Effect of plant extracts and chemical fungicide on viability and percentage of seed-borne fungal infection on calliandra (Calliandra callothyrsus) seed

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Abstract. Calliandra (Calliandra callothyrsus) is one of the potential tree species that can be used as bio-energy resources. Unfortunately, fungi are one of the factors that can infect and decrease the viability of calliandra seeds. Some efforts are needed to improve the viability and inhibit fungal infection through biological or chemical treatments. The study was conducted to determine the effects of various plant extracts and chemical fungicide on the germination capability, as well as the inhibition of fungal infection in calliandra seeds. Parameters observed in this study were seed germination and fungi infection percentage. Seeds were treated by immersing them in hot water (five minutes) followed by chemical fungicides (i.e., benomyl, mancozeb) or plant extracts (i.e., ginger, onion, garlic, turmeric) for one hour. The study design used was a completely randomized design. The results showed that all the treatments could increase seed viability significantly. The treatment with immersion in onion, ginger and mancozeb was more effective on seed germination ability (88.67%, 8.33%, 86%) and reduced in fungal infections (11.33%, 5.33%, 0%). These treatments resulted in a significant difference between seed germination compared with the hot water and turmeric treatments. The best germination and reduction of fungal infection on calliandra seeds were showed on treatment by onion and mancozeb, respectively. The results revealed that garlic, onion, turmeric have a significant effect in inhibiting all fungal species compared to the hot water treatment. Ginger and onion extracts were effective treatments for increasing seed germination and controlling the fungal infection.

1. Introduction
Calliandra (Calliandra callothyrsus) is naturally found in Mexico and Central America, and in 1936 calliandra seeds were sent from Southern Guatemala to Java [1]. Calliandra is the best species compared to other species because it can grow in various altitudes and types of soil and has high living capability. This species has a natural symbiosis with fixation nitrogen bacteria (Rhizobium spp.) and arbuscular mycorrhizal fungi [2]. The benefits of this plant are a potential source of renewable energy, pulp, bee fodder, intercropping, fallow plants, erosion control, land rehabilitation [3], and animal feed [4].

A seed is one of the success keys of the forest plant development program. However, one of the obstacles in the supply of seeds is the infection of pathogenic fungi in both fresh and stored seeds [5]. In stored seeds, the predominant fungus is generally storage fungus. However, there are several types
of field fungus which is carried to the storage area and can survive due to the existence of a surviving structure. Infection of seed-borne pathogenic fungi can cause germination failure, decrease in seed longevity inside the storage, cause diseases in seeds and plants, and also be permanently settled in the soil [6]. Therefore seeds are treated to reduce fungal contamination often before sowing; one of the treatments is the use of chemical fungicides. The active ingredients that are widely used to control fungal pathogens are benomyl and mancozeb. Benomyl and mancozeb are applicable to seed treatment [7]. Based on mobility within the plant, benomyl can be categorized as a systemic fungicide, while mancozeb as contact fungicide [8]. Benomyl and mancozeb are effective in inhibiting mycelium growth and sufficient to stimulate the germination of conidia [9].

However, the use of chemical fungicides can have a negative effect on human health, animals, and also for the environment. Therefore, a low-cost and environmentally friendly alternative that can reduce pathogenic fungal infections is needed that can increase the germination and producing healthy plants. One of the advantages of using plant extracts to control pathogens carried by seeds is its cheapness [10], so reducing the costs. Plants that have the potential to be anti-fungal are ginger, onion, garlic and turmeric [11]. The extract from these plants can inhibit the growth of *Alternaria alternata*, *A. solani*, *Phoma* sp., *Fusarium* sp, and *Aspergillus* sp., and also can increase the germination of peas [12].

The use of plant extracts such as ginger, onion, garlic and turmeric has been carried out in many types of agricultural plants, but there is not much information on the application of ginger, onion, garlic and turmeric on forestry plant seeds. Therefore research on the use of biological fungicides is required and comparison with chemical fungicides is also needed for forest plant seeds. The purpose of this study was to determine the effect of some plant extracts and chemical fungicide on the germination ability and the inhibition of fungal infection to calliandra seeds.

## 2. Experimental Method

### 2.1. Study site

This research was carried out in the pests and diseases laboratory of the Forest Tree Seed Technology Research and Development Center, Bogor, Indonesia (6°35′52.5″S 106°48′39.8″E).

### 2.2. Extract of onion, garlic preparation

Onion and garlic extracts were obtained by grilling the fresh tuber until smooth using mortar followed by the addition of sterile aquades. The seeds were washed with sterile distilled water. Seed treatments were done through the following steps: the seeds were soaked in hot water and left cold for 24 hours; the seeds were soaked in hot water and left cold for 24 hours and then soaked in 10% onion extract solution; the seeds were soaked in hot water and left cold for 24 hours and then soaked in 10% garlic extract solution; the seeds were soaked in hot water and left cold for 24 hours and then soaked in 10% ginger extract solution; the seeds were soaked in hot water and left cold for 24 hours and then soaked in 0.1% benomyl, and the seeds were soaked in hot water and left cold for 24 hours and then immersed in mancozeb 0.1%. The seeds are sown on moist paper media and then put into the germinator chamber.

### 2.3. Observations

Observations were conducted once every two days for one month. Observation parameters included germination and percentage of fungal infections. Isolation of seed-borne fungi was carried out by taking the different fungus colonies that were on the surface of the seeds and then put in 9 cm diameter petri dish filled with PDA and then incubated for seven days. Identification was made by using a microscope preparat by placing one dose of fungi colony in preparat glass, and the observed fungus using the microscope was identified by comparing the morphology of the fungus with the fungus in the fungus identification book [13]. Percentage of fungal infections is calculated using the formula [14]:

\[
\text{Percentage of fungal infections} = \left( \frac{\text{Number of infected seeds}}{\text{Total number of seeds}} \right) \times 100
\]
Percentage of fungal infections = \frac{\text{the number of infected fungal seeds}}{\text{total number of seeds}} \times 100\%

Remarks: The number of infected fungal seeds is a number that represents a part of the fungus that is present on the surface of the seed during germination (1 month)

2.4 Data analysis
The study design used a completely randomized design with six treatments; each treatment consisted of three replications, with each replication consisting of as many as 50 units. The data were analyzed using one-way ANOVA.

3. Result and Discussion
All treatments significantly affected the germination of C. callothyrsus compared to controls (Table 1). Soaking the seeds with hot water could break seed dormancy, so the seed viability is higher than the control. Soaking the seeds in a solution of ginger, shallots, and mancozeb after soaked in hot water and then being left cooled for 24 hours, will produce higher germination than just soaking the seeds in hot water and left to cool for 24 hours. Therefore, these treatments can increase the viability of the seeds compared to just being given hot water. These results were in line with the [15] study, which showed that ginger and mancozeb could increase the germination of Lens arietinum (lentils) and Lathyrus sativus.

Table 1. Calliandra callothyrsus seed germination at several treatments.

| Treatment                                          | Germination (%) |
|----------------------------------------------------|-----------------|
| Control                                            | 58.67<sup>c</sup> |
| Soaked in hot water and left until 24 hours        | 75.33<sup>ab</sup> |
| Soaked in hot water and left until 24 hours and then soaked in turmeric solution 10% for 1 hour | 74.67<sup>b</sup> |
| Soaked in hot water and left until 24 hours and then soaked in ginger solution 10% for 1 hour | 89.33<sup>a</sup> |
| Soaked in hot water and left until 24 hours and then soaked in onion solution 10% for 1 hour | 88.67<sup>a</sup> |
| Soaked in hot water and left until 24 hours and then soaked in garlic solution 10% for 1 hour | 74.67<sup>b</sup> |
| Soaked in hot water and left until 24 hours and then soaked in mancozeb solution 0.1% for 1 hour | 86<sup>ab</sup> |
| Soaked in hot water and left until 24 hours and then soaked in benomyl solution 0.1% for 1 hour | 78<sup>ab</sup> |

Remarks: Numbers followed by different letters indicate a significant difference using the Duncan test at a 95% confidence level.

Ginger and onion treatments can increase the calliandra germination by 10.66% and 10%. According to [10], onion extract can increase the germination of wheat seeds by 3.67%, while ginger extract increases the germination by 9%. Plant extracts' ability to increase seed germination is caused by plant extract capability to suppress the fungi growth that can be found in seed embryos [16]. In addition to that, onion is known to contain auxin, which plays a role in seed germination [17].

Soaking the seeds with turmeric extract does not cause an increase in seed viability; however, it actually decreases the viability if compared with the hot water treatment. It is suspected that there is a growth inhibitory compound contained in turmeric. Turmeric contains diarylheptanoids (demethoxycurcumin, bisdemethoxycurcumin, curcumin), which act as anti-fungus [18]. However, it is also known as growth inhibitors that can inhibit the germination and growth of several types of plants [19].
Soaking the seeds with garlic extract did not increase seed germination and even lowered the germination, presumably because the concentration that had been used was incorrect. [20] reported that exudates produced by garlic roots at concentrations of 0.1 and 0.2 mL- could increase the germination of lettuce seeds while at concentrations of 0.4 and 0.6 mL-, seed germination was decreased.

The identification result showed that there are five seed-borne fungal species of calliandra at the time of storage, including Aspergillus sp., Penicillium sp., Fusarium sp., Cylindrocladium sp. and Botryodiplodia sp. (figure 1). The dominant type of fungus was Aspergillus sp. and Fusarium sp. Conidiophores and conidia forms were one way to identify the genus of fungi. Based on [13], conidia of Cylindrocladium sp. were hyaline, filiform, straight, 1-celled (figure 1a.), Pycnidia of Botryodiplodia sp. were black, single, globose, conidia ovoid, dark and 2-celled at maturity (figure 1b.); conidia of Fusarium sp. were hyaline with macroconidia several celled, typically canoe-shaped, microconidia 1-celled, ovoid (figure 1c). Aspergillus sp. had a hyaline, straight, round vesicle shaped conidiophore at the tip of the conidiophores, bearing phialides at the entire surface, conidia 1-celled, globose/ovoid (figure 1d). Penicillium sp. had conidiophore, hyaline, straight, branched near the apex, ending in a group of phialides, conidia green pale/hyaline, 1-celled, globose/ovoid (figure 1e.). [6] reported that seed-borne fungal species of Dipterocarpaceae were Aspergillus sp., Penicillium sp., Fusarium sp., and Botryodiplodia sp.

Penicillium sp. and Aspergillus sp. are categorized as ubiquitous fungi on tree seeds, fruits, and cones, that act as saprophytes or weak pathogen. They can affect the vigor and viability of seeds that are stored under RH condition while B. theobromae, cause rots in a wide range of host and many Fusarium sp., causing seedlings damping off [6].

Fusarium sp. is a field fungus, but can be carried by seeds and maturate at the storage even in orthodox seeds [6]. [21] reported that Fusarium sp. is one of the fungi that can lessen diseases on calliandra seedling that had rot symptom on stem base or root hypocotyls.

The treatment of seeds soaked in hot water and left to 24 hours has resulted in the highest percentage of fungal infections. It is because hot water can soften the seed coat. High moisture on the seeds due to soaking causes a suitable condition for the growth and development of seed-borne fungi (Table 2). However, further treatment using plant extracts and chemical fungicides prevents or inhibits fungal growth and can even induce seed resistance to fungal infections.
High moisture on the seeds due to soaking causes a suitable condition for the growth and development of seed-borne fungi. However, further treatment using plant extracts and chemical fungicides prevents or inhibits fungal growth and can even induce seed resistance to fungal infections. All the biological and chemical fungicides that were used significantly affected the infection of *Aspergillus* sp. fungi compared to hot water treatment, and also effectively inhibited *Fusarium* sp. Ginger, turmeric, onion and garlic are known to have secondary metabolites that act as anti-fungus.

Garlic, onion, turmeric has a significant effect in inhibiting all fungal species compared to the hot water treatment. Onion and garlic extract significantly suppressed the growth of *Fusarium* sp. compared to ginger extract. Along with [22] reported that the rate of inhibition of fungus growth by ginger is lower than the onion. Based on [12], reported that ginger extract produced the most significant percentage of seeds infected by fungus followed by onion extracts, garlic and turmeric. Meanwhile, ginger contains zingerone, shogaol and gingerol, sesquiterpenoid β sesquiphellandrene, bisabolene, farnesene and monoterpenoid (β phellandrene, cineol and citral) [23].

**Table 2.** Fungi infection percentage carried by calliandra seeds on several treatments after 1 month.

| Treatment | Aspergillus sp. | Fusarium sp. | Penicillium sp. | Cylindrocladium sp. | Botryodiplodia sp. | Total |
|-----------|----------------|--------------|-----------------|---------------------|--------------------|-------|
| A         | 5.33<sup>b</sup> | 4<sup>b</sup> | 1.33<sup>a</sup> | 1.33<sup>a</sup> | 1.33<sup>a</sup> | 13.33  |
| B         | 12.67<sup>a</sup> | 18<sup>a</sup> | 0<sup>a</sup> | 0<sup>a</sup> | 0<sup>a</sup> | 22<sup>a</sup>  |
| C         | 4<sup>b</sup> | 3.33<sup>ab</sup> | 0<sup>a</sup> | 1.33<sup>a</sup> | 0<sup>a</sup> | 8.67<sup>ab</sup> |
| D         | 1.33<sup>b</sup> | 9.33<sup>a</sup> | 0<sup>a</sup> | 0.67<sup>a</sup> | 0<sup>a</sup> | 11.33<sup>abc</sup> |
| E         | 4<sup>b</sup> | 0.67<sup>b</sup> | 0<sup>a</sup> | 0<sup>a</sup> | 0.67<sup>a</sup> | 5.33<sup>ab</sup> |
| F         | 6<sup>b</sup> | 0.67<sup>b</sup> | 1.33<sup>a</sup> | 0.67<sup>a</sup> | 0<sup>a</sup> | 8.67<sup>ab</sup> |
| G         | 0<sup>a</sup> | 0<sup>b</sup> | 0<sup>b</sup> | 0<sup>b</sup> | 0<sup>b</sup> | 0<sup>b</sup> |
| H         | 1.33<sup>b</sup> | 0.67<sup>b</sup> | 0<sup>a</sup> | 0.17<sup>a</sup> | 0<sup>a</sup> | 4.67<sup>ab</sup> |

Remarks: Numbers followed by different letters indicate a significant difference using the Duncan test at a 95% confidence level.

A : control.
B : soaked in hot water and left until 24 hours.
C : soaked in hot water and left until 24 hours then soaked in turmeric solution for 1 hour.
D : soaked in hot water and left until 24 hours then soaked in 10 % ginger solution for 1 hour.
E : soaked in hot water and left until 24 hours then soaked in 10 % onion solution for 1 hour.
F : soaked in hot water and left until 24 hours then soaked in 10 % garlic solution for 1 hour.
G : soaked in hot water and left until 24 hours then soaked in 0.1 % mancozeb solution for 1 hour.
H : soaked in hot water and left until 24 hours then soaked in 0.1 % benomyl solution for 1 hour.

Soaking the seeds in turmeric solution can effectively suppress fungus growth compared to the hot water treatment. Curcumin is one of the compounds of diarylheptanoids which has anti-fungal ability to inhibit the growth of *P. notatum* and *A. niger* by damaging the sandalwood cell wall that caused cell death [24]. Garlic and shallots contain alliin [25], allicin and thiosulfonates [26]. [27] reported that ajoene, which is a derivative of allicin, has the anti-fungal ability by damaging cell walls stronger than allicin.

The fungus growth inhibition mechanism worked due to toxins produced by plant extracts being applied against pathogens [28] and host resistance induction to disease. [29] reported that the presence of hydroxyl groups in phenols was associated with toxicity to microorganisms, namely because hydroxylation increase led to toxicity increase. Ginger extract can induce the production of host defense that compounds such as phytoalexin. It may increase in peroxidase enzymes [30].

Mancozeb treatment has a significant effect on eliminating fungus compared to hot water treatment. Similar results were obtained from the [14] research, which showed that there was no fungal colonization of the seeds added with mancozeb.
The benomyl mode of action is by inhibiting mitosis and cell division [31], while mancozeb inhibits cell metabolism [32]. Furthermore, [9] reported that mancozeb was very effective in inhibiting conidia germination compared to benomyl.

The use of chemical fungicides such as benomyl and mancozeb effectively suppresses fungal growth; besides that, mancozeb also can increase seed germination. However, the use of chemical compounds is harmful to health and also the environment. Because of that, the use of biological pesticides can be used especially for preliminary treatment of seeds because the materials used were cheap, easy to obtain, and only a small amount of plant extracts were needed. The choice of plants as bio-fungicides should also pay attention to other roles than as anti-fungus, which can increase seed germination, not vice versa, which reduces seed germination. Another thing that should be considered in the use of bio-fungicides is the correct concentration, which, in addition to inhibiting growth, can also increase seed viability.

4. Conclusions
The treatments to maintain seed germination and to reduce the percentage of fungal infections were ginger and onion extracts, applied after soaking the seeds with hot water and left cold for 24 hours. Ginger treatment could increase seed germination until 14% and reduce the percentage of fungal infections until 10.67% compared to hot water treatment. Onion treatment could increase seed germination until 13.34% and reduce the percentage of fungal infections until 16.67%.

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References
[1] Duncan M 2001 Botani dan Ekologi Produksi Pemanfaatan Kaliandra (Calliandra callothyrsus) (Eds.) Stewart J, Mulawarman, Roshetko JM, Powell MH Bogor Indonesia: Winrock International, The Taiwan Forestry Research Institute, and Pusat Penelitian dan Pengembangan Kehutanan, Department Kehutanan, Indonesia 63 p
[2] Hendrati R L and Hidayati N 2014 Budidaya Calliandra (Calliandra callothyrsus) untuk Bahan Baku Sumber Energi (Ed.) Na’iem M., Mahfudz, MP, Prabawa SB. Jakarta 1-29 p
[3] Xuan Ty H, Hernawan E, de S Liyanage M, Sila M, Ramdan H, Ginting A N, Hidayat Y, Setijoprodjo A, Roordaert R, Arias R and Duncan M 2001 Pemanfaatan Produksi dan Pemanfaatan Kaliandra (Calliandra callothyrsus) (Eds.) Stewart J, Mulawarman, Roshetko JM, Powell, MH Bogor Indonesia: Winrock International, The Taiwan Forestry Research Institute, and Pusat Penelitian dan Pengembangan Kehutanan, Department Kehutanan, Indonesia 63 p
[4] Peterson R, Palmer B, Shelton M, Merkel R, Ibrahim T M, Arias R, Berhe K and Perera A N F 2001 Produksi hijauan ternak produksi dan pemanfaatan kaliandra (Calliandra callothyrsus) (Eds.) Stewart J, Mulawarman, Roshetko J M, Powell M H Bogor Indonesia: Winrock International, The Taiwan Forestry Research Institute, and Pusat Penelitian dan Pengembangan Kehutanan, Department Kehutanan, Indonesia 63 p
[5] Schmidt L H 2007 Tropical Forest Seed. Berlin Heidelberg: Springer-Verlag Berlin Heidelberg XVIII 409
[6] Sutherland J R, Diekmann M and Berjak P 2002 Forest Tree Seed Health. Rome, Italy: International Plant Genetic Resources Institute 1-74 p
[7] Allen T W, Enebak S A and Carey WA 2010 Evaluation of fungicides for control of species of Fusarium on longleaf pine seed Crop Prot. 23 979–982
[8] Nuraini M N and Latiffah Z 2019 Efficacy of selected fungicides against mycelial growth of Colletotrichum spp. causing anthracnose of chilli Plant Pathol. Quar. 9 43–51
[9] Shin J, Fu T, Park K H and Kim K S 2017 The effect of fungicides on mycelial growth and
conidial germination of the ginseng root rot fungus, *Cylindrocarpon destructans* [10] Hasan MM., Chowdhury SP, Alam S, Hossain B, Alam MS 2005 Antifungal effects of plant extracts on seed-borne fungi of wheat seed regarding seed germination, seedling health and vigour index Pakistan *J. Biol. Sci.* 8 (9) 1284–9

[11] Saha D, Dasgupta S and Saha A 2008 Antifungal activity of some plant extracts against fungal pathogens of tea (*Camellia sinensis*) *Pharm. Biol.* 43 (1) 87–91

[12] Chandel S and Kumar V 2018 Effect of plant extracts as pre-storage seed treatment on storage fungi, germination percentage and seedling vigour of pea (*Pisum sativum*) *Indian J. Agric. Sci.* 8 (11) 1476–1481

[13] Barnett HL and Hunter BB 1998 Illustrated Genera of Imperfect Fungi. 4th Editio. St. Paul: APS Press; 218 p

[14] Pedireddi U R, Rao L S, Choudhary P D, Pallay S, KVVS K, et al 2018 Effect of seed infection on seed quality and longevity under storage of three rice varieties produced at different environments Nematology at IARI View project Studies on Application Of Polymer, Spermine And Scape Regulation On Seed Yield And Quality In *O. J. Pharmacogn. Phytochem.* SP1:3289–98. Available from: http://eands.dacnet. nic.in

[15] Mahal M F 2014 Effects of fungicides and plant extracts on seed germination and seed associated mycoflora of *Lens arietinum* L. and *Lathyrus sativus J.* *Bio-sci.* 22 101–10

[16] Abiamere CO, Nweke FN, Ogbadu LJ, Onyia OC and John CO 2014 Evaluation of *Moringa oleifera*, *Zingiber officinale* (Ginger), *Chromolena odorata* plants extract as seed borne of *cowpea IOSR J. Pharm. Biol. Sci.* 9 (6) 66–7

[17] Kurniati F, Sudartini T and Hidayat D 2017 Aplikasi berbagai bahan zpt alami untuk meningkatkan pertumbuhan bibit kemiri sunan (*Reutealis Trisperma* (Blanco) Airy Shaw) *J. Agro.* IV (1) 40–9

[18] Mioranza T M, Stangarlin J R, Kuhn O J, Portz R L, Balbi-Pena M I, Schwan-estrada K R F, Assi L, Formentini H M, Viecelli C A, Dal'Maso E G and Meinerz C C 2017 Biological properties of turmeric *Sci. Agrar Parana* 16 (1) 1–12

[19] Akter J, Hessain M A, Sano A, Takara K, Islam M Z and De-Xing Hou 2018 Antifungal activity of various species and strains of turmeric (*Curcuma* spp.) Against *Fusarium solani* Sensu Lato *Pharm. Chem. J.* 52 (4) 320–5

[20] Yan-Li Zhou, Yan Wang, Jin-Ying Li, Yan-Jie Xue 2011 Allelopathy of garlic root exudates *J. Appl. Ecol.* 22 (5) 1368–1372

[21] Hidayati N 2018 Identification of Pathogen Causes of Damping off Diseases on Calliandra *Jurnal Pemuliaan Tanaman Hutan* 12 (2) 137–44

[22] Ohunakin A O and Bolanle O O 2017 In vitro antifungal activities of three aromatic plant extracts against *Fusarium oxysporum* Schlechtend. Fr. F. Sp. *Lycopersici* (Sacc.) causal organism of fusarium wilt in tomato *J. Plant. Sci. Agric. Res.* 1 (1) 1-5

[23] Chrubasik S, Pittler M H and Roufogalis B D 2005 Zingiberis rhizoma: a comprehensive review on the ginger effect and efficacy profiles *Phytomedicine* 12 (9) 684–701

[24] Bhawana, Basniwal R K, Buttar H S, Jain V K and Jain N 2011 Curcumin nanoparticles: preparation, characterization, and antimicrobial study *J. Agric. Food. Chem.* 9 (59) 2056–61

[25] Latif M A, Saleh A K M, Khan M AI , Rahman H and Hossain M A 2006 Efficacy of Some Plant Extracts in Controlling Seed-Borne Fungal Infections of Mustard *Bangladesh J Microbiol* 23 (2) 168–70

[26] Harris J C, Cottrell S L, Plummer S and Lloyd D 2001 Antimicrobial properties of *Allium sativum* (garlic) *Applied Microbiology and Biotechnology* 57 (3) 282–6

[27] Yoshida S, Kasuga S, Hayashi N, Ushiroguchi T, Matsuura H and Nakagawa S 1987 Antifungal activity of ajoene derived from garlic *Appl. Environ. Microbiol.* 53 (3) 615–7

[28] Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R and Samiyappan R 2004
Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and *Xanthomonas oryzae pv oryzae* *Physiol. Mol. Plant Pathol.* 65 91–100

[29] Cowan M M 1999 Plant products as antimicrobial agent *Clinical Microbiology Reviews* 12 (4) 564–82

[30] Stangarlin J R, Kuhn O J and Assi L 2011 Control of plant diseases using extracts from medicinal plants and fungi *Science Against Microbial Pathogens Communicating Current Research and Technological Advances* (Ed.) Mendez-Vilas A. Formatex 1033-1042

[31] Yang C, Hamel C, Vujanovic V, Gan Y 2011 Fungicide: modes of action and possible impact on nontarget microorganisms *ISRN Ecology* 130289 8p

[32] Thomson WT 1992 Agriculture Chemicals Fungicides. Thomson Publications Press 181p