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RESEARCH ARTICLE

THE EFFECT OF INDIGENOUS ARBUSCULAR MYCORRHIZAL FUNGI AND CELLULOLYTIC FUNGI TO THE GROWTH AND PRODUCTION OF MAIZE (ZEA MAYS L.) UNDER DROUGHT STRESS IN INCEPTISOL OF ACEH.

Nasrullah[1], Sufardi[2], Ashabul Anhar[3] and Fikrinda[2].

1. Department of Magister Agroecotechnology, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh Indonesia.
2. Department of Soil Science, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh Indonesia.
3. Department of Agrotechnology, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh Indonesia.

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Abstract

The function of indigenous arbuscular mycorrhizal fungi (AMF) has been used to improve soil marginal quality in increasing agriculture yield, meanwhile the combination from indigenous cellulolytic fungi was not investigated yet especially in Inceptisol of Aceh with the limitation of soil water. This research was aimed to know the effect of AMF and indigenous cellulolytic fungi to the growth and production of maize in drought stress in Inceptisol. The research was conducted by using polybag experiment at Inceptisol media from University Farm Station 2 Ie Seuum Krueng Raya (Aceh Besar). The research was used randomized complete block design with nine treatments combination of two factors with three replications. The first factor examined was indigenous AMF inoculation that consists of without AMF, *Gigaspora gigantea* inoculation and *Gigaspora gigantea+Acaulospora tuberculata* combination inoculation, and the second factor was indigenous cellulolytic fungi dose, it was 0, 10, and 20 ml per polybag. The result of the study show that the inoculation of AMF indigenous *Gigaspora gigantea* and the combination of *Gigaspora gigantea+Acaulospora tuberculata* give significant effect, compare with control in increasing dry root weight, root volume, and root colonization, that could influence the enhancement of cob without cornhusk, seed weight and 100 seed weight each plant in Inceptisol with drought stress condition (50% field capacity). The inoculation of AMF 10 g per polybag and combining with cellulolytic fungi 10-20 ml per polybag could increase maize production in drought stress condition from 22,3 g become 49,6 g per plant.

Introduction:-

Maize was one of the important and popular crops as food and animal feed. However, the availability yield of maize was not enough yet in Indonesia, which proved from the data that import has been started from 2013, 2014, and 2015 each one was 3.194.419 ton, 3.175.362 ton and 3.500.104 ton (Badan Pusat Statistik, 2017). The

Corresponding Author:-Nasrullah.
Address:-Department of Magister Agroecotechnology, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh Indonesia.

extensification activity like extend the field area from maize was going especially outside from Java Island (Kementerian Pertanian, 2016). One of the available marginal field was Inceptisol field. The total area of Inceptisol outside from Java was around 52 million ha (Hidayat and Mulyani, 2005). Aceh has Inceptisol around 3,16 million ha (Subagyo *et al*., 2000).

Inceptisol in Aceh was not utilized optimal because the limitation of soil water in dry season, and low precipitation beside low soil fertility status (Muyassir *et al*., 2012). The availability of water is really important to support plant physiology activity. The drought stress condition was impact to plant growth, biomass production, decreasing of cel expension, and decreasing of photosynthesis production (Taiz and Zeiger, 2002). In other words, drough stress was the important abiotic factor which correlated with low of soil water availability and effect to the hampered plant growth (Liu *et al*., 2012).

One of the strategy to solving the availability of soil water in Inceptisol was inoculation of arbuscular mycorrhizal fungi (AMF). AMF was fungi which live in soil and able to do symbiosis mutualism with host plant. Mycorrhizal was not only increasing nutrition transfer rate, but also increasing the endurance in biotic and abiotic stress condition (Smith and Read, 2008). AMF was able to improving soil aggregate (Leifheit *et al*., 2013) and increasing soil organic (Daynes *et al*., 2013). Each AMF has the different function in increasing plant growth (Tian *et al*., 2004). So, the selection of AMF isolates and matched with plant was needed.

The other strategy in increasing maize growth and yield production in Inceptisol was the application of cellulolytic microorganism especially from the fungi group. Cellulolytic microorganism was able to degradation cellulose become the simpler compound. Cellulolytic fungi is microorganism produce cellulase that degrade cellulose by hydrolysing the b-1, 4-glycosidic linkages of cellulose (Behera *et al.*, 2017). AMF colonization and rhizosfer microorganisms in soybean root was synergize to increasing plant growth and yield production (Bertham *et al*., 2005). The combination inoculation of 30 AMF spore and *Pseudomonas fluorescens* 10^9 cfu ml^{-1} was able giving the optimal result to P uptake and maize growth in Andisol (Musafa *et al*., 2015). The other research was shown that AMF and *Trichoderma* spp. was able to increasing plant growth and resistance to biotic and abiotic stress in some plants (Buysens *et al*., 2016). This research was aimed to know the effect of indegenous AMF and cellulolytic fungi dose inoculation to maize growth and yield production in Inceptisol of Aceh with drought stress condition.

Material and Method:-

The research was conducted in Field Experimental Faculty of Agriculture, Universitas Syiah Kuala, soil analysis was done in Soil and Plant Research Laboratory BPTP Aceh and root infection by AMF analysis was done in Soil Biology Laboratory of Faculty of Agriculture, Universitas Syiah Kuala. The top soil layer Inceptisol (0-20 cm) was taken from University Farm Station 2 Ie Suum, Mesjid Raya sub-district, Aceh Besar district (Indonesia). The maize seed variety was Bisi 2 with production 13 ton ha^{-1} dry shelled. Indigenous AMF *Gigaspora gigantea* and *Acaulospora tuberculata* was selected from maize root in previous research (Fikrinda *et al*., 2016; Rahmiyana, 2017), and indigenous cellulolytic fungi (A.32) was from Fikrinda's collection. Compost used in the research was from Soil Department Faculty of Agriculture Universitas Syiah Kuala with organic C 12,73% (high), N-total 0,56% (high), ratio C/N 22,73 (high), P_2O_5 total 0,12% (high), K_2O total 1,08% (high), pH 7,5 (base). Beside that, Urea was used as basic fertilizer with 45% N, 36% P_2O_5, and 60% K_2O.

The research was used randomized complete block design with 2 factors. First factor was AMF variety, consist of 3 rates; without AMF (0 g per polybag); 10 g per polybag *Gigaspora gigantea* and 10 g per polybag *Gigaspora gigantea+Acaulospora tuberculata*, and second factor was combined with 3 rates dose cellulolytic fungi factor, it was 0, 10, and 20 ml per polybag, so it was 9 combination treatments (Table 1.).

Table 1:-The treatment combination of the inoculation of AMF (*Gigaspora gigantea* and *Gigaspora gigantea+Acaulospora tuberculata*) and the dose of cellulolytic fungi

Combination of treatments	
M1 =	Without AMF and without cellulolytic fungi
M2 =	Without AMF + 10 ml per polybag cellulolytic fungi
M3 =	Without AMF + 20 ml per polybag cellulolytic fungi
M4 =	*Gigaspora gigantea* (10 g per polybag) and without cellulolytic fungi
M5 =	*Gigaspora gigantea* (10 g per polybag) + 10 ml per polybag cellulolytic fungi

M6 =	*Gigaspora gigantea* (10 g per polybag) + 20 ml per polybag cellulolytic fungi
M7 =	*Gigaspora gigantea+Acaulospora tuberculata* (10 g per polybag) and without cellulolytic fungi
M8 =	*Gigaspora gigantea+Acaulospora tuberculata* (10 g per polybag) + 10 ml per polybag cellulolytic fungi
M9 =	*Gigaspora gigantea+Acaulospora tuberculata* (10 g per polybag) + 20 ml per polybag cellulolytic fungi

Inceptisol from the top layer (0-20 cm) was cleaned from grass and air dried for a week. Compost with the dose 10 t ha^{-1} (5 g kg^{-1} soil) was given 1 week before the seed planted with mixed the soil until homogen and filled to polybag with 15 kg soil first. AMF was given in spore form which contain *Gigaspora gigantea* and *Acaulospora tuberculata* with dose 10 g per polybag. AMF was placed in plant hole and mixed with maize seed, while cellulolytic fungi inoculan was applied 0, 10, and 20 ml each plant hole based on the treatments. Basic fertilizer like Urea, TSP, and KCl were given with dose 300 kg ha^{-1},100 kg ha^{-1} and 100 kg ha^{-1} each fertilizer and applied in the begining of planting, except for Urea fertilizer which applied 2 steps, it was given 50% in the beginning of planting and other 50% given when 15 days after planting (DAP).

The addition of water in planting media was done by scaling the polybag every day to considering water needed and added up to 50% field capacity. The metodology for the application of field capacity or water stress is decribe by Tobar *et al.* (1994) and Meddich *et al*. (2000) *in* Meddich *et al.* (2015). Polybag was scaled first and soil sample around 13 kg was filled up to the polybag. The value get was P1. And then, soil was watered until saturated and left alone around 48 hours. After 48 hours the soil was scaled again to get the P2 value. The result 0,50 x (P2-P1) was water needed for 50% field capacity. The status water treatment was done 4 weeks after planting season until harvest. The handling activity was watering and managing the pest. Watering was done in the afternoon based on the treatments (keep on 50% field capacity).

Plant parameters were P uptake (wet destruction method), root dry weight and root volume, percentage of root colonization by FMA (using the method of Brundrett *et al*., 1996), cob diameter, cob weight without cornhusk, seed weight per plant and 100 seed weight. The data from the research was analysed used F test at 5%, and if there is significant effect, the test was continued use LSD test at 5%.

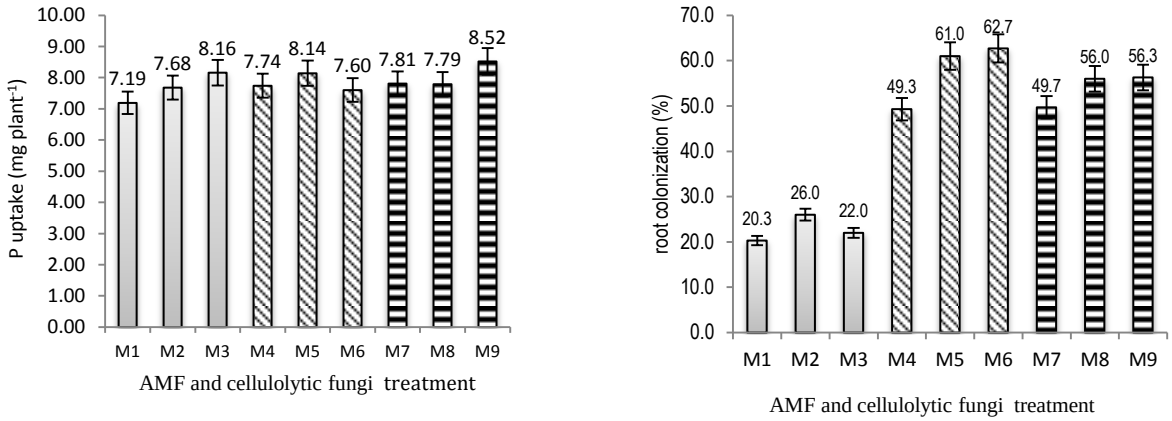
Results and Discussion:-

Soil characteristic

The first soil chemistry analysis were shown that soil pH 6.03 was rather acid (Electrometric method), organic C (Walkley and Black method) was low (1.20%), total N (Kjeldahl method) was low criteria (0.14%), C/N ratio was low criteria (8.57), P available (Bray I method) was medium (16.66 mg kg^{-1}), exchange K (*1N* NH_4OAc pH7 method) was very high (2.06 cmol kg^{-1}), and CEC (*1N* NH_4OAc pH7 method) was low criteria (7.00 cmol kg^{-1}). The result was indicated that Inceptisol in University Farm Station 2 Ie Suum, Mesjid Raya sub-district, Aceh Besar district (Indonesia) was low fertility criteria and having some soil chemistry problems like medium acid, low organic matter, and low N nutrient. Beside that, water availability was low and become the problem in Inceptisol of Aceh.

P Uptake and Root Colonization

The result of research was shown that the combination inoculation AMF and cellulolytic fungi was not effect significantly to P uptake maize. The Picture 1 was shown that the lowest mean of P uptake maize was control treatment (M1), and the highest P uptake was *Gigaspora gigantea+Acaulospora tuberculata* and cellulolytic 20 ml per polybag treatment (M9), eventhough it was not effect significant statistically like in the Picture 1. It was correlated with Karnilawati (2013) research that giving mycorrhizal was not effect to P uptake maize in Andisol. Meanwhile, there is an increasing P uptake maize in treatment combinations AMF and cellulolytic fungi compare with control. The increasing of P uptake was caused by AMF activity in increasing nutrients availability and root development (Smith *et al*., 2010). P uptake maize was correlated with P soil available. Morgan *et al.* (2005) was explained that mycorrhizal would support plant growth in low nutrient P and N condition, and symbiosis between plant with mycorrhizal would advantegous to plant growth in acid soil and low nutrients. But, species from *Gigaspora* genus was able to solving high nutrients environment (Liu *et al*., 2012).



Picture 1:-P uptake and root colonization maize in various treatment combination AMF variety and cellulolytic fungi dose

The research was shown that soil P available (P_2O_5) before the treatment was 16,66 mg kg^{-1} and it was medium criteria. The soil P available indicated that it was enough to necessity of maize in vegatative phase, so AMF role in increasing P soil available was low and also P uptake maize low. The result was correlated with many results from other researchers, which said that AMF was increasing plant growth and nutrient uptake in soil which has low nutrient (Marschner, 2012). Andrade *et al*. (2015) was reported that AMF was not effected to P plant contain in soil with medium fertility status.

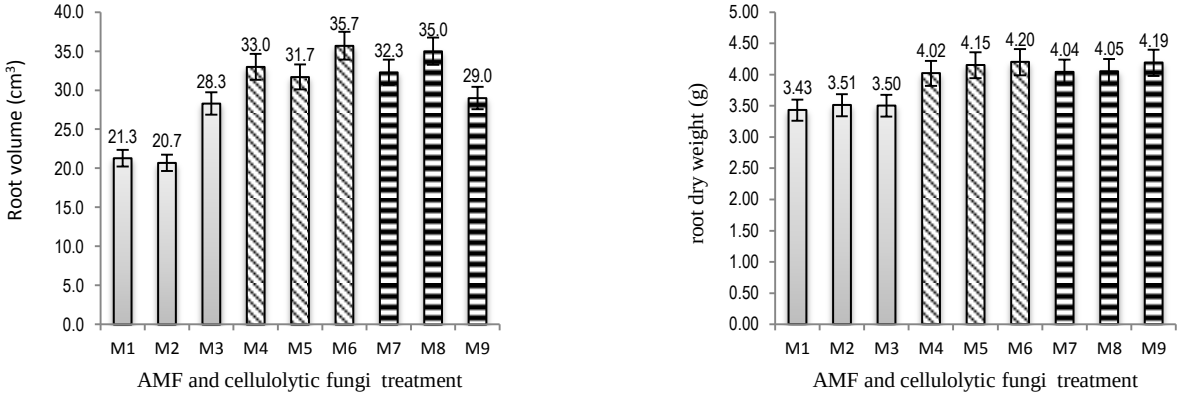
Picture 1 was shown that the combination of AMF and cellulolytic fungi was significantly effect to maize root colonization in drought stress condition. The result showed that the root colonization by AMF was medium criteria, based on criteria by Rajapakse and Miller (1992). It was caused the high soil potassium and soil phosphate in medium criteria (Table 2.). Smith and Read (2008) said that the high soil potassium content will decressing colonization degree. When the soil P was high in intensif planting system, it always harm the activity and AMF function (Plenchette *et al*., 2005). Picture 1 was shown that the lowest mycorrhizal infection degree in maize root in control treatment (M1), and the highest was M6 with *Gigaspora gigantea* and 20 ml per polybag treatment. It shown that AMF inoculation was able to increasing root colonization by AMF in the low water condition. The difference root colonization between AMF was indicated by the matching level AMF to host plant. Every AMF strain has different ability in increasing plant growth (Tian *et al*., 2004). Silva *et al.* (2014) said that AMF colonization in rhyzospher zone *Eucalyptus* was dominated by AMF variety *Gigaspora margarita* it was 40%. The level of AMF activity was depend on enviroment factor, compability host plant, and mycorrhizal variety (Chapman *et al*., 2011). The availability AMF colonization was determine the symbiosis corelation AMF and the host plant. Root infection degree by mycorrhizal become one of the indicator to knowing the matching between AMF and a plant species. Nasution *et al*. (2014) said that if the infection degree was high it would indicate the mycorrhizal activity was active and expanding root absorption area to water and nutrients. The treatment without AMF was shown there is root colonization by AMF. The root colonization was indicated caused by indigenous AMF from the soil.

Variable Growth of Maize

The inoculation of mycorrhizal combined with cellulolytic fungi dose was given the significant effect to root volume, root dry weight, and root infection. The Picture 2. was shown that the lowest root volume in without AMF and cellulolytic fungi 10 ml per polybag treatment (M2). The highest root volume was *Gigaspora gigantea* and 20 ml per polybag treatment (M6). It was indicated that AMF application was able to increasing maize root volume in Inceptisol with water limitation (50% field capacity). This fact was shown that AMF and cellulolytic fungi to root volume was depended on AMF variety. Both of two AMF treatments that tried was able to increasing plant ablity to absorb water through the increasing effectivity from root hydrolic conductivity (Abdel-Salam *et al*., 2018). Yusrizal (2017) research was found that there is the different soybean root volume caused by AMF *Gigaspora* and combination *Glomus+Gigaspora* treatment with control. While, the cellulolytic fungi given was not consistant to maize root volume in Inceptisol with soil water availability 50% field capacity.

The AMF inoculation was effecting to maize root absorption. Jannah (2011), AMF inoculation in plant would giving the positive respond, where it would created external AMF hyphae so it would expanding water absorption area and

nutrients in soil. AMF could increased plant root availability through expanding the mycelium or hyphae which having the important role based ecology in plant nutrient uptake (Cavagnaro *et al*., 2006). It was one of the caused maize that innoculated AMF was able growth and production in drought stress plant media condition (50% field capacity). Kilham (2014) said that the hyphae size more smooth than root hair was possible the hyphae penetrate to soil micro pores, so the hyphae can absorb water when soil water was very low. Sastrahidayat (2011) mentioned that AMF was having high auxin which support root growth. AMF interaction with maize was spreading root absorption zone through the external hyphae so it was able to serve nutrients and water for plant. Labidi *et al*. (2015) said that the elongation of external hyphae was caused emerge the root tertiary and increasing nutrient uptake was high, so the plant metabolysm process was not hampher in drought stress condition.



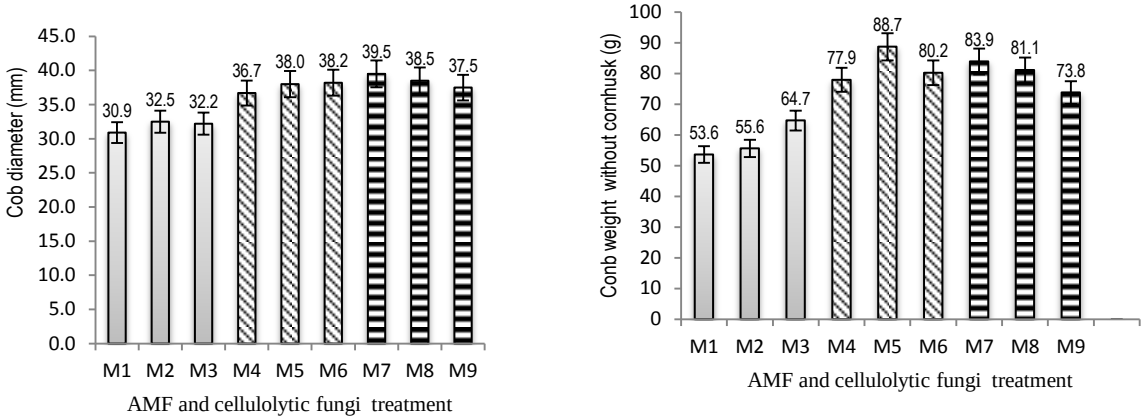
Picture 2:-Root volume and root dry weight maize in various treatment combination AMF variety and cellulolytic fungi dose

In the Picture 2 was shown that the lowest dry root weight found in without AMF combination and without cellulolytic fungi treatment (M1). While, the highest dry root weight was found in *Gigaspora gigantea* and 20 ml per polybag treatment (M6). AMF application 10 g per polybag and combined with cellulolytic fungi was effected to dry root weight *Gigaspora gigantea* and *Gigaspora gigantea+Acaulospora tuberculata* combinations. AMF was able to serve host plant mineral nutrients and water as the material to change with photosynthesis product (Smith and Read, 2008) especially through the external hyphae. Nurhayati (2012) was explained that the main function hyphae was water absorption from soil and produced phosphate enzyme and would change accumulate P in external hyphae become polyphosphate compound.

Result and Production of Maize

The Picture 3 and 4 was shown that the combination AMF variety and cellulolytic fungi given various effect to maize production parameter in drought stress condition. The lowest cob diameter was without AMF and without cellulolytic fungi treatment (M1). While, the combination treatment cellulolytic fungi 10-20 ml per polybag and without AMF was able increased cob diameter, eventhough it was not effect statistically. While, the AMF combination and cellulolytic fungi (M4-M9) was able increasing cob diameter compare control. The biggest cob diameter was found in combination treatment *Gigaspora gigantea+Acaulaspora tuberculata* and without cellulolytic fungi (M7) was significantly effect to without AMF and cellulolytic fungi treatment (M1-M3). It was informed that AMF inoculation was able increasing cob diameter in drought stress condition. The symbiosis of AMF with root and able increasing N, P, and K nutrient, increasing soil water using, increasing osmotyc pressure of plant cell in soil with low water containt so the plant will maximize CO_2 utilization and energy from sun to increasing vegetative rate and production (Thangadurai and Mohammed, 2010).

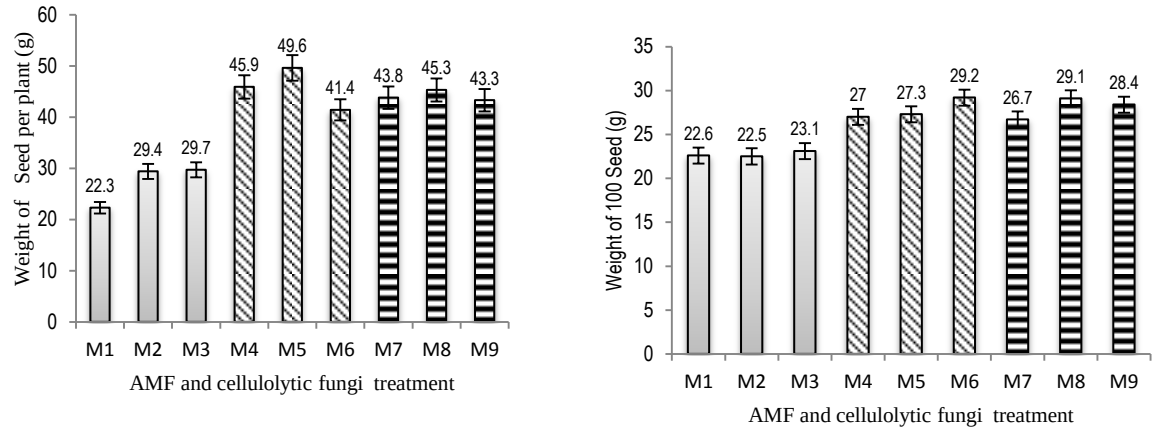
And then, if maize was inoculated with AMF *Gigaspora gigantea* variety and *Gigaspora gigantea+Acaulospora tuberculata* (M5-M9) can increasing cob diameter bigger than control treatment. It shown that AMF inoculation was good combination with cellulolytic fungi or not (M4 and M7) was able plant growth in drought condition so increasing maize production. If compare between 2 kinds of AMF which tried, there is a different in giving the treatment to maize production eventhough a little. The different response was indicated because the different function and mycorrhizal function to host plant (Bakony *et al*., 2018). Ningrum *et al.* (2013) said that the AMF application and combination of bokashi and AMF was giving the bigger value of cob diameter than control without AMF.



Picture 3:-Cob diameter and cob weight without cornhusk in various treatment combination AMF variety and cellulolytic fungi dose

The Picture 3. was shown that the lowest of cob weight without corn husk was M1 treatment, it was without AMF and without cellulolytic fungi. While, the cob weight without cornhusk shown in M5 treatment, it was AMF *Gigaspora gigantea* and cellulolytic fungi 20 ml per polybag treatment, but the result statistically was not different with others treatment result, except treatment which is not inoculated AMF (M1-M3).

According to Picture 4 could seen that the lowest seed weight per plant was control treatment (M1) and the highest in M5 treatment it was *Gigaspora gigantea* and cellulolytic fungi 10 ml per polybag. The lowest weight of 100 seed was M2 (combination without AMF and cellulolytic fungi 10 ml per polybag), while the highest in M6 it was AMF *Gigaspora gigantea* and cellulolytic fungi 20 ml per polybag. While, the result was not different statistically with the result from others treatments except treatment without AMF with variety doses cellulolytic fungi (M1-M3). Based on the data, it could that AMF inoculation was able to increasing yield production and maize production in marginal soil. The weight of 100 seed was described the size accumulation result of photosynthesis to the seed where to produced photosynthate it was needed P compound with high energy in ATP form (Hodiyah, 2007). The result from Sufardi *et al*. (2018) shown that AMF *Gigaspora sp* inoculation 10 g per polybag and combine with 100 g cow manure per polybag (20 t ha^{-1}) soybean yield can increased up to 1,70 t ha^{-1}. Moelyohadi *et al*. (2012) said that maize was given compost mycorrhizal was given the best effect to growth and yield of maize in marginal dry land with harvest yield around 8,57 t dry shelled ha^{-1}.



Picture 4:-Seed weight per plant and 100 seed weight various treatment combination AMF variety and cellulolytic fungi dose

Picture 4 shown that there is the difference between treatment which inoculated AMF and non AMF in cellulolytic fungi doses various. The combination treatment without AMF and cellulolytic fungi dose (M1-M3) was seen as treatment with the lowest yield, while the treatment combination inoculation AMF *Gigaspora gigantea* or mixing

Gigaspora gigantea+Acaulaspora tuberculata could increasing maize yield in drough condition (50% field capacity). It was correlated with root ability in absorb the water and soil nutrients where the AMF application started shown the effect in the generative phase. It was correlated with Aguilar and Barea (2015) said that the water absorption and nutrients by root will increase with AMF and fungi getting the organic compound from host plant. The research of Singh *et al*. (2012) was reported that when plant was water defficient in generative phase, the plant energy from photosynthesis result will more use to create pod caused by maristem cel was not active splitting, so it could decreasing plant dry weight. The result from Abdel-Salam *et al*. (2018) shown that the AMF colonization can protecting rose from tissue distruction caused by drought stress. Sastrahidayat *et al*. (2001) was reported that AMF inoculation *Gigaspora margareta* was able giving the true contribution in increasing cob weight and dry shelled weight. According to the data maize in the marginal field with drought stress condition it was 22,26 g become 49,60 g plant^{-1}.

Meanwhile the AMF combination and cellulolytic fungi (M4-M9) was able to increasing cob dry weight without cornhusk and seed dry weight per plant, if compare with combination treatment without AMF and cellulolytic fungi (M1-M3). Cellulolytic fungi inoculation was not able consistantly increasing yield of maize in this research. At the end, the result from this research shown that the indigenous AMF colonization can protect maize from drought stress and able increasing the yield compare with control in Inceptisol Aceh.

Conclusion:-

The AMF inoculation indigenous *Gigaspora gigantea* was giving the best effect compared with combination of *Gigaspora gigantea* and *Acaulospora tuberculata* in increasing root dry weight, root volume, and percentage of root colonization, followed by the increasing of cob without cornhusk, seed weight per plant and 100 seed weight in Inceptisol with drought stress condition (50% field capacity). The AMF inoculation 10 g per polybag was combined with 10-20 ml cellulolytic fungi per polybag in drought stress condition was able increased yield maize from 22,3 g become 49,06 g per plant.

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