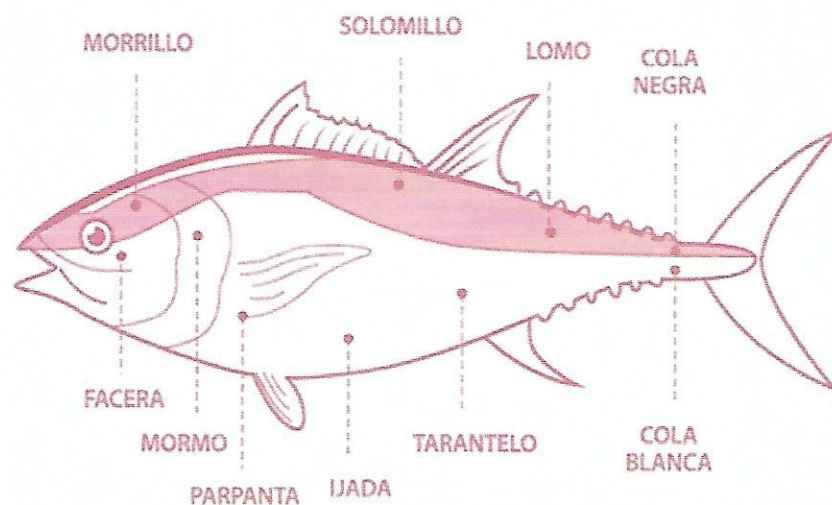


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Eka Susila, Aswaldi Anwar, Auzar Svarif and Agustian
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RESEARCH ARTICLE

SELECTION OF SIX TYPES OF ISOLATES OF INDIGENOUS ARBUSCULAR MYCORRHIZAL FUNGI FOR GROWTH, YIELD AND ESSENTIAL OIL CONTENT OF SHALLOTS (*Allium ascalonicum* L).

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Abstract

To test the level of compatibility, it is necessary to test the effectiveness of the type of Arbuscular Mycorrhizal Fungi (AMF) with shallots on the greenhouse scale, which can be seen from the growth and the results as well as answering the question of the compatibility level of isolate / type AMF to improve the quality of shallots based on essential oil content produced. The objectives of the study were (1) to find compatible indigenous AMF type for shallots on growth and yield, (2) to see the effect of indigenous AMF on shallots bulbs quality from the essential oil content. The potted study was prepared using Completely Randomized Design, with one factor of mycorrhizal (M) with 6 type (*Scutelospora sp1*, *Glomus sp1*, *Glomus sp2*, *Glomus sp3*, *Gigaspora sp1*, *Glomus sp4*) and 1 treatment without inoculation of AMF. The similarity of variance between treatments was tested with Duncan's Multiple Range Test (DMRT) at a 5% significance level. The results of the study showed that (1) All types of AMF tested were able to increase colonization, plant height and shallots yield, but were not significantly different to the number of tillers and number of leaves, (2) Inoculation of all types of AMF can increase the essential oil content of shallots bulbs. Conclusion: There were differences in the effectiveness between the six isolates of AMF on root colonization, plant height, yield and essential oil content of shallots. *Glomus sp1*, *Glomus sp2* and *Glomus sp3* were potential isolates to be applied to shallots cultivation.

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Introduction:-

Shallots (*Allium ascalonicum* L) are one type of commodity that have significance for the community, both seen from their economic value and nutritional content. A distinctive smell is one of the markers that the shallots plant produces essential oils. According to Lancaster and Boland [1] shallots can stimulate the release of tears if the shallots is slashed on the skin and chemical compounds that emit a distinctive smell. Chemical substances that can

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stimulate tears are called lacrimators, whereas the peculiar smell of shallots is caused by volatile components (essential oils). Essential oils are produced by flavor biochemical processes, in which flavors have precursors or basic ingredients that react with specific enzymes of shallots which then produce various types of chemicals such as lacrimators, essential oils, pyruvic acids, and ammonia efficacious for various diseases [1]. The active ingredients of essential oils consist of, methylalliin, cycloalkin, kaemferol, quercetin, and floriglusin.

Shallots are cultivated on dry land in the dry season. Ironically, shallots have a short root that cannot stand the drought conditions. The Directorate General of Horticultural Production Development [2] states that shallots crops are not drought-resistant because of their short roots. Currently the production center of shallots in West Sumatra is in the highlands with irrigation that relies on rainfall and watering by humans, unlike in Java (Brebes and Cirebon) which uses a system of *leb* (water inundation) on shallots cultivation that cause maximum production. The development of shallots in the lowlands became one of the alternatives to increase production from West Sumatra. The problem is that the dry lowlands of West Sumatra tend to have an acid reaction or a low pH which is caused by the wet climate [3].

In acid soils, the availability of Phosphor (P) element is low because it is bound by Aluminium (Al) and Ferro (Fe) elements. Phosphor on the plays a role in improving shallots root development, so it can simplify and accelerate the absorption of nutrients. Phospore also plays a role in improving the quality and yield of plants by reducing weight loss of shallots bulbs. For plants grown in dry and acidic soils, the presence of ArbuscularMycorrhizal Fungi (AMF) is advantageous since in addition to increasing the availability of P element, it can improve the ability of plants to grow and survive in limited water conditions. The presence of AMF can improve and increase the water absorption capacity of host plants. If the needs of plants in the form of nutrients and water are met, metabolism increases and primary and secondary products will also increase. Further research is needed to see the effectiveness of AMF in improving the quality of tubers through producing essential oils produced.

Inoculation of AMF originating from the plant itself (*indigenous*) is believed to be more able to adapt to the plant. Susilaet al[4], reported 6 isolates of AMF derived from the shallots rhizosphere abundant presence (Isolation Frequency>40% and Relative Abundant > 3% [5]) See Figure 1. The compatibility between AMF and host plant is one of the factors in getting maximum benefit from AMF activity[6]. AMF effectiveness testing is often seen from plant growth and yield. Answering the question of the effect of AMF presence and the level of compatibility of AMF type on improving bulbs quality through the essential oil content has not been reported. The objectives of this study were 1) looking for indigenous AMF type that were compatible on shallots plant for AMF colonization, growth and yields, and 2) etamining the effect of indigenous AMF type on the essential oil content produced.

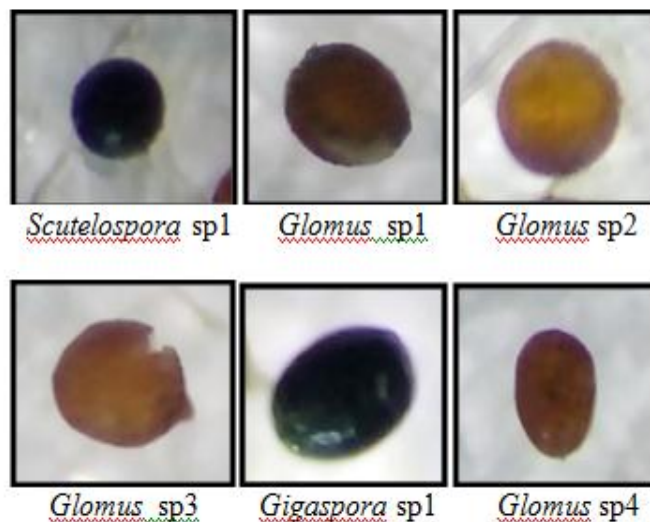


Figure 1:-AMF spores derived from shallots rhizosphere with abundant presence (magnification 100 x)

Material and Methods:-

Origin of isolate AMF indigenous (Exploration of AMF):-

Sample had previously been collected from 3(three) locations of centers of shallot cultivation in West Sumatra : Alahan Panjang [4], in the district of Solok ($100^{\circ}46'58.7''$ E- $1^{\circ}04'23.5''$ S), Saniang Baka [4] in the district of Solok ($100^{\circ}31'32.2''$ E- $0^{\circ}42'55.7''$ S) and Kambang [4], in the district of Pesisir Selatan ($100^{\circ}42'00.0''$ - $1^{\circ}42'00.0''$ S). See Figure 2.

The spores of the soil samples were isolated using wet sieving methods [5]. A soil sample of 20 g with 3 replications added 150 mL of water and was stirred for 2 minutes. The suspension was then allowed to stand for 10 s, and the soil fraction was filtered using a 60, 125, 300 and 500 μ m sized sieve. Spores trapped in each filter were picked and filtered on filter paper by water spraying. They were then transferred by spraying sterile water to a petridish for spore type and morphology based on publication by Invam [7] and Brundrett [8].



Figure 2:-Map of sampling location (source: Google map, 2017). (a) Saniang Baka, in the district of Solok (b) Alahan Panjang in the district of Solok (c) Kambang, in the district of Pesisir Selatan

Trapping and Breeding of Single Spore AMF:-

The morphologically identified genus AMF was selected based on the presence of [5], bred to be propagated inoculants. Spores of AMF were taken with a pasteur pipette. Each target species in the spore suspension on a petridish in pipette and collected on a watch glass. A total of 30 spores in a suspension of + 1.0 mL were taken with a pipette, transferred to a tube of micro tube (1.5 mL). Then stored for 2-3 days in the freezer before use.

Breeding of AMF species using 200 mL volume pot culture on sterile sand medium using corn plant as a host. The media use fine river sand (0.5 to 1.0 mm) amount of 200 g, saturated with 50 mm sodium citrate solution (pH 8.0) and autoclave at 121°C for 1 hour to sterilization. Pots were filled with sterile sand medium prior to inoculation and planting. Planting hole made in the middle of the media using sterile glass rod as deep as 6-7 cm and filled with sterile cotton at the bottom of the hole.

The corn seeds were rinsed with 10% sodium hypochlorite (NaOCl) for 10 minutes and rinsed with sterile water, then added to the damp cloth for 5 days. One maize seed sprout was inoculated with a spore suspension using a pipette. The roots of the sprouts (radicles) were arranged so that the root tips are accumulated on the sterile cotton, and the spore suspension was placed along the roots with the pipette carefully. In principle, the spores stick to the roots and accumulate at the root tips on sterile cotton. Sprouts of corn seeds that have been planted were covered

with sterile sand media, so the shoots (plumula) appear on the surface of the media. Then micro tubes were rinsed with sprayed sterile water for watering planting media.

Plants are maintained for 6 weeks by sterile water spraying and nutrient solution. The nutrient solution was given after 2 weeks of age using 100 ml of Hyponex nutrient solution once a week until the age of 6 weeks. At the end of the culture period, 6 weeks observed root colonization with staining method [9], and spore breeding with microscope observation (spore density on the media by Dandan and Zhiwei[5].

Experiment Design and Statistical analysis:-

The study was conducted in the greenhouse of Universitas Andalas Faculty of Agriculture. The test was conducted in pot of diameter 15 cm filled with a media mixture of zeolite and sand (1: 1) as much as 4.0 kg which have been sterilized. The experiment used a Completely Randomized Design (CRD) consisting of one factor of selected AMF isolates based on the presence of spores (Isolation frequency > 40% and Relative abundance >3%) and one control (no inoculation of AMF).

1. Control (no inoculation of AMF)
2. Isolate single spore indigenous 1 = *Scutelospora sp1*
3. Isolate single spore indigenous 2 = *Glomus sp1*
4. Isolate single spore indigenous 3 = *Glomus sp2*
5. Isolate single spore indigenous 4 = *Glomus sp3*
6. Isolate single spore indigenous 5 = *Gigaspora sp1*
7. Isolate single spore indigenous 6 = *Glomus sp4*

All treatments in this experiment were repeated 3 times, in which one treatment unit per replicate consisted of 2 plant samples. The total number of experimental plants is $7 \times 3 \times 2 = 42$ pots. Data were analyzed using statistical analysis (ANOVA) with Statistical Tool for Agricultural Research (STAR) program. The result of variance was continued by Duncan's Multiple Range Test (DNMRT) at 5% level.

Trial Implementation:-

The inoculum AMF comprises sand, zeolite, spore, hyphae and root pieces from unequal AMF isolates, depending on the density of the spores per gram of inoculum, so standardization is made so that the inoculum of each given isolate has a relatively equal spore density, ie ± 100 spores gram^{-1} of inoculant.

The seed bulbs used were the varieties of Brebes that were ordered at Indonesian Vegetable and Fruit Research Institute (BalitsaLembang). Bulbs were selected which appeared solid and had been stored ± 3 months. Uniform bulbs size with tuber diameter $\pm 1.5 - 2$ cm and weight ± 5 g. The medium is a mixture of zeolite and sterile sand (1: 1) dried and sterilized. A medium of 4.0 kg, placed in pot $d = 15$ cm. The pots were arranged in a greenhouse according to the floor plan and labeled.

Before planting a cropping hole as deep as 7 cm was made. Into the planting hole included inoculum (river sand, spores, hyphae, and root pieces of host) from each of the AMF isolates that have been standardized according to treatment (Table 2). Further on the hole (above inoculum) planted shallots seed bulbs. Previously seedlings were soaked with sodium hypochlorite (NaOCl) 1.0% for 10 minutes and washed with sterile water. *Ajir* is installed 1 week after planting (wap) with a distance of 5 cm from the sample plant and marked 5 cm from the soil surface to help observe the height of the plant.

Media in pots were watered daily using sterile water. Planting media maintained 70% capacity to hold water. Pest and disease control is carried out if the plant shows symptoms of attack, physical control is done. If the attack can no longer be physically controlled, it is chemically done at the recommended dose.

Observation Parameters:-

AMF colonization on root samples of shallot crops:-

Staining technique was used to observe AMF colonization in root plant samples using methods by [9], [10].

Table 1:-Observation of root colonization

Observation Parameters	Description
Percentage of AMF infection (PI) ; Koramik and Mc.Graw [9]	$\% \text{ PI} = \frac{\text{Number of infection roots}}{\text{Total number of root instances}} \times 100\%$ Category : 0 - 5% (very low) 6 - 26% (low) 26 - 50% (moderate) 51 - 75% (high) 76 - 100% (very high)
Intensity of AMF infection ; Travelot <i>et al</i> [10]	$\% \text{ M} = (95 n_5 + 70 n_4 + 30 n_3 + 5 n_2 + n_1)/N$ With : M= Percentage of AMF infection intensity N= Total number of roots observed n1-5= Number of infected root specified based on the class of intensity (class 0-5) Class 0 = 0%(not infected) 1 = 1 % (infected, very low) 2 = 5-10 % (infected, low) 3 = 11–50 % (infected, moderate) 4 = 51 – 90 % (infected, high) 5 = > 90 % (infected, very high)

Growth and yield: Data collected in the quantitative data in the form of data result of symbiotic ability (compatible) isolate indigenous AMF with shallots seen from plant height, number of leaves, number of seedlings observed at age 45 **Days After Planting** (DAP). For the number of tubers and fresh weight of bulbs crops observed during harvest.

Essential oil content of tubers (%); The content of volatile oil (%), the isolation of essential oils using the method of water distillation. Isolation procedure; Guenther¹¹ which was modified. Shallots coated tuber (500 g) that has been washed in chopped with a size of 20-30 cm. Then put in the distillation flask, add aquadest and then heated at a temperature of 100°C (4-6 hours). The resulting of distillate in the form of essential oil and water then added chloroform to form two phases namely water and chloroform that binds oil. Water is removed from the measuring flask using a separation funnel and evaporated at 40 ° C to evaporate chloroform. Then all that is left is pure essential oil. The yellow oil after being separated from the water is then shaken with a bit of anhydrous Na₂SO₄ crystals. The oil obtained was encrusted in a dark glass bottle and then calculated the essential oil content by the formula :

$$\% \text{ essential oil content} = \frac{a}{b} \times 100\%$$

With, a = The weight of the oil produced
 b = Sample weight

Result and Discussion:-

AMF colonization (%) :-

From the statistical analysis and further test with Least Significant Different (LSD) at 5% level (Table 2), there was a significant difference in both the percentage of roots and the intensity of infection due to the inoculation of AMF compared with the treatment without AMF inoculation (Table 2). Isolate AMF type *Glomussp* showed a higher infection percentage and infection intensity, and greatest potential to be an inoculum for shallots. The lowest root colonization is indicated by the treatment without AMF. Although pot experiments have been performed in a sterile state, there is still a root infection by the AMF, but in a low percentage. Experiments in sterile soil pots still allow the existence of AMF in it, therefore soil sterilization must be repeated. In addition, other factors can cause AMF sources such as watering water containers, seeds and so on. The author in Safitri [12] reported still found an infection in the root of tomato plant on treatment without AMF as much as 26.67%.

Table 2:-The colonisasi AMF with plant root

Treatment	Root colonization (%)	
	Root Infection	Intensity of Infection
<i>Glomus sp2</i>	90.000 a	45.000 a
<i>Glomus sp1</i>	86.667 ab	45.667 a
<i>Glomus sp3</i>	85.000 ab	37.000 b
<i>Scutelospora sp1</i>	85.000 ab	32.667 bc
<i>Glomus sp4</i>	80.000 ab	26.667 cd
<i>Gigaspora sp1</i>	73.333 b	24.333 d
Without AMF	20.000 c	7.000 e
	CV= 10.69	CV=11.89

The numbers followed by the same letters are not significantly different by DNMRT test at 5% level

AMF colonization on the roots of shallots plants with high and low categories can be seen in Figure 3.

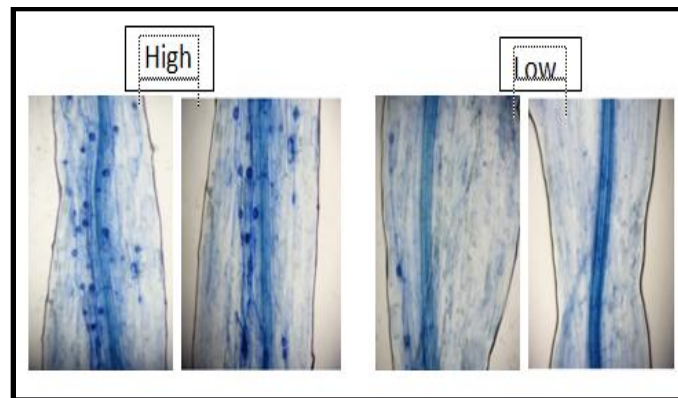


Figure 3:-Colonization of root with high and low intensity (magnification 100 x)

Spore density (spores):-

AMF inoculation may significantly increase the density of spores on plants with the medium than without AMF inoculation. Likewise, the different types of AMF are different from each other except *Glomus sp4* and *Gigaspora sp1*. This means that there are differences in spore growth of each type of AMF that leads to a difference in compatibility between the type of AMF with shallots plants. High spore density is dominated by *Glomus sp* (Figure 4). Respectively, the spore density in every 50 g of soil samples was found in the medium inoculated by isolate *Glomus sp1*, *Glomus sp2* and *Glomus sp3*. Sundariet al [13] states that AMF from *Glomus* type has a high adaptability compare to another types. The same study reported by Mbogneet al [14] *Glomus* type of the highest relative abundance. Seen in the Table 3.

Table 3:-Spore density in 50 g soil sample.

Isolat of AMF	Spore Density in 50 g soil sample
Glomus sp1	232.00 a
Glomus sp2	80.33 b
Glomus sp3	50.33 c
Scutelospora sp1	35.00 d
Glomus sp4	20.67 e
Gigaspora sp1	18.00 e
Without of AMF	5.00 f
CV=10.12	

The numbers followed by the same letters are not significantly different by DNMRT test at 5% level

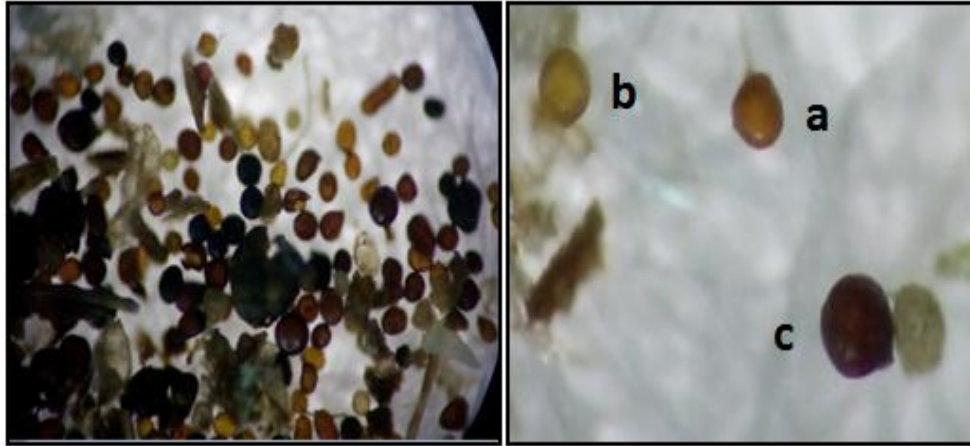


Figure 4:-Spora density under of microscope (magnification 100 x) : a) *Glomus sp1* (globose,brown) b) *Glomus sp2* (globose, yellow) c) *Glomus sp3* (globose, reddish brown).

Table 4:-Growth and yield of shallots are inoculated indigenous AMF on 45 DAP .

Isolat of AMF	Plant height	Number of tillers	Number of bulbs	Number of leaves	Weight of bulbs
	---cm---	---tillers---	---bulbs---	---leaves---	---g---
<i>Scutelospora sp</i>	42.17 a	6.33	6.83 ^{bc}	19.50	41.00 ^{bc}
<i>Glomus sp1</i>	42.83 a	6.83	8.50 ^a	21.00	47.53 ^{ab}
<i>Glomus sp2</i>	42.33 a	7.17	9.17 ^a	23.00	42.80 ^{ab}
<i>Glomus sp3</i>	43.17 a	6.33	7.83 ^{ab}	19.67	43.07 ^{ab}
<i>Gigaspora sp</i>	42.17 a	5.67	6.17 ^c	18.67	40.22 ^{bc}
<i>Glomus sp4</i>	40.50 ^{ab}	6.67	8.00 ^{ab}	21.33	41.97 ^{abc}
Without AMF	37.00 ^b	5.88	6.00 ^c	18.83	37.03 ^c
	CV=4.88	CV= 15.14	CV= 11.08	CV= 12.75	CV=7.74

The numbers followed by the same letters are not significantly different by DNMR test at 5% level

AMF inoculation significantly increased plant height ($p = 0.029$), number of tubers ($p = 0.0023$), and the fresh weight of tubers ($p = 0.047$) compared with no AMF inoculation (Table 4). This is because AMF has a phosphatase enzyme capable of liberating P and other elements previously unavailable become available in the soil. The high growth rate of plants is an indication of an efficient process of photosynthesis. The process of photosynthesis in a light reaction produces energy in the form of Adenosintriphosfat (ATP) and NADPH compounds. ATP is an energy source to perform various metabolic processes in the plant body. The availability of nutrients P will affect the formation of ATP. The presence of mycorrhiza can increase the absorption of nutrients, especially element P. Increasing the P content in plant tissue can accelerate cell division, especially in plant meristem tissue, resulting in further growth. The highest plant height is dominated by *Glomus sp*. In line with the research of Budiarmoko [15] states that the inoculation of AMF; *Glomussp* type can increase teak plant (*Tectonagrandis*) height by 6.99-47.59%. Likewise, the highest number of tubers dominated by *Glomussp* type, were significantly different from *Scutelospora sp1*, *Gigaspora sp1* and without of AMF treatment. The number of tillers and the number of leaves did not show any significant difference compared with the AMF-inoculated treatment. This is due to an increase in nutrient status favorable to plants by AMF, one of which is due to the volume of soil being explored the external hyphae of AMF increased by 5-200 x compared without AMF [16]. However, because the research was conducted in pots, the exploration of soil volume by external AMF hyphae was also limited, so it was not able to contribute P to increase the growth of number of tillers and number of leaves.

The content of the essential oil of shallots (%):-

Statistical analysis and DNMRT 5% test showed that inoculation of different types of indigenous AMF was significantly different from the content of essential oil of shallots in 50 g of the ingredients compared with no AMF inoculation. This means that AMF inoculation affects the production of essential oils on shallots. This proves that AMF supplements plant nutrients through absorbs and mineral translocations and induces changes in secondary metabolism that lead to increased secondary compounds [6]. Recently there has been more interest on the extraction of essential oil of shallots one of them due to their antioxidant activity and antimicrobial properties [17],[18], so it is necessary to conduct further research on the chemical composition of essential oils produced from the inoculated shallots of AMF as seen in Figure 5.

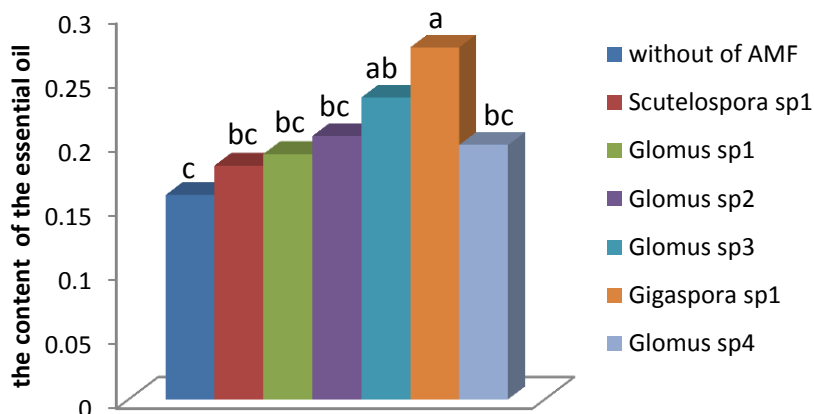


Figure 5:-The content of essential oil of shallots in 50 g ingredient

Essential oil extracts of shallots can be used as natural antimicrobial additives for incorporating in various food products. It is recommended that essential oils of *Allium ascalonicum* may be a new potential source as antimicrobial agents and natural antioxidants applied in food systems [19].

Conclusion and recommendation:-

The results of the study showed that (1) All types of AMF tested were able to increase colonization, plant height and shallots yield, but not significantly different to the number of tillers and number of leaves, (2) Inoculation of all types of AMF can increase the essential oil content of shallots bulbs.

Further research is needed on the role of AMF on the production of essential oils, especially on the components of the essential oils on shallot plants.

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