International Journal of Agriculture System
Vol. 7 Issue 1, June 2019
Nationally Accredited Journal Decree No. 48a/E/KPT/2017
P-ISSN: 2337-9782, E-ISSN: 2580-6815. DOI: 10.20956/ijas.v7i1.1779

Enhance content of leaf chlorophylls and the primary root diameter of shallot (*Allium cepa* L.) with seed coating by rot fungi

Iradhatullah Rahim[1*], Syamsiar Zamzam[1], Suherman[1], Syamsia[2], Selis Meriem[3], Yunarti[4], Andi Nasruddin[5]

[1] *Department of Agrotechnology, Universitas Muhammdiyah Parepare, Indonesia.*
[2] *Department of Agrotechnology, Universitas Muhammadiyah Makassar, Indonesia.*
[3] *Department of Biology, Alauddin Islamic State University, Makassar, Indonesia.*
[4] *Department of Agribusiness, Agricultural Polytechnic State of Pangkep, Pangkep, Indonesia.*
[5] *Department of Plant Pest and Disease, Hasanuddin University, Indonesia.*

[*] *Corresponding author's e-mail: iradhat76@gmail.com*

How to Cite: Rahim, I., Zamzam, S., Suherman, Syamsia, Meriem, S., Yunarti., and Nasruddin, A., (2019). Enhance content of leaf chlorophylls and the primary root diameter of shallot (*Allium cepa* L.) with seed coating by rot fungi. *Int. J. Agr. Syst.* 7 (1): 18-26

ABSTRACT

Shallot production is affected by the cultivation techniques, one of them is seed coating. The technique is a practice by covering seeds by using particular substances such as growth regulators, micronutrients, fungicides, or antioxidants that increase seed vigor in the field. Micro nutrients and growth regulators can be collected from rot fungi. This study aimed to determine the leaf chlorophyll content and root diameter of shallot plants with seed coating used 4 superior rot fungi isolates that applied using single and combination isolates. The research was arranged in an experiment using a Randomized Block Design consisting of 11 treatments repeated 3 times so that there were 99 experimental units. Leaf chlorophyll content was measured by SPAD-502 plus chlorophyll meter, while the length and width of the leaf was measured by Leaf Area Meter. The results showed that seed coating with combination of Tramella sp + Pleurotus sp isolates gave the best response to the leaf chlorophyll content and the primary root diameter of the shallot. The highest content of leaf chlorophyll was 0.029 nm with a primary root diameter of 1.33 nm. Seed coating with a combination of rot fungi isolates is likely one of the techniques to increasing plant growth and chlorophylls content.

Keywords:
Chlorophylls; seed coating; rot fungi ;micro nutrient;vigor; growth regulator; leaf area

1. Introduction

Shallot (*Allium cepa sp*) is one of the horticultural commodities that has a high economic value. Aside from being a cooking spice, this plant is used as spices and medicine. Essential oil content of shallot cure some health problems. Production of Indonesian shallots reached 1,470,155 tons in 2017. Although there was a growth of 1.61% per year, the productivity of shallots in Indonesia decreased. In 2014 the productivity reached 10.22 tons.ha^{-1}.y^{-1} to 9.31 tons.ha^{-1}.y^{-1} in 2017 (Agricultural Ministry, 2018). Shallot yield was influenced by cultivation techniques, one of them was seed enhancement. It was an effort that can be done to improve seed quality and vigor. Seed treatment play a vital controlling to plant growth and production. According to Taylor et al. (1998), there are three techniques that can be used to improve seed quality, namely pre-sowing hydration treatment, seed conditioning, and coating technology.

The use of seed coating in the seed industry is strongly effective to improve seed morphological features, increase seed storage, and reduce the higher risk for contracting from plant that grow near each other. Moreover this practice also can be used to carry some additives such as antioxidants, anti-microbes, repellents, antagonistic microbes, and growth regulators (Ilyas, 2012). Using chemical substances continually in maintaining plants and seeds induces environmental problems. On the other hand, potential biological agents are being developed to date.

The rapid development of life science and agriculture effect to create agricultural technology by utilizing biological agents, such as bacteria (Plant Growth Promoting Rhizobacteria/PGPR) and fungi. Based on research by Iswati (2012), PGPR showed an important role in tomato plants growth particularly to stimulate the growth of stems, leaves, and roots. Several findings also showed that specific fungi and bacteria can enhance plant growth and control particular diseases by producing plant growth hormones and other metabolites compounds. Seed of long bean with coating treatment by *Methylobacterium spp*. TD-L2 isolates still had high viability up to 12 weeks of storage, with germination of 90.3% (Sari, 2009). A similar result also found in in seed bean with coating treatment of *Methylobacterium spp*. that had not shown a decrease viability significantly with period of 20 weeks with and average germination of 93% (Yuningsih, 2009). Seed of hybrid rice coated with *Bacillus subtilis* (Tirawati, 2012) and *Pseudomonas fluorescens* (Krisnandika, 2012) also had a high viability and vigor with storage period to 15 weeks. Seed coating using sodium alginate and xanthan gum mixed with 3 isolates of actinomycetes, Bacillus, and Pseudomonas improved the health and viability of chili seeds over a storage period of 5 months (Tefa et. Al., 2016)

As well as rot fungi, such as *Tremella sp*, produce enzyme of peroxidase that used in textile bioremediation processes, delignification, and decolorization of wood pulp, and anti-cancer therapy (Webster & Weber, 2007). *Pleurotus sp* can produce Indole Acetic Acid (IAA) and GA3 hormones of 1,794 and 2,551 µgL-1, respectively (Rahim, Nasruddin, Kuswinanti, Asrul, & Rasyid, 2018). Those potency lead rot fungi as a primary substance to increase growth, specially to enhance chlorophylls content. Hence, we use rot fungi by coating of shallot seed, and mixing them with bentonite, kaolinit, and CMC.

2. Materials and Method

2.1. Materials and Treatment

The seed shallots of Bima variety used in this study taken from farmer. The rot fungi isolates of *Mycena sp, Lycoperdon, Tramella sp* and *Pleurotus sp* were collected from Plant Biotechnology Laboratory Hasanuddin University. Other substances used were bentonite, kaolinite, CMC (Carboxy-Methyi-Cellulose), alcohol, spiritus bunner, aquades, rice flour, heat resistant plastic, labels and polybags. The tools used were digital scales, autoclaves, laminar air flow, SPAD-502 plus chlorophyll meter, digital microscope endoscope 1000 x 8 LED (Light Emitting Diode) magnifier, Leaf Area Meter, petri dish, spatula, and gutters.

This study was arranged in an experiment using a Randomized Block Design with treatment of seed coating using combination of rot fungi isolates,in a single and in mixture isolates. There were the coating treatment:

1. Isolate of *Mycena sp* (M)
2. Isolate of *Lycoperdon sp* (L)
3. Isolate of *Tramella sp* (T)
4. Isolates of *Pleurotus sp* (P)
5. Combination of M + L isolates
6. Combination of M + T isolates
7. Combination of M + P isolates
8. Combination of L + T isolates
9. Combination of L + P isolates
10. Combination of T + P isolates
11. Without seed coating or control treatment.

Each treatment of isolates was grown in the rice flour and inoculated for 7 days at room temperature. Seed shallots with uniform size were dipped into CMC then rolled on the medium of fungi flour mixed with kaolinite and bentotite in a ratio of 1: 1: 1. The coated seeds were planted in the polybags with medium of soil and sterilized manure.

2.2. Shallot Growth Measurement

The height of plant, leaf number, and leaf area of shallot were measured at 8 weeks after planting. Leaf Area Meter was used to measure the length and width of leaves from three different size from small, medium, and large using. The leaf area was measured by multiplying the leght of leaves to its width (Sitompul and Guritno, 1995).

2.3. Leaves Chlorophyll Measurement

The chlorophyll contents were measured using S*PAD-502 Plus Chlorophyll Meter* by attaching the tools to the middle leaf blade. This conducted at 60 days after planting. The amount of leaf chlorophyll were measured with formula $Y = 0.0007x - 0.0059$, Y is a content of leaf chlorophyll and x is a value of counted results from *SPAD-502 plus chlorophyll meters* (Farhana et al, 2007).

2.4. Measurement of Primary Root Diameter

The primary root measured was the main root that grew from the base of the stem, which continues to grow elongated and enlarged which can support plant growth as anchors. In its development, this root is branching into lateral roots. The largest primary root were and put into water in the plastic then measured with a Digital Microscope Endoscope Camera Magnifier 1000 x 8 LED. The diameter was measured at 60 days after planting.

2.5. Data Analysis

Data obtained from all the analysis conducted in this study were analyzed statically using analysis of variance (ANOVA).

3. Results and Discussion

3.1. Shallot Growth

Plant height, number, and leaf area of shallot which rot fungi coating shown in Table 1.

Table 1. The growth of shallot with rot fungi coating

Isolate of Rot Fungi	Sprout speed (days)	Plant height (cm)	Number of leaves (blade)	Leaf area (cm^2)
Isolate of *Mycena sp* (M)	6.11[c]	39.60	30.78[b]	2.76
Isolate of *Lycoperdon sp* (L)	6.33[c]	37.41	27.78[a]	2.67
Isolates of *Tramella* sp (T)	7.21[c]	37.63	29.78[a]	2.80
Isolates of *Pleurotus sp* (P)	6.78[c]	38.18	28.44[a]	2.56
Isolates M + L	7.21[c]	38.46	29.00[a]	2.74
Isolates M + T	5.00[a]	38.74	26.56[a]	2.55
Isolates M + P	7.22[c]	37.63	27.67[a]	2.38
Isolates L + T	7.23[c]	38.04	27.00[a]	2.58
Isolates L + P	6.00[c]	38.19	30.11[b]	2.64
Isolates T + P	5.44[b]	39.61	30.44[b]	2.87
Without Rot Fungi Isolate	7.23[c]	36.82	25.11[a]	2.45

Description: The numbers followed by the same letter did not significantly different at the level of α = 0.05 in the HST test.

The treatment without rot fungi isolate showed a lowest of height, leaf number, and also a slowest sprouting speed among other treatments of seed coating with rot fungi (Table 1). It showed that rot fungi affects plant growth as found in bacteria. Kumar et al., 2012 explained that most of Rhizobacteria species could increase plant growth by producing IAA hormones and provide other nutrients. The growth hormones is also released by rot fungi and this related positively to plant growth. It means that the provision of rot fungi isolates by coating the seeds can increase plant growth, especially sprout speed and number of leaves. When the number of leaves increases, it can be predicted that there will be an increase in plant metabolic activity, especially photosynthesis.

3.2. Content Chlorophyll of Shallot

Analysis of variance showed that the treatment of shallot seed coating with fungi isolates did not significantly affect the chlorophyll content of leaf shallots aged 60 days after planting. Combination treatment of *Tremella sp* and *Pleurotus sp* (T + JT) isolates showed the highest leaf chlorophyll (Figure 1).

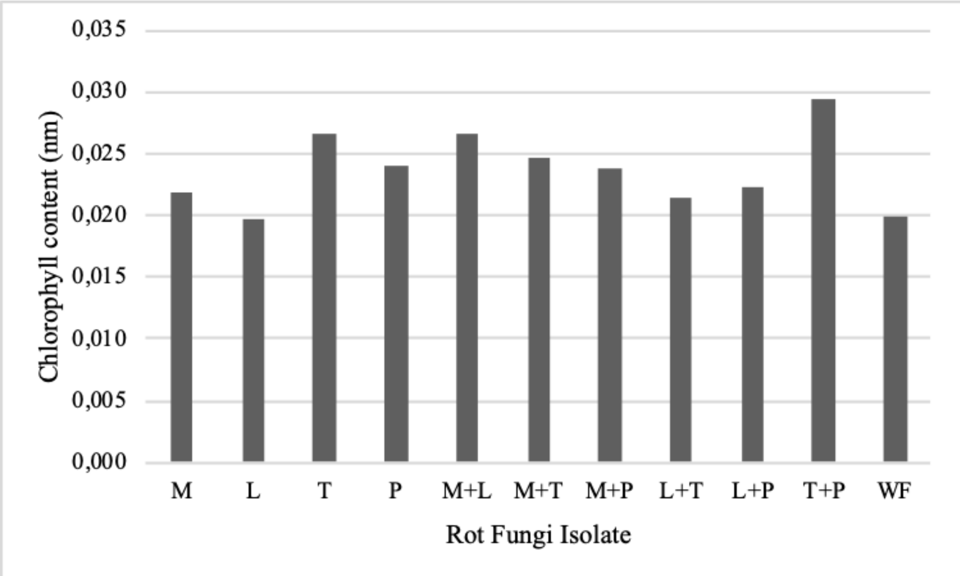

Figure 1. Chlorophyll content of leaves of shallot plants coated with rot fungi. M = Mycena sp, L = Lycoperdon sp, T = Tremella sp, P = Pleurotus sp, WF = Without Fungi.

Figure 1 showed that chlorophyll contents between treatment of seed coated with a single isolate and combination rot fungi isolates were similar. Chlorophyll is a green pigment stored in chloroplasts. This organelle is almost found in parenchyma tissues of palisade and spongy of vascular plants. The pigments of chlorophyll, carotenoids, and xanthophyll in chloroplast were mainly composed in thylakoid membranes (Salisbury and Ross 1992). The chlorophyll affect plant growth and its crop production. The higher chlorophyll content, the higher its production and therefore lead to enhance plant's growth.

Combination of Tremella + Pleurotus (T+P) isolates showed a highest chlorophyll contets 0.029 nm (Figure 1). This concentration was higher than chlorophyll of leaf base of Gandasuli, which was 0.026 nm (Pratama & Laily, 2015). In this findings, the highest chlorophyll contents from combination of T+P isolates are driven by high IAA hormone release from *Tremella sp* was 2,444 μgL^{-1} and *Pleurotus sp* was 1,794 $\mu g\ L^{-1}$ (Iradhatullah, Kuswinanti, Asrul, & Rasyid, 2015). This combination caused the IAA hormone content to be high compared positively correlated with chlorophyll content. Indeed, IAA hormone induces plant's growth (Rao, 2010). Moreover, *Tremella sp* and *Pleurotus sp* are potential to dissolve phosphate, 2,510 μgL^{-1} and 3,094 μgL^{-1} respectively (Asrul, Rahim, Kuswinanti, Rasyid, & Nasruddin, 2018).

The reduced of chlorophyll contents might caused by inhibited chlorophyll synthesis, declined rubisco enzyme, and inhibitory activity of nutrient absorption, in particular nitrogen and magnesium which play a main role in chlorophyll synthesis. The concentration of this pigment can be used as a valuable indicator to evaluate the metabolic imbalance between photosynthesis and primary production at limited water condition (Nio and Banyo, 2011). So that if there is an increase in chlorophyll concentration, the photosynthesis process will run according to the plant needed. This increase can occur by adding rot fungi isolates to plant media.

3.3. Diameter of Shallot Plant Primary Root

Roots is a vertical organ of plant growing downward in the media. Root is a main vegetative organs that supply water, minerals, and other important substance for growth and plant development. In accordance with Sitompul and Guritno (1995), the role of root is as important as plant crown. The crown supply carbohydrates through photosynthesis and root supply nutrients and water for plant metabolism. The potential of plant to absorb nutrients can be determined by measurement of root poriferation, root length, fresh weight of root, and its dry weight. Potassium plays in growth and development of plant roots. Leiwakabessy, et al (2003) indicates that element of enough potassium will enhance root's growth. The high availability of potassium for plants support root development. The high availability measurement of primary root diameter was to determine the ability of root growth of shallots with seed coating by rot fungi isolate (Figure 2). The average primary root diameter (mm) of shallots was shown in Figure 3.

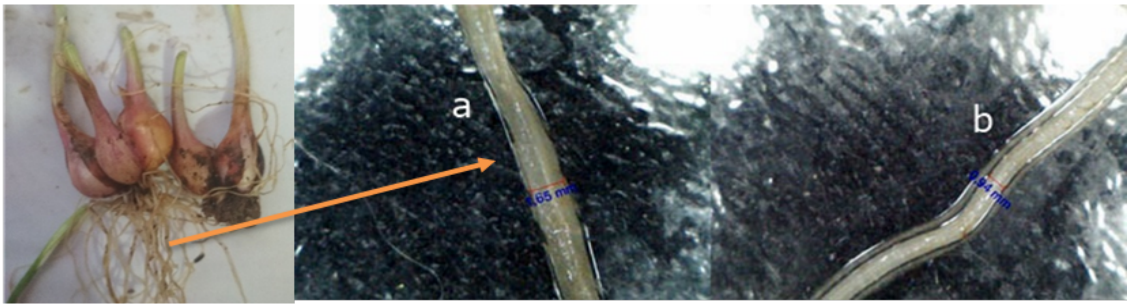

Figure 2. The primary root diameter (mm) of shallots was high with the seed coating by rot fungi Tremella+Pleurotus isolates (a), and the low in without rot fungi (b).

Analysis of variance indicated that there were not statistically difference to primary root diameter of shallots, otherwise showed positive responds at all treatments. Duruoha, Piffer, & Silva (2007) also researched on root diameter of corn with variant bulk density excluding fertilizer. The diameter at density of 1.20 $g.cm^{-3}$ was 1.48 mm and density of while in 1.40 $g.cm^{-3}$ and 1.60 $g.cm^{-3}$ density yielded diameters of 1.37 mm and 1.17 mm respectively. The diameter of those are lower than the diameter of primary diameter of the root of shallot in this study, in which was 1.33 mm. This measure was found in shallot root with combination of T + P fungi isolates, there were *Tremella sp* and *Pleurotus sp* (Figure 2). That combination isolates showed a highest root diameter than a single isolate. This was similar to finding of Pan & Dam, (2012), that a consortium or combination of 3 isolates types enhance fastest decomposition than a single bacterial isolate.

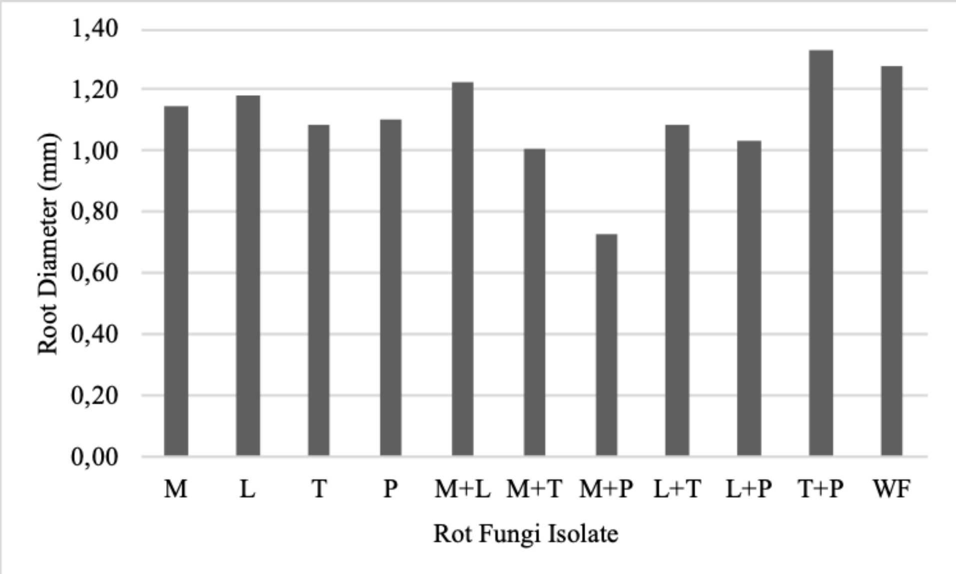

Figure 3. The average primary root diameter of shallots in various isolates of rot fungi with seed coating at 60 days after planting.

The use of seed coating to improve seed germination requires adhesives and additives that able to be fused, in condition there are no negative impacts on germination. The main adhesives materials used to covering seed are diatomaceous earth, charcoal, methylethyl cellulose, arabic gum, and polyvinyl alcohol (Kuswanto, 2003), carboxyl methylcellulase (CMC), alginate (Zahran *et al.*, 2008), and chitosan (Zeng *et al.*, 2012). Those substances are mixed into fungi flour to provide adhesion to seeds. This could increase the potential of rot fungi as seed coating enhance plant growth.

4. Conclusion

We investigated that there were increase in chlorophyll content of leaves and increase in primary root diameter of shallot with seed coating through flour of sot fungi isolates. The flour referred to combination of *Tremetes sp* and *Pleurotus sp*.

Acknowledgements

We thank to DRPM Dikti that funded this research. Thanks to students of Agrotechnology UMPAR were support this research in the field. Thanks also to Muh. Akhsan Akib for providing tool-lendings and Siti Halima Larekeng for facilities in Laboratory of Biotechnology and Tree Breeding, Faculty of Forest Hasanuddin University.

References

Agricultural Ministry Republic of Indonesia, 2018. The agricultural data of the last 5 years. http://www.pertanian.go.id/home/?show=page&act=view&id=61

Asrul, L., Rahim, I., Kuswinanti, T., Rasyid, B., & Nasruddin, A. (2018). Effect of Cocoa Pod Husk Compost Produced Using Rot Fungi on the Growth of Cocoa Seedlings. *Online Journal of Biological Sciences*, *18*(1), 69–73. https://doi.org/10.3844/ojbsci.2018.69.73.

Duruoha, C., Piffer, C. R., & Silva, A. S. (2007). Zea mays. *Irriga, Botucatu*, *12*(1), 14–26. https://www.researchgate.net/publication/267919161_Corn_root_length_density_and_root_diameter_as_affected_by_soil_compaction_and_soil_water_content.

Farhana, M.A, M.R Yusop, M.H. Harus, A.K. Din, 2007. Performance of Tenera Population for The Chlorophyll Contents And Yield Component. Proceedings of The PIPOC 2007. International Palm Oil Congress (Agriculture, Biotechnology & Sustainability). Malaysia Palm Oil Board. Malaysia. Vol. 2 : 701-705

Ilyas, 2012. Seed science and technology: Theory and results of research. (In Bahasa Indonesia). Bogor: IPB Press. 138 p.

Iswati R. 2012. Effect of dose of PGPR formula from bamboo roots on tomato plant growth (Solanum lycopersicum syn). (In Bahasa) JATT 1 (1): 9-12.

Iradhatullah, R., Kuswinanti, T., Asrul, L., & Rasyid, B. (2015). Growth Rate and Indole Acetic Acid Production of Several Fungal Rot Isolates, *4*(6), 2013–2015.

Krisnandika AAK. 2012. Utilization of Pseudomonas fluorescent RH-4003 and ascorbic acid bacteria to maintain the viability of hybrid rice seeds during storage. Scription. (In Bahasa Indonesia). Bogor Agriculture University, Bogor.

Kumar, A., Kumar, A., Devi, S., Patil, S., Payal, C., & Negi, S., 2012. Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: an in vitro study. *Recent Research in Science and Technology*, *4*(1), 1–5. Retrieved from http://recent-science.com/.

Kuswanto, H. 2003. Seed Processing, Packaging and Storage Technology (In Bahasa Indonesia). Kanisius. Yogyakarta.

Leiwakabessy, F.M., U.M. Wahjuddin, and Suwarno. Soil Fertilizer. (in Bahasa). Bogor Agricultural University.

Pan, I., & Dam, B. (2012). Composting of common organic wastes using microbial inoculants, 127–134. https://doi.org/10.1007/s13205-011-0033-5.

Pratama, A. J., & Laily, A. N. (2015). Analysis of Chlorophyll Content of Gandasuli Leaves (Hedychium gardnerianum Shephardex Ker-Gawl) at Three Different Dev. In *Seminar Nasional Konservasi dan Pemanfaatan Sumber Daya Alam* (pp. 216–219). Semarang: UNS Semarang.

Nio and Banyo. 2011. Leaf Chlorophyll Concentration as an Indicator of Water Deficiency in Plants. Scientific Journal of Science Vol. 11 No. 2.

Rahim, I., Nasruddin, A., Kuswinanti, T., Asrul, L., & Rasyid, B. (2018). Utilization of Cocoa Pod Husk Waste Composting by Tremella Sp and Pleurotus Sp as A Medium to Growth of Cocoa Seedling. *IOP Conference Series: Earth and Environmental Science*, *156*(1). https://doi.org/10.1088/1755-1315/156/1/012012.

Rao, S. (2010). Soil Microorganism and Plant Grow. Jakarta: UI Press.

Sari, P.E. 2009. Effect of composition of coating materials and Methylobacterium spp. the seed storage and vigor of long bean seeds (Vignasinensis L.). [Scription]. (In Bahasa). Bogor Agricultural University.

Salisbury, F.B. and Ross, C.W. (1992). Plant Physiology. 4th Edition, Wadsworth Publishing, Belmont.

Sitompul, S., and Guritno, B., 1995. Plant physiology and plant development. (in Bahasa). Raja Grafindo Persada, Jakarta.

Taylor, A.G., P.S. Allen, M.A. Bennet, K.J. Bradford, J.S. Burris, and M.K. Misra. 1998. Seed Enhancements. Seed Science Research 8:245-256. https://doi.org/10.1017/S0960258500004141.

Tefa, A., E.Widajati, M. Syukur, and Giyanto., 2016. Use of Probiotic bacteria to suppress *Colletotrichum acutatum* infections and improve chili seeds quality during storage. Savana Cendana. Jurnal Pertanian Konservasi Lahan Kering. Vol.1 No.01.

Tirawati. 2012. Coating of seeds with Bacillus subtilis AB89 and tocopherol to maintain the viability of hybrid rice seeds during storage. [Scription]. (In Bahasa). Bogor Agricultural University.

Webster, J., & Weber, R. (2007). *Introduction to Fungi* (Third). Camridge University Press.

Yuningsih AFV. 2009. Effect of Application of *Methylobacterium spp*. on Viability of Beans (*Phaseolus vulgaris* L.) during Storage. Scription (In Bahasa Indonesia). Bogor Agriculture University, Bogor.

Zahran, E.; Sauerborn J.; Elmagid, A.Abd. ; Abbasher, A.A.; Müller-Stöver, D. 2008. Granular formulations and seed coating: delivery options for two fungal biological control agents of *Striga hermonthica*. J. Plant Dis. Plant Protect. 115 (4) p.18-185.

Zeng D., Xinrong L. and Renjie. 2012. Application of Bioactive Coatings Based on Chitosan for Soybean Seed Protection. International Journal of Carbohydrate Chemistry. Vol.2012.