causes toxic effects such as teratogenic, carcinogenic, immunotoxic, and mutagenic effects (Garcinuno *et al.* 2004). One of the ways to overcome this residual fungicide problem is the use of bioremediation. Bioremediation is a method for

<sup>1</sup> Graduate School, Phytopathology Department, Faculty of Agriculture, IPB University, IPB Darmaga Campus, Bogor 16680

<sup>2</sup> Phytopathology Department, Faculty of Agriculture, IPB University, IPB Darmaga Campus, Bogor 16680

<sup>3</sup> Indonesian Soil Research Institute, Indonesian Agency for Agricultural Research and Development, Cimanggu, Bogor 16114

\* Correspondence Writer: Email: jeanmanalu@gmail.com

removing pollutants from contaminated environments by utilizing environmentally friendly microorganisms (Finley *et al.* 2010). In the soil environment, there are different types of microorganisms, such as fungi that participate actively in all the processes of transformation of organic matter (Spagnoletti and Chiochio 2019). Dark septate endophyte fungi (DSE) is a group of endophytic fungi that have melanized hyphae, form the dark colonies on various agar media, are able to colonize plant roots without causing disease symptoms, and increasing plant growth (Jumpponen 2001; Khan *et al.* 2017; Surono & Narisawa 2017).

Several studies have reported the potential of DSE fungi in agriculture. Mahmoud and Narisawa (2013) reported that *Scolecobasidium humicola* plays a role in increasing the growth of tomato plants in abiotic stress. Tellenbach *et al.* (2013) reported that *Phialocephala europaea* could inhibit the growth of *Phytophthora citricola*. Besides, *Phialocephala fortinii* also could degrade C, N, and P organic compounds (Caldwell *et al.* 2000; Tang & Malik 2001). DSE fungi were reported to be found in conditions with heavy metals contamination (Tang & Malik 2001) and this condition is caused by the melanin in DSE hyphae is the most important component of the cell wall to decrease the toxicity of heavy metal (Ban *et al.* 2012). Khan *et al.* (2017) reported the presence of secondary metabolites production such as IAA which made endophytic fungi could survive in the environmental stress conditions caused by the contamination of heavy metals. At present, information about the ability of DSE fungi to degrade pesticides has not been reported, specifically the mancozeb fungicide. There is a limited information about the effects of pesticides especially for fungicide mancozeb on DSE. Thus, the objective of this study was to obtain DSE fungal isolated from chili roots collected from contaminated environment and to evaluate the tolerance of DSE fungal to mancozeb by *in vitro* test.

## MATERIALS AND METHODS

This study was conducted at the Plant Mycology Laboratory of IPB University and Soil Biology Laboratory of Soil Research Center, starting from December 2018 until June 2019. Root tissue samples were collected from Ci anjur and Bogor regions. Dithane-45 which contained 80% mancozeb was used for this study.

### Isolation of Dark Septate Endophyte Fungi

Isolation of dark septate endophyte was carried out using modification of the surface sterilization method of Surono & Narisawa (2017). The root samples were washed with tap water to remove debris and then cut into approximately 10 mm segments. The segment root samples were washed by a solutions of sodium hypochlorite (1% available chlorine) for 1 minute, followed by twice using of 0.005% solutions of Tween

20, and rinsed three times using sterile water using a vortex mixer. The sterile root segments were dried overnight, then planted into 50% corn meal agar medium. The selected fungal isolates were dark and slow-growing. The fungi were grown on corn meal malt yeast agar (CMMYA), malt yeast agar (MEA), and oat meal agar (OMA). The fungal candidates were used for the pathogenicity test.

### The Effect of DSE Fungal Isolates Test on Chilli Seeds

The sterilization of chilli seed surface was carried out using modification of Surono & Narisawa (2017) method. Chilli seeds were sterilized by 70% ethanol for 1.5 minutes and a solutions of sodium hypochlorite (1% available chlorine) for 1 minutes, and rinsed three times using sterile water, then dried overnight. The sterilized seeds were transplanted on oatmeal agar in a sterile culture bottle which had been inoculated by the fungal isolates. Seeds transplanted onto non-inoculated medium were used as a control and incubated for two weeks at room temperature. There were three replicates for each treatment, including the control. Observation parameters included germination percentage, plant height, root length, and dry weight.

### Effect of Mancozeb on DSE Fungal Mycelial Growth

This test was carried out using modification of the method of Malandrakis *et al.* (2018). There were 9 isolates selected from the previous test. This test was conducted to determine the ability of DSE fungal isolates to grow on PDA medium containing mancozeb with respective concentrations of 100, 200, 400, and 800 ppm. PDA medium that were not mixed with mancozeb were used as a control. There were three replicates for each treatment, including the control. Inoculations were carried out using a 5-mm mycelial plug cut from the edge of 7 days old DSE fungal isolates colonies grown on PDA medium. The cultures were incubated for 14 days at room temperature in the dark condition. The effect of mancozeb on the growth of DSE fungal isolates were expressed in the percent inhibition. Percent inhibition was calculated by the formula: % inhibition = colony diameter of untreated control - colony diameter of mancozeb treated / colony diameter of untreated control x 100%.

### Measurement of DSE Fungal Mycelial Biomass

This test was carried out using a modification of method used by Garraway & Evans (1991). Five selected DSE fungal isolates were used to know their capabilities to grow on malt extract broth medium supplemented with mancozeb. The selected DSE fungal isolates were transferred into 30 ml malt extract broth medium containing mancozeb with respective concentrations of 100, 200, 400, and 800 ppm at pH 7 in 100 mL sterile culture bottle. The cultures were incubated in a rotary incubated shaker with 115 rpm at room temperature for 14 days. The grew DSE fungal mycelial were separated from the liquid medium by

filtering the mycelial using Whatman No. 1 filter paper. The filtered DSE fungal mycelial were dried in an oven at 60°C for 24 hours. The dry weights of DSE fungal mycelia were calculated by the formula: dry weight = weight of filter paper with dry weight DSE fungal mycelial - weight of filter paper. The effect of mancozeb on the growth of DSE fungal was determined by using the dry weight of the DSE fungus mycelium obtained. Percent inhibition was calculated by the formula: % inhibition = mycelial biomass of untreated control - mycelial biomass of treated with mancozeb / mycelial biomass of untreated control x 100%.

**Statistical Analysis**

A two-way analysis of variance (ANOVA) with DSE fungal isolates and mancozeb concentrations (100, 200, 400, and 800 ppm) as factors was performed on the data obtained on growth parameters and effective concentration (EC 50) using SAS 9.4. Significant differences (P<0.05) between the mean values of different treatments were compared and evaluated using Tukey test.

**RESULTS AND DISCUSSION**

**Isolation of DSE Fungi**

Thirteen darkly-pigmented fungal isolates were obtained from 864 root segments of chilli plants grown on environments contaminated by pesticides. There were nine fungal isolates obtained from Bogor region, meanwhile there were four fungal isolates obtained from Cianjur region (Table 1).

The number of isolates obtained from the isolations of chili root originated from Cianjur and Bogor could be influenced by the environmental conditions of the origin of the sample which had been exposed to the use of pesticides. The presence of endophytic fungi in nature can be influenced by the use of pesticides (Stuart *et al.* 2010). Nettles *et al.* (2016) reported that the use of pesticides on corn and soybean plants influenced the

abundance of genera and endophytic species populations.

**Effect of DSE Fungal Isolates on the Growth of Chilli Seeds**

Chilli seeds germinated in DSE fungus isolate colonies showed the growth responses of seeds that grew normally and abnormally. This study showed 7 isolates were able to promote 100% seed germination, namely DS14, MM11, GA11, MM15.2, CO19, GA7.1, and MM12. MM18 isolate showed that the per centation of seed germination was slightly higher than control plant that was 89%, whereas MM15.1 showed that per centation of seed germination was similar to control plant that was 78%. GA7.2 was suspected as a potential endophytic fungi to promote seed germination. Therefore, only nine isolates (DS14, MM11, GA11, MM15.2, CO19, GA7.1, MM12, MM18, and MM15.1) that were processed and used for further experiments (Table 2).

**Effect of Mancozeb on the Growth of DSE Fungal Isolates**

Medium supplemented with mancozeb influenced the mycelial growth of DSE fungal isolates. Observation of the diameters of DSE fungal isolates colony showed that the higher the concentration of mancozeb on PDA medium, the higher the percentage of growth inhibition of DSE fungal isolates. Figure 1

Table 1 The total of DSE fungal isolates obtained from the root of chili plant

Location	∑ Root segment	∑ Isolate
Sarongge Kidul, Cianjur Region	144	0
Galundra, Cianjur Region	144	4
Nyalindung, Cianjur Region	144	0
Sukagalih, Bogor Region	144	6
Citeko, Bogor Region	144	2
Darussalam, Bogor Region	144	1
Total	864	13

Table 2 Seed germination rates (%), root length (cm), plant height (cm), and dry weight (mg) of chilli seeds inoculated with different DSE fungal isolates

Isolates	Seed germination rates (%)	Root height (cm)	Plant height (cm)	Dry weight (mg)
DS14	100.00*	0.56*	2.71*	0.9*
MM11	100.00*	0.42*	2.39*	1.5*
GA11	100.00*	0.35*	1.53*	0.6*
MM15.2	100.00*	0.32*	1.34*	0.9*
Co19	100.00*	0.30*	1.14*	0.6*
GA7.1	100.00*	0.98*	1.79*	1.3*
MM12	100.00*	0.58*	2.00*	0.8*
MM18	89.00*	0.81*	2.09*	1.0*
MM15.1	78.00*	0.94*	1.63*	1.2*
GA7.2	67.00**	0.34*	0.78*	0.6*
Co1	22.67**	0.22*	0.34**	0.2**
MM10	11.33**	0.03*	0.11**	0.0**
GA11	0.00**	0.00*	0.00**	0.0**
Control	78.00*	0.85*	2.86*	1.7*

Description: <sup>1)</sup> Those with the same letter were not significantly different (P<0.05) after Tukey's Honestly Significant Difference test.

showed that the growth of DS14 and GA7.1 were inhibited 100% at each concentration. It was indicated that both isolates were very sensitive to all concentrations of mancozeb. GA11 was only able to grow on medium supplemented with 100 ppm mancozeb with growth inhibition percentage was 78.86% on 14 days of incubation period. MM11 was able to grow on medium supplemented with 100 and 200 ppm mancozeb with the percentages of growth inhibition were 14.15 and 28.79%, respectively, on 14 days of incubation period.

MM15.1 and MM12 showed that mycelial growth were inhibited less than 50% when mancozeb concentrations were 100–400 ppm, whereas at the concentration of 800 ppm the growth inhibition was above 50%. These results indicated that these two DSE fungal isolates were resistant to mancozeb concentration up to 400 ppm (Figure 1). Malandrakis *et al.* (2018) reported that the nonpathogenic *Fusarium solani* endophytic fungi was insensitive to mancozeb at a concentration of 100 µg/mL. Spagnoletti and Chiocchio (2019) also reported DSE fungi *Alternaria alternata* and *Cochliobolus* sp. were tolerant to carbendazim fungicide concentration of 181.25 ppm.

The results of probit analysis showed that MM15.1 had the higher IC<sub>50</sub> value than the other isolates and it showed that MM15.1 was resistant to mancozeb. In contrast, CO19 had the lowest IC<sub>50</sub> value indicating that this DSE fungi isolate was very sensitive to mancozeb (Table 3).

The morphology of colonies of DSE fungal isolates were observed when they were cultured on PDA medium supplemented with mancozeb for two weeks. There were a lot of changes in morphology of DSE fungal isolate in the presence of mancozeb in the medium compared to the mancozeb-free control (Figure 2). Figure 2 showed the colony of MM15.1 cultured on control medium without mancozeb

changed from dark to white. The same result was also demonstrated by Wyss *et al.* (2004) that the imidazoline herbicide group caused changes in the color of the fungus colony of *Phomopsis amaranthicola*, in addition to the undergoing a change in color it also changed the shape of hyphae and conidia. The inhibition of mycelial growth on solid and liquid medium of mancozeb can be caused by the presence of both manganese and zinc in the formulation and it could exert neurotoxic effects (Brody *et al.* 2013).

Table 3 The IC<sub>50</sub> (ppm) of mancozeb for DSE fungal isolates

Isolat	IC50
MM15.1	848.18
MM12	605.16
MM15.2	216.72
MM11	209.22
MM18	129.70
GA11	96.45
CO19	16.55

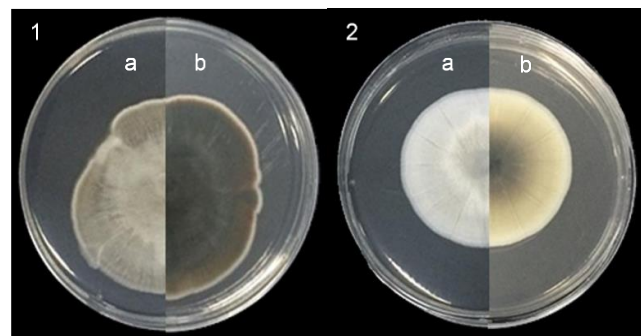


Figure 2 Morphology of MM15.1 grown on PDA-medium supplemented with mancozeb. (1a) front appearance, (1b) back appearance at 0 ppm, (2a) front appearance, and (2b) back appearance at 100 ppm.

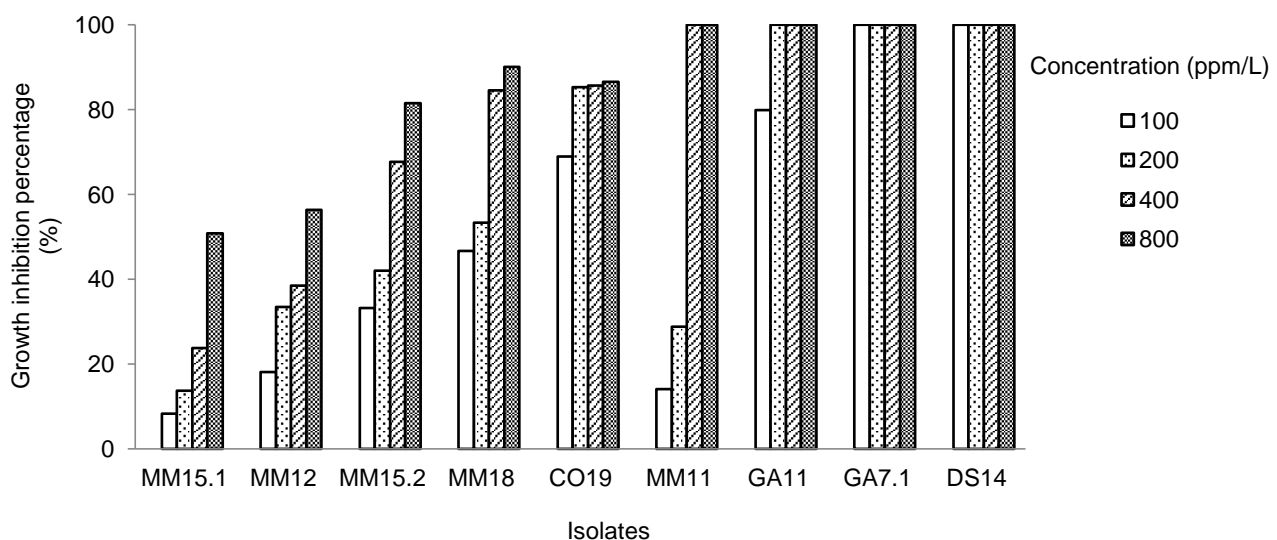


Figure 1 Growth inhibition percentage (%) of DSE fungal isolates on PDA medium supplemented with mancozeb for 14 days of incubation period.

### Mycelial Biomass of DSE fungal isolates

Five DSE fungal isolates (MM15.1, MM12, MM15.2, MM18, and CO19) showed their abilities to grow on PDA medium supplemented with mancozeb from the lowest to the highest concentrations. These five DSE fungal isolates were selected to test the ability of their growths on malt extract liquid medium supplemented with mancozeb. This test showed that the presence of mancozeb on liquid medium also affect the DSE fungal isolates growth that caused the reduction of the biomass.

Based on the measurement of the biomass of DSE fungal isolates from liquid medium supplemented with mancozeb, it was obtained as many as two isolates of DSE fungus namely MM15.1 and MM12 which their mycelial growths were inhibited under 50% with inhibition percentage of 37.33% and 45.56%, respectively, at concentration of 100 ppm (Figure 3). DSE fungi are more tolerant to different types of stress than the other soil fungi. This could be due to the fact that the chemical compounds act effectively on the mycelium, while spores and chlamydospores are more resistant to these compounds (Spagnoletti & Chiochio 2019).

### CONCLUSION

Based on the results of the study, DSE fungi can be isolated from the chilli roots collected from environment which are exposed to the use of pesticides. Seven isolates of DSE fungus can be used as a growth promoter for chilli plants. The addition of mancozeb to the PDA medium and Malt Extract Broth (MEB) medium affected the growth of DSE fungal isolates mycelial and the color changes of DSE fungal isolates. M M15.1 and MM12 isolates can be the potential DSE fungi to degrade residue of mancozeb. This test will be continued to our further research using GC analysis to know the degradation of mancozeb.

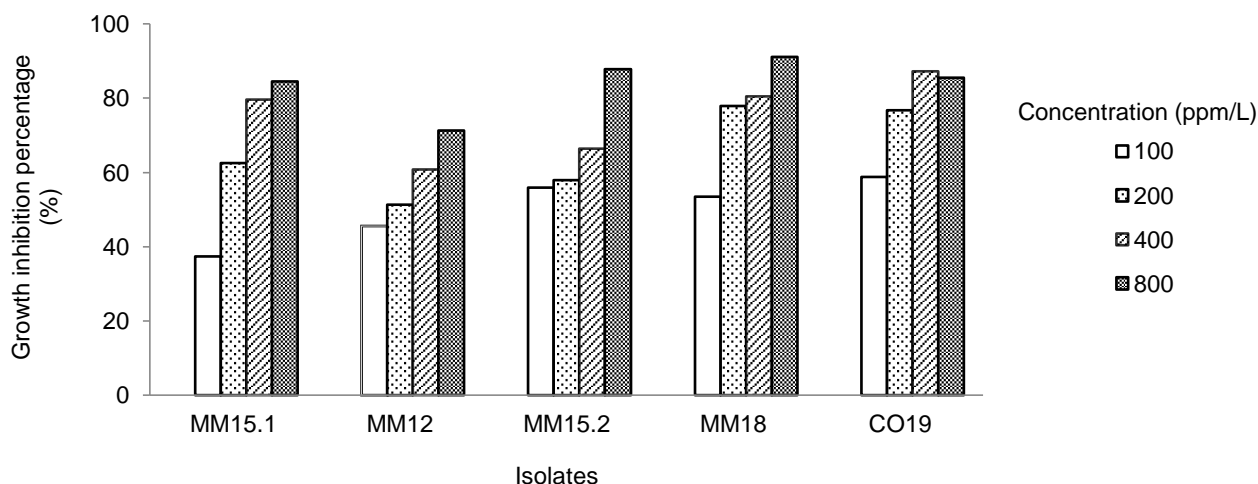


Figure 3 Growth inhibition percentage (%) of DSE fungal isolates grown on PDA-medium supplemented with mancozeb for 14 days of incubation periode.

### REFERENCES

- Ban Y, Tang M, Chen H, Xu Z, Zhang H, Yang Y. 2012. The response of dark septate endophytes (DSE) to heavy metals in pure culture. *PLOS ONE*. 7(10): 1–11. <https://doi.org/10.1371/journal.pone.0047968>
- Brody AH, Chous E, Gray JM, Pokyrwka NJ, Raley-Susman MK. 2013. Mancozeb-induced behavioral deficits precede structural neural degeneration. *NeuroToxicology*. (34): 74–81. <https://doi.org/10.1016/j.neuro.2012.10.007>
- Caldwell BA, Jumpponen A, Trappe JM. 2000. Utilization of major detrital substrates by dark septate, root endophytes. *Mycologia*. 92(2): 230–232. <https://doi.org/10.1080/00275514.2000.12061149>
- Carniel LSC, Niemeyer JC, de Oliveira Filho LCL, Alexandre D, Gebler L, Kaluberg-Filho O. 2019. The fungicide mancozeb affects soil invertebrates in two subtropical Brazilian soils. *Chemosphere*. 232: 180–185. <https://doi.org/10.1016/j.chemosphere.2019.05.179>
- Černohlávková J, Jarkovský J, Hofman J. 2008. Effect of fungicides mancozeb and dinocap on carbon and nitrogen mineralization in soils. *Ecotoxicology and Environmental Safety*. 72: 80–85. <https://doi.org/10.1016/j.ecoenv.2008.07.001>
- Cycoń M, Piotrowska-Seget Z, Kozdrój J. 2010. Responses of indigenous microorganisms to a fungicidal mixture of mancozeb and dimethomorph added to sandy soils. *International Biodeterioration & Biodegradation*. 64: 316–323. <https://doi.org/10.1016/j.ibiod.2010.03.006>
- Demanou J, Sharma S, Dörfler U, Schroll R, Pritsch K, Njine T, Bausenwein U, Monkiedje A, Munch JC, Schloter M. 2006. Structural and functional diversity of soil microbial communities as a result of

- combined applications of copper and mefenoxam. *Soil Biology & Biochemistry*. 38: 2381–2389. <https://doi.org/10.1016/j.soilbio.2006.03.007>
- Fatma F, Verma A, Kamal A, Srivastava A. 2017. Monitoring of morphotoxic, cytotoxic and genotoxic potential of mancozeb using *Allium* assay. *Chemosphere*. 30: 1–7.
- Finley SD, Broadbelt LJ, Hatzimanikatis V. 2010. In silico feasibility of novel biodegradation pathways for 1,2,4-trichlorobenzene. *BMC Systems Biology*. 4(7): 1–14. <https://doi.org/10.1186/1752-0509-4-7>
- Garcinuño RM, Ramos L, Fernández-Hernando P, Cámara C. 2004. Optimization of a matrix solid-phase dispersion method with subsequent clean-up for the determination of ethylene bisdithiocarbamate residues in almond samples. *Journal of Chromatography*. 1041: 35–41. <https://doi.org/10.1016/j.chroma.2004.04.043>
- Garraway MO dan Evans RC. 1991. *Fungal Nutrition and Physiology*. Florida (FL): Krieger Publishing Company. p 231.
- Jumpponen A. 2001. Dark septate endophytes- are they mycorrhizal?. *Mycorrhiza*. 11: 207–211. <https://doi.org/10.1007/s005720100112>
- Khan AR, Ullah I, Waqas M, Park GS, Khan AL, Hong SJ, Ullah R, Jung BK, Park CE, Ur-Rehman S, Lee IJ, Shin JH. 2017. Host plant growth promotion and cadmium detoxification in *Solanum nigrum*, mediated by endophytic fungi. *Ecotoxicology and Environmental Safety*. 136: 180–188. <https://doi.org/10.1016/j.ecoenv.2016.03.014>
- Mahmoud RS, Narisawa K. 2013. A new fungal endophyte, *Scolecobasidium humicola*, promotes tomato growth under organic nitrogen conditions. *PLOS ONE*. 8(11): 1–8. <https://doi.org/10.1371/journal.pone.0078746>
- Malandrakis A, Daskalaki ER, Skiadac V, Papadopoulou KK, Kavroulakis N. 2018. A *Fusarium solani* endophyte vs fungicides; compatibility in a *Fusarium oxysporum* f.sp. *radicis-lycopersici*-tomato pathosystem. *Fungal Biology*. 122: 1215–1221. <https://doi.org/10.1016/j.funbio.2018.10.003>
- Monkiedje A, Spiteller M, Olusoji M. 2002. Soil quality changes resulting from the application of fungicides mefenoxam and metalaxyl in sandy loam soil. *Soil Biology and Biochemistry*. 34: 1939–1948. [https://doi.org/10.1016/S0038-0717\(02\)00211-0](https://doi.org/10.1016/S0038-0717(02)00211-0)
- Nettles R, Watkins J, Ricks K, Boyer M, Licht M, Atwood LW, Peoples M, Smith RG, Mortensen DA, Koide RT. 2016. Influence of pesticide seed treatments on rhizosphere fungal and bacterial communities and leaf fungal endophyte communities in maize and soybean. *Applied Soil Ecology*. 102: 61–69. <https://doi.org/10.1016/j.apsoil.2016.02.008>
- Spagnoletti FN, Chiocchio VM. 2019. Tolerance of dark septate endophytic fungi (DSE) to agrochemicals *in vitro*. *Revista Argentina Microbiology*. 1–7. <https://doi.org/10.1016/j.ram.2019.02.003>
- Stuart RM, Romão AS, Pizzirani-Kleiner AA, Azevedo JL, Araújo WL. 2010. Culturable endophytic filamentous fungi from leaves of transgenic imidazolinone-tolerant sugarcane and its non-transgenic isolines. *Arch Microbiology*. 192: 307–313. <https://doi.org/10.1007/s00203-010-0557-9>
- Surono, Narisawa K. 2017. The dark septate endophytic fungus *Phialocephala fortinii* is a potential decomposer of soil organic compounds and a promoter of *Asparagus officinalis* growth. *Fungal Ecology*. 28: 1–10. <https://doi.org/10.1016/j.funeco.2017.04.001>
- Szolar OHJ. 2007. Environmental and pharmaceutical analysis of dithiocarbamates. *Analytica Chimica Acta*. (582): 191–200. <https://doi.org/10.1016/j.aca.2006.09.022>
- Tang MVJY, Malik R. 2001. Heavy metal tolerance of fungi. *Minerals Engineering*. 14(5): 499–505. [https://doi.org/10.1016/S0892-6875\(01\)00037-1](https://doi.org/10.1016/S0892-6875(01)00037-1)
- Tellenbach C, Sumarah MW, Grünig CR, Miller JD. 2013. Inhibition of *Phytophthora* species by secondary metabolites produced by the dark septate endophyte *Phialocephala europaea*. *Fungal Ecology*. 6: 12–18. <https://doi.org/10.1016/j.funeco.2012.10.003>
- Worthing CR. 1991. *Mancozeb*. In *the pesticide manual*. C.R. Worthing, ed. Lovensham, Suffolk, Great Britain, pp. 529–530.
- Wyss GS, Charudattan R, Roskopf EN, Littell RC. 2004. Effects of selected pesticides and adjuvants on germination and vegetative growth of *Phomopsis amaranthicola*, a biocontrol agent for *Amaranthus* spp. *Weed Research*. 44: 469–482. <https://doi.org/10.1111/j.1365-3180.2004.00425.x>