Response of Growth of Garlic Towards *Aspergillus niger* and *Fusarium* sp. Inoculant

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Abstract. Garlic is one of the main commodities in Indonesia.Various constraints in the cultivation system such as the condition of the land and attacks of diseases such as root wilting are problem of increasing the productivity of this plant. This study aims to obtain information about the effect of giving inoculants of *Aspergillus niger* and *Fusarium sp* on the growth of garlic in different media planting until the age of 20 days. Garlic bulb were obtained from local farmers, with a size of weight 6-7 gram/bulb. The experiment was carried out at the greenhouse scale using a completely randomized design with 5 replications. Treatment includes the type of planting media, soil, sand and soil + sand (ratio1:1), three type of inoculant, *Aspergillus niger, Fusarium sp* and mixing *Aspergillus niger* + *Fusarium sp* and control. Inoculants are given 10 mL (5 x 10^{10} CFU/mL) in liquid on pots containing 300 grams of planting media. Observations are carried out after 20 days planting. The results showed that *Aspergillus niger* and *Aspergillus niger* + *Fusarium sp* inoculants had a positif significant effect on the growth of garlic in all treatment media soil, sand and soil + sand. *Fusarium sp* inoculant inhibits growth of 30% - 60%, *Aspergillus niger* inoculant can increase growth of 40% - 90% when compared to controls on all planting media. The use of *Aspergillus niger* inoculant can overcome diseases caused by *Fusarium sp*, it can even increase the growth of 30% - 40% in sand and sand + soil media planting.

1. Introduction

Garlic or *Alium Satuvum* L. is one of the important plants in Indonesia. This bulb plant is widely used as a spice in a variety of processed foods and also used as a base for herbal medicine [1-7]. In 2014, this planted area in Indonesia was 1,913 ha with a production of 8.83 tons ha^{-1} in total production in 2014 was 16,893 tons. The consumption of garlic per capita per year in Indonesia is predicted to increase in line with the increase in population [8]

The increaseing of garlic production is closely related to the availability of land and the presence or absence of plant pests and diseases. One of the diseases that is often found in the field of garlic is stem or root rot caused by microorganisms, both by fungi, bacteria and viruses . One of the fungi that causes stem or root rot is *Fusarium* This fungus lives cosmopolite on the soil and can attack on roots, bulb and stems of garlic plants [4,9-12]. As a result the plant will wither and end in death.

The efforts to overcome the disease caused by *Fusarium sp* usually use by chemical, extract herbal and microbe [10, 11, 12, 13]. Besides being harmful to health, the use of chemicals can also result in damage to the balance of ecosystems that have been formed previously. For this reason, it is necessary to continue to innovate technology that is easy, not expensive and environmentally friendly in overcoming diseases of garlic caused by *Fusarium sp.* The one effort that can be done is the use of functional microbes that are endemic to the soil, such *as Aspergillus niger.* Until now, there isn't reported using *Aspergillus niger* as a biocontrol for plant diseases caused by *Fusarium*

In the preliminary experiments in the laboratory we found that *Aspergillus niger* has the ability to inhibit the growth of *Fusarium sp*. However, further research is still needed at the greenhouse scale. Another advantage of *Aspergillus neger* is the ability of this fungus to produce secondary metabolit like IAA which is needed by plants [14], and increase plant germination [15]. This study aims to determine the effect of using inoculant of *Aspergillus niger* and *Fusarium sp* on garlic plants until the age of 20 days. The experiment was carried out in a greenhouse using a plastic pot with a capacity of 350 grams. Experiments using a randomized design complete with factorial. The main factors are inoculants, namely *Fusarium sp* (A), *Aspergillus niger* (B), mixing *Fusarium sp and Aspergillus niger* with a ratio of 1:1 (C) and control (K). The second factor is the type of planting medium, namely sand (P), soil (T) and a mixture of sand + soil (PT) with a ratio of 1:1. Observations were made on the 20th day after planting, with parameters of pH and EC of planting media, and vegetative parameters which include plant height, root length, wet and dry weight of the upper plants and roots.

2. Materials and Methods

2.1. Materials

Aspergillus niger, Fusarium sp., potato dextrose agar (PDA), distillated water, molasses, plastic pod, sand and soil were used as materials in this study.

2.2. Methods

Aspergillus niger and *Fusarium sp* used in this study were obtained from our own culture collection. These isolate are the result of fungi selection soil and garlic disease. The isolate were cultivated in PDA and incubated for 96 hours, at pH 7, and 30^{o}C. After incubation, 20 mL of distillated water was added, and cultivated in 200 mL molasis media at room temperature for 7 days (until population 5 x 10^{10} CFU/mL). This suspension was used as inoculants.

The experiment was carried out at the greenhouse scale by using a completely randomized design with 5 replications. Treatment includes the type of planting media, soil, sand and mixing soil + sand (ratio 1:1), three type of inoculant, *Aspergillus niger, Fusarium sp* and mixing *Aspergillus niger + Fusarium sp* (ratio 1:1) and control. Inoculants are given 10 mL (5 x 10^{10} CFU/mL) in liquid on pots containing 300 grams of planting media, and each pot is filled with one bulb of garlic. Garlic were obtained from local farmers, with a size of weight 6-7 g/bulb. Observations are carried out after 20 days planting. Observation parameters included the pH and EC of the planting medium, plant height and root length, wet and dry weight of the upper plant/vegetative and the roots.

2.3. Measurement of parameters

pH and EC measurements were carried out directly on the planting media after 20 days planting by using the Hanna model HI 991301 pH / EC / TDS and temperature devices. Measurement of plant height and root length is carried out directly. The plant is cleaned from the planting media by washing it with running water. Measurement of plant height and root length is done with a ruler. After that the plants are

separated from the upper part of plant and the roots, then dropped to find out the weight. To find out its dry weight, this part of the plant was oven at 80^0C for 24 hours.

3. Results and Discussion

The results of pH and EC planting media by using inoculant are shown in Figures 1 and 2. Using of the inoculant formula give the affects on pH of media planting in sand and sand + soil, but no effect on soil planting media. While the EC value changes with used of inoculant formulas on all planting media treatments. Generally the EC value decreases in all planting media when compared with EC in the control treatment, except in treatment A, sand media planting where EC increased significantly when compared to controls. The highest EC value is shown in the treatment of the inoculant *Aspergillus niger* (B) formula in soil, which is equal to 0,57 mS. While the lowest value was indicated by the treatment of the same inoculant for sand planting media, which is 0,12 mS (Figure 2). The soil media has a higher EC value, this condition is possible because the soil is able to maintain its moisture. Changes in pH and EC are still very small, this is probably due to the relatively short planting period (20 days). EC influences plant growth through nutrient absorptions by roots. Usually this effect will be seen in the EC value values above 1 mS [16-18].

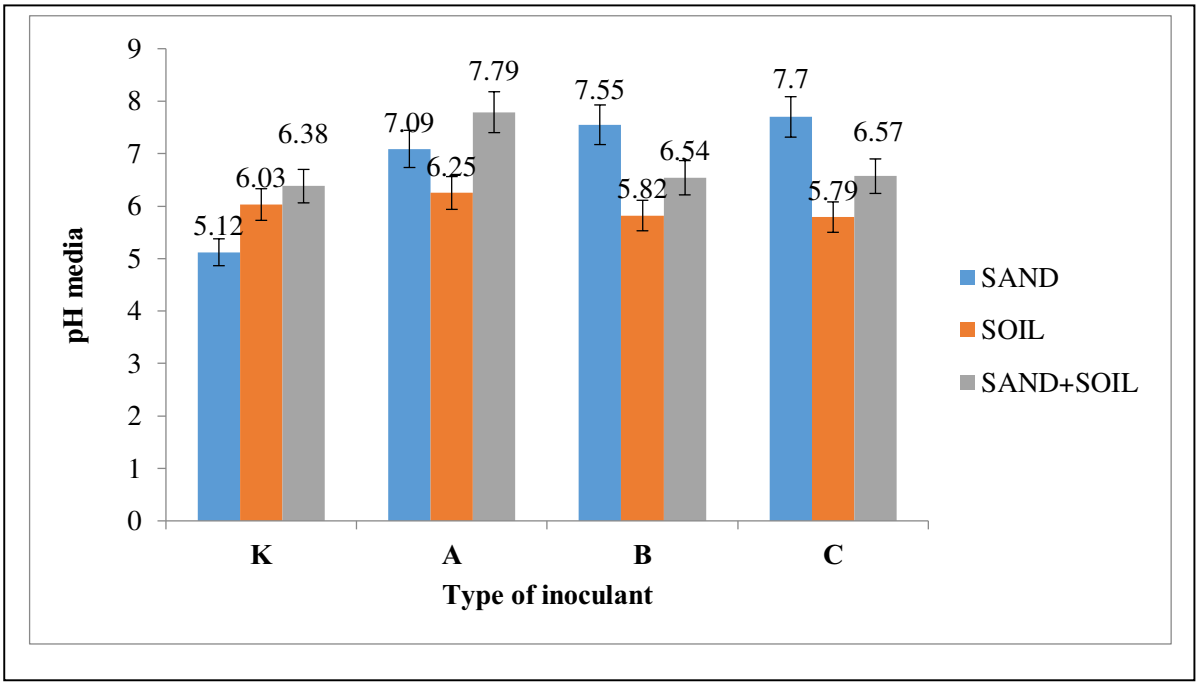

Figure 1. The effect of inoculants on the pH media planting of garlic after 20 days planting (**K**: control, **A**: *Fusarium sp*, **B**: *Aspergillus niger*, **C**: *Fusarium sp* + *Aspergillus niger*).

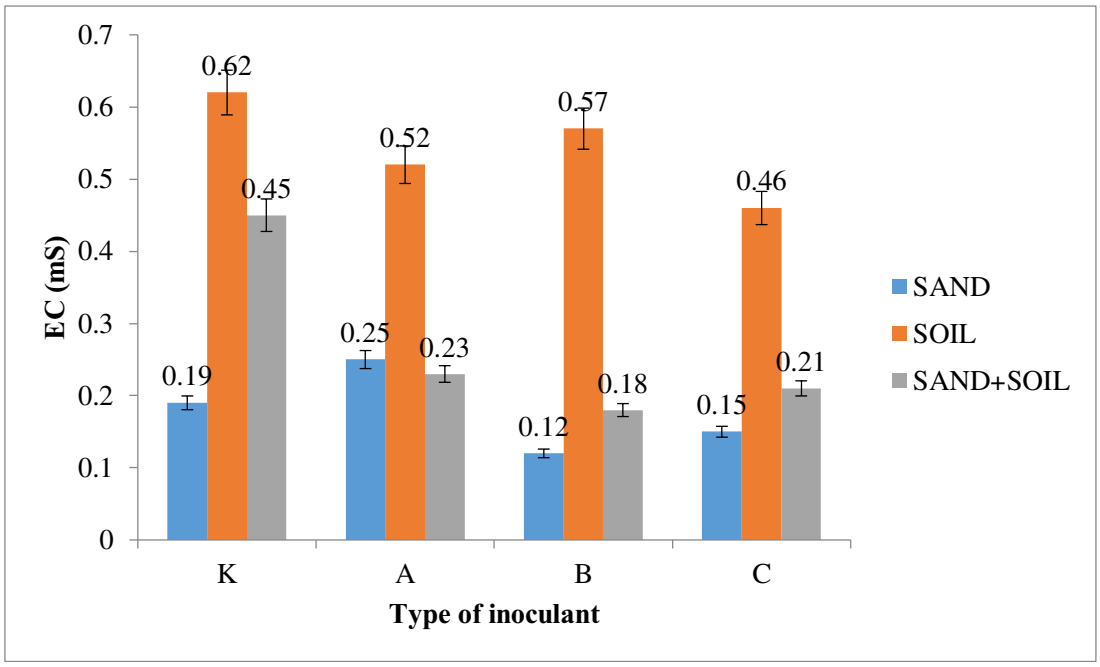

Figure 2. The effect of inoculants on the pH media planting of garlic after 20 days planting (**K**: control, **A**: *Fusarium sp*, **B**: *Aspergillus niger*, **C**: *Fusarium sp* + *Aspergillus niger*).

In Figure 3 shows the influence of *Fusarium sp* and *Aspergillus niger* on garlic after 20th day planting in all growing media. The results of examination of root conditions in the growing media showed damage to the garlic bulb inoculated with *Fusarium sp*. In general, the results of visual observations of vegetative growth shows the influence on the using of *Fusarium sp* and *Aspergillus niger* inoculants both singly and in mixture on all types of planting media (Figure 3). A very significant effect can be seen by using *Fusarium sp* inoculants, where plants are stunded growth in all planting media. The real effect is shown on the media planting soil + sand, where plant die after 20th day planting (Figure 3 and 4).

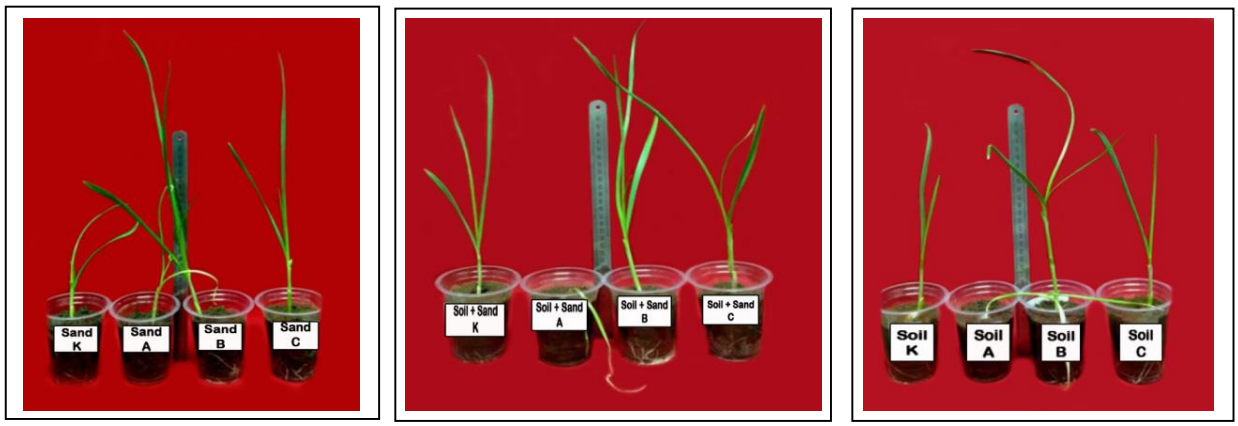

Figure 3. Condition of garlic plant after 20 days in 3 different (sand, soil and soil+sand) media planting and 4 different inoculants (**K**: control, **A**: *Fusarium sp*, **B**: *Aspergillus niger*, **C**: *Fusarium sp* + *Aspergillus niger*).

Figure 4. Condition of garlic plant after 20 days planting in soil + sand media planting (**K**: control, **A**: *Fusarium sp,* **B**: *Aspergillus niger* and **C**: *Fusarium sp + Aspergillus niger*).

The results on vegetative parameters plant height, root length, wet and dry weight of the upper plants and roots are presented in Figures 5 to 10.

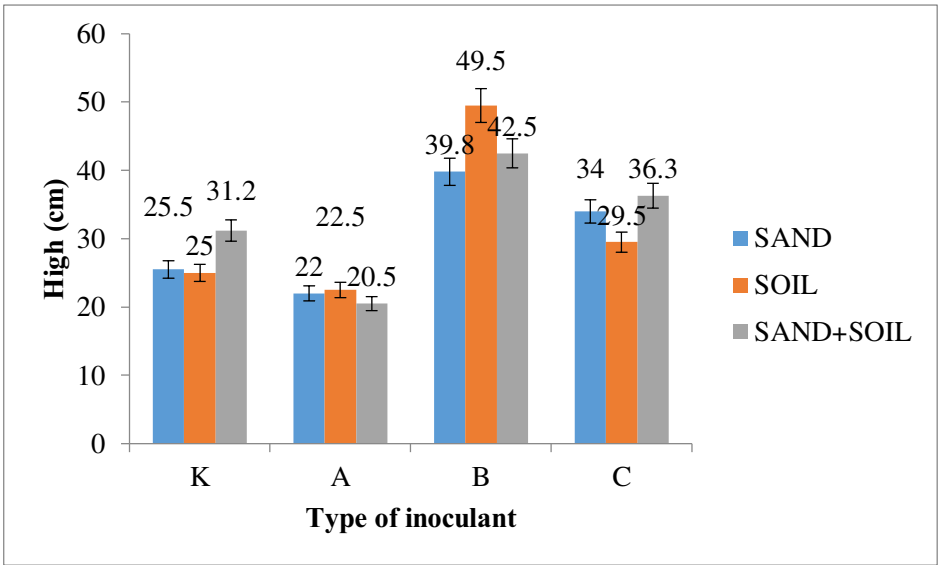

Figure 5. The effect of inoculants on the plant height of garlic after 20 days planting (**K**: control, **A**: *Fusarium sp*, **B**: *Aspergillus niger*, **C**: *Fusarium sp + Aspergillus niger*).

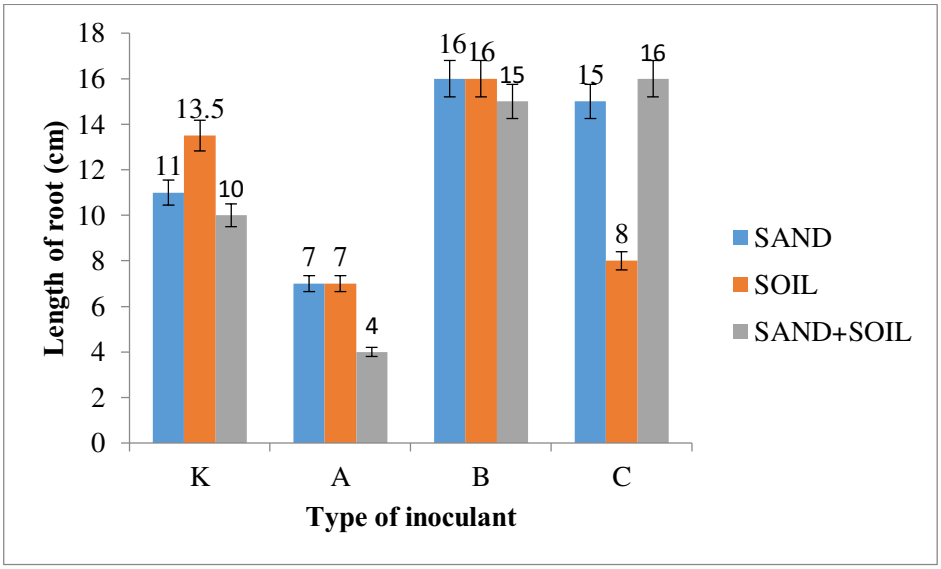

Figure 6. The effect of inoculants on the length of root garlic after 20 days planting (**K**: control, **A**: *Fusarium sp*, **B**: *Aspergillus niger*, **C**: *Fusarium sp + Aspergillus niger*).

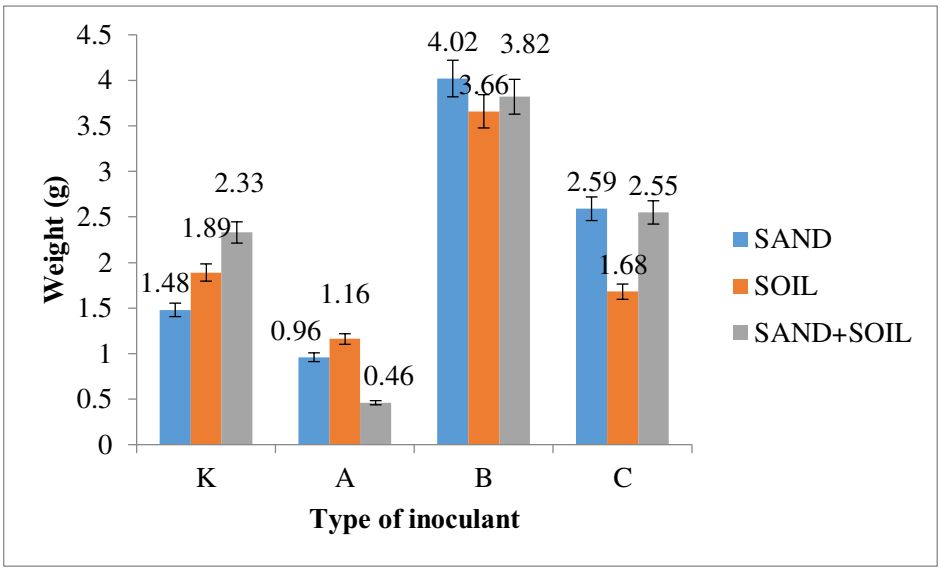

Figure 7. The effect of inoculants on the wet weight of the upper plant of garlic after 20 days planting (**K**: control, **A**: *Fusarium sp*, **B**: *Aspergillus niger*, **C**: *Fusarium sp* + *Aspergillus niger*).

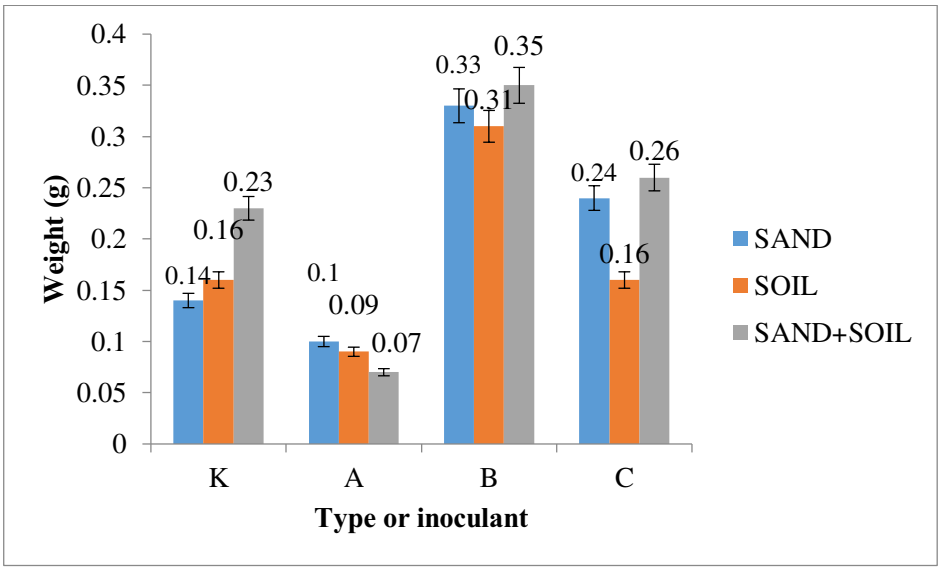

Figure 8. The effect of inoculants on the dry weight of the upper plant of garlic after 20 days planting (**K**: control, **A**: *Fusarium sp*, **B**: *Aspergillus niger*, **C**: *Fusarium sp* + *Aspergillus niger*).

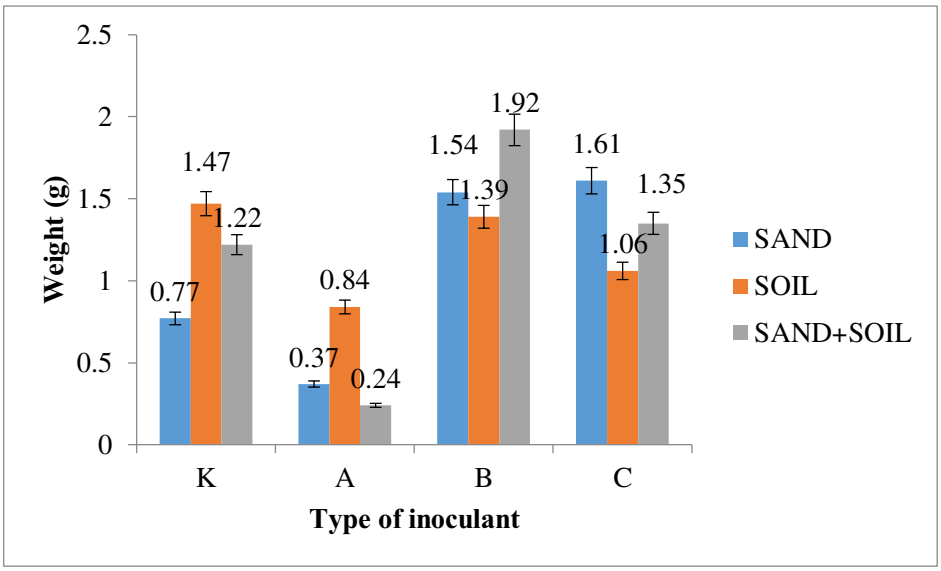

Figure 9. The effect of inoculants on the wet weight of roots garlic plants after 20 days planting (**K**: control, **A**: *Fusarium sp*, **B**: *Aspergillus niger*, **C**: *Fusarium sp + Aspergillus niger*).

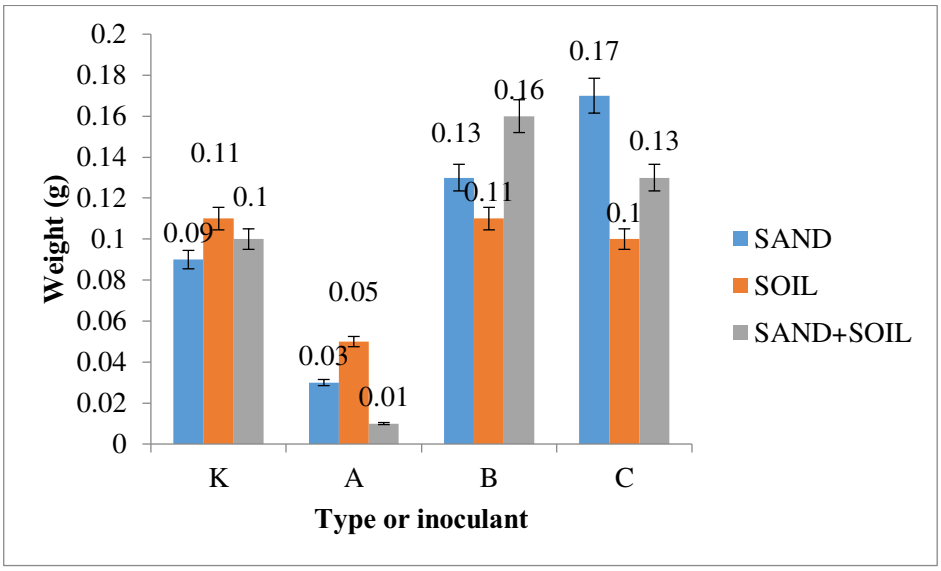

Figure 10. The effect of inoculants on the dry weight of the roots garlic plants after 20 days planting (**K**: control, **A**: *Fusarium sp*, **B**: *Aspergillus niger*, **C**: *Fusarium sp + Aspergillus niger*).

The inoculants of *Aspergillus niger*, have a positive influence on the vegetative growth of garlic on all the planting media tested. This positive influence is also shown in all planting media given *Aspergillus niger* + *Fusarium sp*. The influence of giving *Aspergillus niger* and *Fusarium sp* inoculant on a mixture of soil and sand was showing in Figure 4. The *Fusarium sp* inoculant had a significant effect on the height of garlic plants in the age of 20 days, namely growth inhibition when compared with the control and other treatments for all planting media. The addition of *Aspergillus niger* inoculants to growing media containing *Fusarium sp* had a significant impact, especially on wet weight and root dry weight in sand and sand + soil mixed media (Figure 7, 8, 9 and 10). This condition indicates that the inoculant *Aspergillus niger* can be used to increase the ability of germination and plant resistance to diseases caused by *Fusarium*. The using of *Aspergillus niger* inoculants can spur growth of germination and vegetative plants [15]. The results showed that *Aspergillus niger* and *Aspergillus niger* + *Fusarium sp* inoculants had a positif significant effect on the growth of garlic in all treatment media soil, sand and soil + sand. *Fusarium sp* inoculant inhibits growth of 30% - 60%, *Aspergillus niger* inoculant can increase growth of 40% - 90% when compared to controls on all planting media. The use of *Aspergillus niger* inoculant can overcome diseases caused by *Fusarium sp*, it can even increase the growth of 30% - 40% in sand and sand + soil media planting.

Garlic extract has compounds that can inhibit the growth of fungi, bacteria and virus [1, 4, 9, 10]. One of the causes of wilt disease in horticultural plants includes *Fusarium* group [10-13]. To overcome the *Fusarium* attack biologically, the *Trichoderma* group is commonly used [13, 19, 20]. The utilization of *Aspergillus niger* as an alternative to controlling wilt caused by fusarium in garlic plants has never been done. The results of this study indicate that Aspergillus niger has the potential to control wilt in garlic. Another advantage of using Aspergillus niger is its ability to produce growth hormones such as IAA [15]

4. Conclusion

Fusarium sp has a negative effect on the growth of garlic in all planting media, but *Aspergillus niger* gives a positive response and can reduce the negative impact on the attack of *Fusarium sp.*

5. References

[1] Irkin R and Mihriban K. 2007. Control of *Aspergillus niger* with garlic, onion and leek extracts. *Afr. J. of Biotech*. **6**(4): 384-87

[2] Kumar K.P.S., Dedjit B., Chiranjib, Pankaj T., Rakesh K. 2010. *Alium sativum* and its health benefits: An overview. *J. Chem. Pharm. Res*., **2**(1): 135-46.: 656-62

[3] Bisen P.S. and Mila E. 2016. Nutritional and therapeutic potentioal of Garlic and Oniuon (Allium sp.). *Curr. Nutri. & Food Sci*. **12**: 190-199

[4] Alzowahi F.A.M., Ahmed AT. Amani AS and Kadam T.A. 2013. The inhibitory effects of garlic extract and its fractions against some *Enterobacteriaceae* sp isolated from sprouted Mung bean. *Int. J. Curr. Microbiol. App. Sci.* **2**(7): 104-15

[5] Gebreyohannes G. And Mebrahtu G. 2013 Medicine values of garlic: A review. *Int. J. of Med. and Medic. Sci.* **5**(9): 401-408

[6] Queiroz Y.S., Ishimoto E.Y., Bastos D.H.M., Geni R.S. and Elizabeth A.F.S.T. 2009. Garlic (*Alium sativum* L.) and ready-to- eat garlic products: *In vitro* antioxidant activity. *Food Chem.* **115**: 371-74

[7] Neeraj S., Kaura S., Dilbaghi N., Parle M. And Pal. M. 2014. Garlic: A pungent Wonder from Nature. *Int. Res. J. Pharm*. **5**(7): 523-29

[8] Kementerian Pertanian, Direktorat Jenderal Hortikultura 2015. Statistik produksi hortikultura tahun 2014. Direktorat Jenderal Hortikultura, Kementerian Pertanian. h. 52

[9] Dugan F.M. 2007. Diseases and disease management in seed garlic: problems and prospects. *The Am. J. of P. Sci. and Biotec*. **1**(1): 47-51
[10] Palmero D., Miguel D.C., Walid N., Laura G., Alejandra C., Stephen W., Maria T.GJ. and Julio C.T., 2012. *Fusarium proliferatum* isolated from garlic in Spain: Identification, toxigenic potentioal and pathogenicity on related *Allium* species. *Phytop. Mediter.* **51**(1): 207-18
[11] Amini J. And Dzhalilov F.S. 2010. The effects of fungicides on *Fusarium oxysporum f.sp.lycopersici* associated with Fusarium wilt on tomato. *Journal of Plant Protection Research* **50**(2): 272-78
[12] Chand S.K., Satyabrata N. and Rai K.J. 2016. Regulation of miR394 in response to *Fusarium oxysporum f. Sp. Cepae* (FOC) infection in Garlic (*Allium sativum L.*). *Frontiers in Plant Science 7*: Article 258
[13] Yeole G.J., Kotkar H.M., Teli N.P and Mendki P.S. 2016. Herbal fungicide to control *Fusarium* wilt in Tomato plants. *Biopestic. Int.* **12**(1): 25-35
[14] Sugiharto A. 2012. Isolasi dan seleksi kapang holotoleran serta aplikasinya pada tanaman padi (*Oryza sativa* L.) varietas ciherang. Thesis. pp 74
[15] Rahmansyah M., Arwan S. and Titi J. 2017. Pengaruh inokulan *Aspergillus niger* terhadap pertumbuhan kecambah sorgum tercekam kekeringan dan pertumbuhannya di lapangan. *Pros. Sem. Nas. Masy. Biodiv. Ind.* **3**(3): 426-32
[16] Ludwig F., Dirceu M.F., Poliana RD.M and Roberto L.V.B. 2013. Electrical conductivity and pH of the substrate solution in gerbera cultivars under fertigation. *Hort. Bras.* **31**: 356-60
[17] Pandey C.B., Praveen K and Chaudhari S.K. 2017. Root exudates reduce electrical conductivity and water potential of rhizospheres and facilitate non-halophytes to survive in dry land saline soil. *Trop. Eco.* **58**(4): 705-16
[18] Samarakoon U.C. Weerasinghe P.A. and Weerakkhody W.A.P. 2006. Effect of electrical conductivity (EC) of the nutrient solution on nutrient uptake growth and yield of leaf Lettuce (Lactuca sativa L.) in stationary culture. *Trop. Agr. Res.* **18**: 13-21
[19] Sundaramoorthy S. and Balabaskar P. 2013. Biocontrol efficacy of *Trichoderma spp*. Against wilt of tomato coused by *Fusarium oxysporum f. Sp. lycopersici*. *Journal of Applied Biology & Biotechnology.* **1**(3): 36-40
[20] Raihan A., Momena K., Mohsina ME.F., Ataur R., Jamal U. And Shamin M.N. 2016. *Trichoderma* suppresses pathogenic *Fusarium* causing tomato wilt in Bangladesh. BMRJ. **14**(4): 1-9