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Isolation and collection of arbuscular mycorrhiza (AM) fungi from ten species of weed in Yezin agricultural university campus

Hlaing Hlaing Htay, Mya Win and Soe Win

Abstract
In the present, rhizosphere soil samples were collected from ten species of weeds. That marginal soil was taken in different locations from Yezin Agricultural University Campus from June to December, 2018. The survey was the isolation of arbuscular mycorrhizal fungi (AMF) spores' density and colonization of the ten species of roots was studied in Poaceae and Cyperaceae families. The experiments were carried out to isolate the AMF from ten species of root and rhizosphere soil from weeds. The eight species of weeds included in Poaceae families and two in Cyperaceae families. In this experiment, AMF and plant root hairs its play important roles in plant water uptake and absorb nutrients in soil. The relative of endomycorrhizae and a root hair were served in plant water relations and it's were grown with or without inoculation of the AMF that were control drought conditions and water stress. All species of rhizosphere soils were observed the hyphae colonization percent and spores' numbers. Arbuscular Mycorrhizal fungi colonization percent in roots was calculated by grid-line intersection method. The collected AMF spore number and populations used to count by spore descending method. In this experiment were showed the highest AMF percent that have been found in *Echinochloa colona* L. Link (92.62%). The lowest AMF colonization percent is *Sporobolus diander* (Retz.) P. Beauv (30.86). The highest number of AMF spores was found in *Leptochloa chinensis* (L.) Nees (25.71). The lowest number of AMF were *Scirpus grossus* L. (5.14). The largest size of AMF spores was observed in *Echinochloa crus-galli* (L.) Beauv. (443.0µm) and the smallest AMF spores' size were found *Saccharum spontaneum* L. (49.08µm). Arbuscular Mycorrhizal fungi of vesicle and arbuscules were found in all ten species have been studies. This study result has highlighted the practical situations where AMF have a significant impact in restoring or maintaining soil health and fertility. Although, they probably represent the most significant plant microbe symbiosis of the complex microbial interactions that occur in the rhizosphere soil. In this experiment result, AMF and plant root hairs its play important roles in plant water uptake and absorb nutrients in soil, drought conditions and water stress.

Keywords: Arbuscular mycorrhizal fungi (AMF)

Introduction
In the world, a major threat of confronting of environmental degradation is uncontrolled use of chemical fertilizers contributes largely to the deterioration of the environment through depletion of fossils that generation of carbon dioxide and contamination of water resources. It leads to damage of soil fertility due to imbalanced use of fertilizer that has adversely impacted agricultural productivity and soil quality and has caused soil degradation (Anonymous 2008) [5]. Currently, there is growing realization that adoption of ecological and environment protection and sustainable farming practices can only reverse the refuse trend in the global productivity (Wani *et al*. 1995) [61]. The distribution of certain mycorrhizal fungal species has been related to phosphorous level, soil pH, soil disturbance, salinity (Abbot and Robson 1991), vegetation or hydrologic condition of the soil (Ingham and Wilson 1999) [25].
Among the mycorrhizal fungi and vascular plants that are found highly evolved by mycorrhizal symbiosis in mutually beneficial relationship. The benefit of mycorrhizal fungi to plants is primarily attributed to their ability to increase plant uptake of phosphorous (P) and other soil nutrients. Agronomic practices such as crop rotation, fertilization and tillage affect the extent of arbuscular mycorrhiza (AM) colonization and nutrient uptake of crops. Proper management of AM fungi has the potential to improve the profitability and sustainability of agricultural systems. These fungi are very important constituent of plant soil microbe system and can adapted to a wide range of environments. They are found in soils with very different water establishment including very arid habitats.

Mycorrhizae establish symbiotic relationships with plant and it plays an essential role in plant growth, disease protection and soil quality (Muthukumar and Udaiyan 2000) [37]. The deficiency of phosphorous is widespread in tropical soils and under that conditions the AM fungi contributes the phosphorous uptake to plants (Smith *et al*. 2003) [45]. Plantation of seedlings inoculated with AM fungi that provide favorable soil condition for naturally growing vegetation in the overburden diminishes. The extra-radical mycelium of AM fungi acts as an extension of the host root system, absorbing and providing nutrient to the plant (Smith and Read 2008) [46]. It was recommended that plant growth in waste lands could be effectively improved by integrating AM fungi (Nicolson 1967) [41].

Weeds are an important variable in organic crop production that are economically and weeds may serve to maintain diversity and agronomically benefit taxa of AM fungi (Vatovec *et al*. 2005) [60]. It was observed that the density of AM fungi spores increased significantly with increasing weed species number (Chen *et al*. 2004) [12].

Arbuscular mycorrhizal fungi (AMF) are the major component of soil and land plant (Smith and Read 2008) [46] that fungi (AMF) symbiosis is the most widespread type of mycorrhizal association. It is estimated in about 250,000 of plants species, including vegetables, crops, herbs and trees (Koide and Dickie 2002) [30].

More than 80% of the vascular flowering plants can be colonized by arbuscular mycorrhizal fungi (AMF), while only a few plant families do not form (Harrier 2001) [22]. The soil microorganism components are arbuscular mycorrhizal fungi, which affect plant development and minerals uptake strongly (Tahat *et al*. 2008b) [58]. Plant roots are influenced the physical, chemical and biological conditions of the soil in the rhizosphere (Gregory 2006; Smith and Read 2008) [17, 46]. The rhizosphere soil area is highly dynamic interactions and communications between plant roots system and the pathogens and other beneficial microbes (Hirsch *et al*. 2003) [21].

Soil microorganisms are dominant in the biogeochemical cycling of both inorganic and organic nutrients in the soil and in the maintenance of soil quality. In particular that microbial activity in the rhizosphere is a major factor which determines the availability of nutrients to plants that has a significant influence on plant health and productivity. The basic principles of rhizosphere microbial ecology are including the function and diversity of the microorganisms that reside there is necessary before soil microbial technologies can be applied (Bolton *et al*. 1992) [8].

The mycorrhiza act as bio protectants against pathogens and toxic stresses which these benefits are conferred through abiotic and biotic interactions in the rhizosphere. Soil-plant-microbe interactions are complex and many ways in which the outcomes can influence plant health and productivity (Kennedy 1998) [28]. Arbuscular mycorrhizal fungi (AMF) have been shown to have considerable significance in the maintenance of soil health and fertility, and several groups of beneficial rhizosphere microorganisms. Between the interaction rhizobia bacteria and the roots of leguminous plants has been well researched (Brockwell *et al*. 1995) [11], but for the mycorrhizal relationship it has only recently become a significant topic of research (Smith and Read 1997) [53].

The selection of appropriate adapted strains of AMF is very important. Application of mycorrhizal technologies then requires knowledge of the biodiversity across and within the species involved. This then needs to be interpreted in terms of a predictor of potential mycorrhizal effectiveness (Kuhn *et al*. 2001) [31]. Mycorrhizal technology is aimed at restoring the inoculum potential of AMF in problem soils. This may be achieved through bioaugmentation, by inoculating soils with AMF or by using transplanted seedlings that already have the appropriate AMF in their roots. Alternatively, indigenous but depleted populations of AMF may be restored by the use of a mycotrophic cover crop that stimulates the build-up of inoculum such that subsequent crops or plant communities gain the benefits (Dodd *et al*. 1990) [14].

Arbuscular mycorrhizal fungi, belonging to the phylum Glomeromycota are a main component of soil microbiota and probably represent the most important terrestrial symbiosis (Schwarzott and Walker 2001) [49].

Arbuscular mycorrhizal fungi (AMF) are improving the assimilation and transportation of nutrients from the soil to host plants that form trophic root associations with plants (Lopez-Gutierrez *et al*. 2004) [32]. Strongly increase the absorbing surface are due to their capacity to form a lot of branched extracellular structures. At the same time, the fungal hyphae produce alkaline and phosphatases acid, which dissolve insoluble phosphates, making them available to the plants (Smith and Smith, 1996; Sato *et al*. 2015) [48, 51]. Mycorrhizal associations produced by glomeromycota fungi are known as arbuscular mycorrhiza or vesicular-arbuscular mycorrhizas and are abbreviated as VAM. The occurrence and physiological importance of vesicular-arbuscular mycorrhizal (VAM) fungi in agriculture, horticulture and forestry have been extensive studies throughout the world (Brundrett 2002) [9].

The AM fungi may provide benefits to plants other than in phosphorous uptake and has not been widely addressed that are recognized to be capable of reducing pathogenic infection (Schonbeck 1979 and Dehne 1982) [47]. It is increasing the uptake of poorly mobile nutrients such as zinc (Gildon and Tinker 1983) [16] or probably improving plant water relations (Allen and Allen 1986) [4]. Similarly, a few studies have tracked individual fungi through time as environments change and these kinds of studies are necessary to understanding of the dynamics of mycorrhizal symbioses. These fungi are well known to improve plant growth on nutrient-poor soils and enhance the uptake of P, Cu, Ni, Pb and Zn (Khan *et al*. 2000) [29].

Arbuscular mycorrhizal fungi (AMF) invade cortical cells intracellularly, intracellularly and form clusters of finely divided hyphae known as arbuscules in the cortex. They also form membrane-bound organelles of varying shapes known as vesicles outside and inside the cortical cells. Outside the root in the soil extensive, branched, external mycelium grows from the infection units (Smith and Read 2008) [46].

Arbuscular mycorrhizal fungi are mutualistic symbiosis formed between the roots of 60% of all plants species and soil borne fungi in the order Glomales (Trappe 1987) [56], there by extensions of plant root system. In this paper, observation and determination of colonization of arbuscular mycorrhizal fungi in collected roots, isolation of spore from rhizosphere soil and measuring the size comparing the collected spore from selected weeds.

Materials and Methods

Selected of sites and collected weeds

The study was conducted during 2018, June to December at

Yezin Agricultural University Campus. In this study, eight species of weeds collected include Poaceae families and two species in Cyperaceae families were determined. The identification of the collected weeds was identified according to the thesis of IRRI (2010), CIBA-GEIGY-2 (1981), CIBA-GEIGY-3 (1981), Lwin Mar Saing (2010) [34], Zaw Myo Tun (2010), Sandar Htwe (2014) [44] and some available literatures.

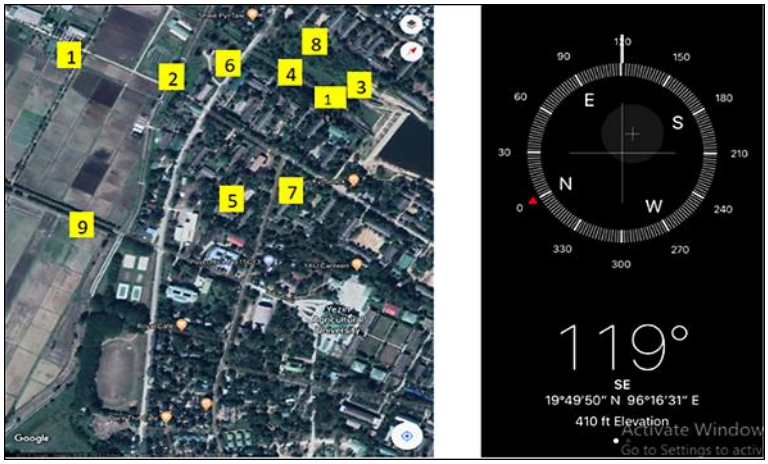

Fig 1: Ten species root and rhizosphere soil sample collected area of Yezin Agricultural University Campus Area. (Source: Google map data, 2018)

Collection of samples

Roots and rhizosphere soil samples was collected from the ten species of plant's rhizosphere which were growing various location of Yezin Agricultural University (YAU) Campus (June to December, 2018). In this collection procedure, about 100g of soil was collected by digging with a soil borer about 10-15cm. The grass roots samples were cut into approximately 2-3cm long pieces. The collected soil and roots samples were packed in airtight plastic bags for further study.

Collection of ten species of weed samples

In this research, ten species of weed sample were collected from different locations of Yezin Agricultural University Campus for isolation of spore and determination of infectivity rate.

Screening of mycorrhiza fungi and isolation of spores

Spore was isolated from the rhizosphere soil by their floating adhesion technique (Sutton and Barron, 1972) and by wet sieving method (Gerdemann and Nicolson, 1064). Rhizosphere soil of were collected weeds sample from depth of 10-15 cm after the surface soil has been scraped and discarded from different location in Yezin Agricultural University Campus. In this procedure, four sieves (750µm, 100µm, 50 µm) were used for this experiment. The soil sample (50g) was placed in a Blender. For this type of rhizosphere soil, about 500ml of water was needed to add and blended at high speed for this approximately five seconds. The soil suspensions were poured through a stack for approximately five seconds. The stream of tap water is added to facilitate the movement of spores. The organic debris from the top sieve was discarded. The material that remains in the 50 µm, 100 µm and 250 µm aperture sieves was transferred to petridishes and examined under microscope. The mycorrhiza spores were carefully collected with the fine forceps. Most sand was remained in the blender.

Assessment of root colonization and spore population

For the assessment of mycorrhizal colonization, firstly the ten species of roots samples were stained and the percentages of vesicular mycorrhizal colonization were estimated by grid line method (International Culture Collection of Vesicular Arbuscular Mycorrhiza IVAM).

Fig 2: Show the procedure of spore collection

Staining procedure for grass roots

The collected root samples were washed in water to remove adhering soil and sand. About 3cm of root fragment samples were cut and boiled in 10% KOH at 100 ℃ for 3 minutes (depending on the hardness of root fragments) to remove cytoplasmic contents from cell. Older thicker roots require longer incubation times. Browning of the isolation is an indication of the clearing process. Then the roots were washed with water stained with ink-vinegar method for about 4 minutes. The stained roots were washed with water and examined under the light microscope for mycorrhizal infection. Then determination the root infection rate was carried out by Grids-line method (INVAM Method). The stains were prepared by mixing water, glycerin and lactic acid in proportion of 1:1:1: (v/v/v).

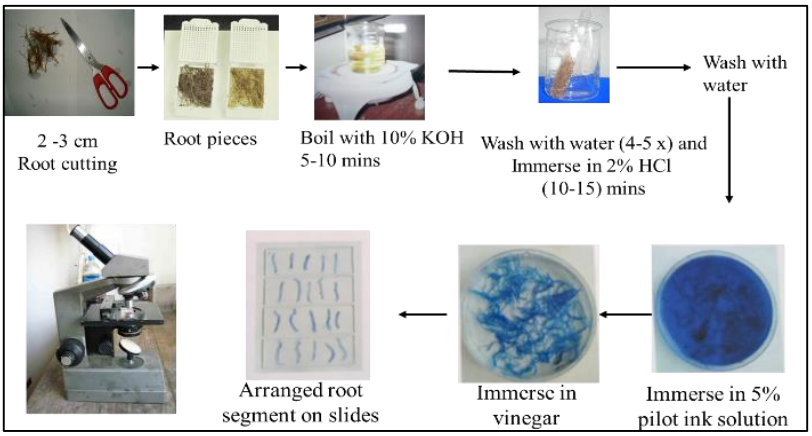

Fig 3: Observation and determination the colonization of VAM fungi in collected grasses roots

Preparation of acid fushin solution

Acid fuchsin reagent

Ingredient	Composition
Distilled water	300ml
Glycerin	300ml
Lactic acid	300ml
Acid fuchsin	0.15g

The composition of 10% KOH solution was as follows

Ingredient	Composition
KOH	10 gm
Distilled water	90 ml

The composition of HCl acidified was prepared as follows

Ingredient	Composition
HCL	2 gm
Distilled water	98 ml

Ink-vinegar stain solution

Ingredients	Composition
Pilot Fountain ink	1ml
Vinegar	99 ml

Procedure for grid-line intersection method (Newman, 1966)

A grid line was marked on the bottom of the petridish to form 0.5-inch squares. Twenty fragments of roots sample (3cm) were randomly spread out in a plastic petridish. Then vertical and horizontal gridlines were scanned under the microscope and the presence or absence of infection was recorded at each at point where the roots and mycorrhizal infected root to intersect on the line.

The formula for the gridline intersection method.

Root colonization % = (Intersection of infected roots x 100)/Total number of intersection roots.

Result

Collection of ten weed sample

The study was conducted during 2018 June to December at Yezin agricultural university campus. In this study, ten species of two weed families were selected. The plants were studies for association of arbuscular mycorrhizal (AM) fungi. They are (1) *Cynodon dactylon* (L.) Pers, (2) *Eleusine indica* (L.) Gaertn, (3) *Echinochloa colona* (L) Link, (4) *Echinochloa* (L.), (5) *Sporobolus diander*, (6) *Saccharum spontaneum* (L.), (7) *Leptochloa chinensis* (L.), (8) *Scirpus grossus* (L.), (9) *Kyllinga brevifolia* (Rottb), (10) *Imperator cylindrica* (L.) P. Beauv. They were collected from different location of Yezin agricultural university campus for isolation of spores and determination of infectivity rate.

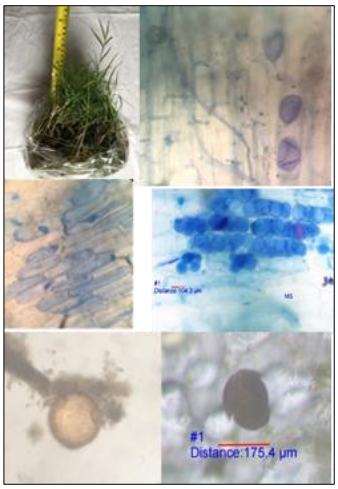

Fig 4: Show the spore vesicles and hyphae in root 40x Cynodon dactylon (L.) Pers.

Scientific Name - *Cynodon dactylon* (L.) Pers.
Local Name - Mye-Sa-Myet
English Name - Bermuda grass
Family - Poaceae

This species can be distinguished by its stoloniferous culms. Leaf sheath with bearded auricle and orifice, the inflorescence composed of three to four digitate spikes bearing purplish green spikelet.

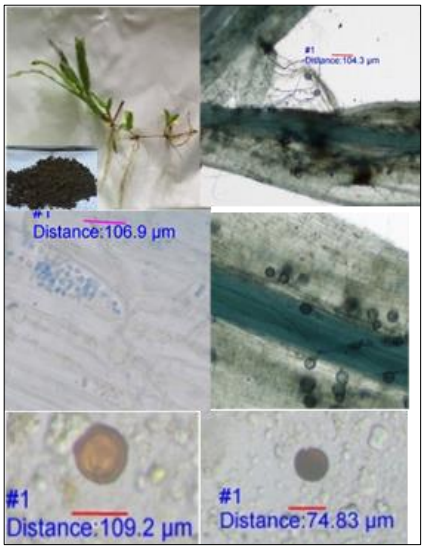

Fig 5: *Eleusine indica* (L.) Gaertn.

Scientific Name - *Eleusine indica* (L.) Gaertn.
Local Name - Sin ngo myat
English Name - Goose grass, yard-grass
Family - Poaceae

The outstanding feature that help for easy identification of the species are tufted habit, chartaceous leaf sheath with scarious margins, digitately arranged inflorescence, with an extra one below the terminal cluster, linear ascending spikes terminating in a spikelet, very closely imbricate spikelet, scabrid lemmas and paleas winged, light orange-brown ellipsoid grain with obliquely striated lines.

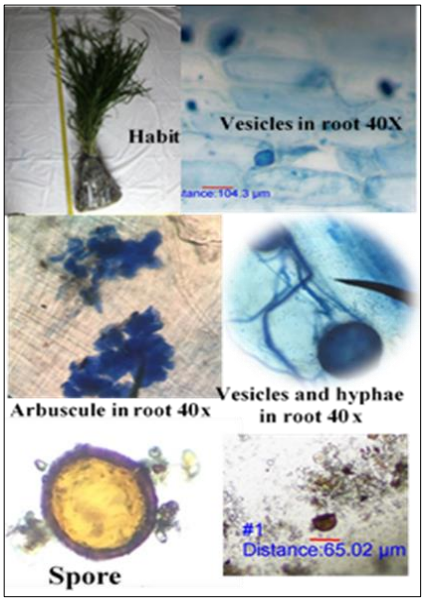

Fig 6: *Echinochloa colona* (L.)

Scientific Name - *Echinochloa colona* (L.) Link
Local Name - Bae-Sa-Myet
English Name - Bermuda grass
Family - Poaceae

The outstanding features of this species are tufted annuals habit, ligule absent, represented by a discovered zone, erect inflorescence with racemosely arranged six to ten spikelet dense racemes and neatly four rows of ovate-acute awnless paired spikelets.
Scientific Name - *Echinochloa crus-galli* (L.) Beauv.
Local Name - Myat Thee
English Name - Not known
Family - Poaceae

The characters that help for the easy identified of this species are strongly nerve leaf sheath chartaceous with narrow scarrious margin, ligule represented by liac colored zone, erect inflorescence composed of six to ten simple ascending racemes along a central axis, paired, untidily rowed spikelets upper floret perfect, elliptic.

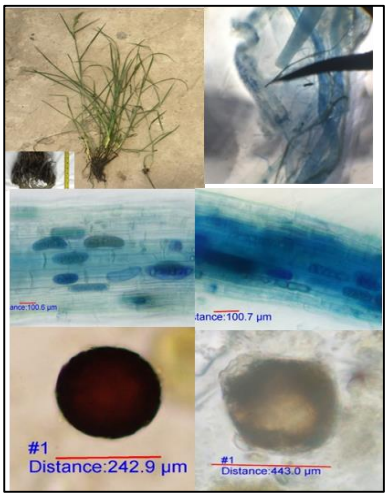

Fig 7: *Sporobolous diander* (Retz.) P. Beauv.

Scientific Name - *Sporobolus diander* (Retz.) P. Beauv.
Local Name - Myat Khar
English Name - lesser drop seed, two anther smut grass
Family - Poaceae

The outstanding features the species are weekly tufted habits, ciliolate membranous ligule, contracted panicle with crowed lanceolate-oblong grayish green spikelets, two stamens with creamished tinged-purple anther and obovate-oblong coarsely pitted grain.

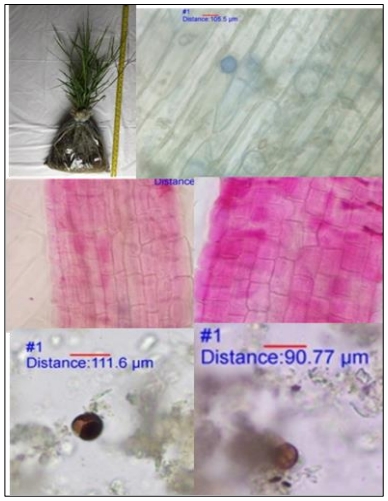

Fig 8: *Saccharum spontaneum* L.

Scientific Name - *Saccharum spontaneum* L.
Local Name - Kaing-myat
English Name - Kans grass
Family - Poaceae

Perennial tall reed like grass, culums rhizomatous caestipose, recemes, plume-like panicle, paired spikelets, callus beard with silky white hairs are outstanding features of the species.

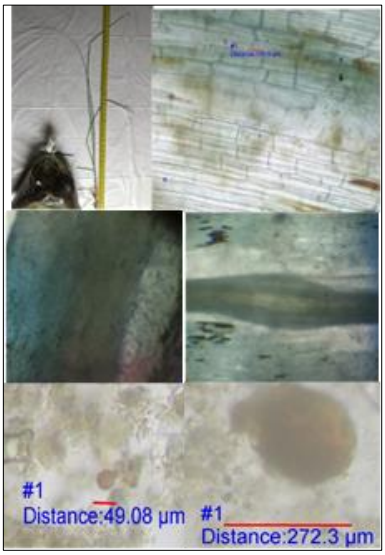

Fig 9: *Saccharum spontareum* L.

Scientific Name - *Leptochloa chinensis* (L.) Nees.
Local Name - Daung mee puan
English Name - Chinese sprangletop,
Family - Poaceae

The outstanding characters of perennial, tufted, erect and slender sometimes with reclining stems, aquatic-wet to flooded, lowland, high competitiveness, leaf smooth, linear, long, inflorescence narrowly ovate, loose panicle, many spike-like slender branches, racemes slender, each with two rows of spikelets, purplish or green and four to six flowers.

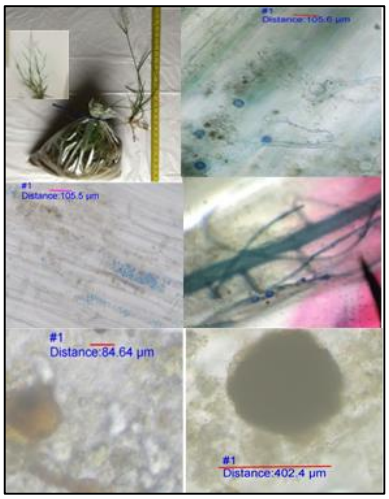

Fig 10: *Leptochloa chinensis* (L). Nees

Scientific Name - *Scirpus grossus* L.
Local Name - Watt Lar
English Name - Club-rush
Family - Cyperaceae

The outstanding characters of that species are perennial, grass like species, tubers, stolons and seeds, grass like leaves, erect sedge, rough to touch and inflorescence dense, terminal corymb, fifteen-centimeter-long.

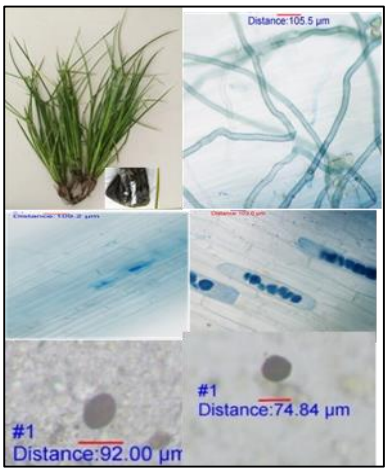

Fig 11: *Scripus grossus* L.

Scientific Name - *Kyllinga brevifolia* (L.) Rottb
Local Name - Than Htut Myat
English Name - Green kyllinga
Family - Cyperaceae

The outstanding characters of that species are perennial, rhizome, stems reach six inches, several erect stems to heights up to about half a meter, often much shorter, inflorescences of a few spikelets, cluster of flowers arranged on a stem, dark green leaves in color, glabrous, no auricles, ligule present, produced at the end of the triangular stems, roots are dense system of rhizome, red to purple color.

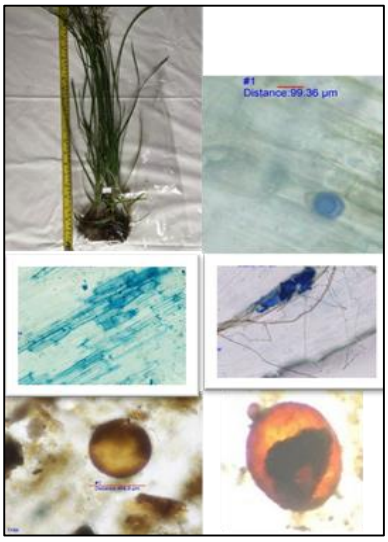

Fig 12: *Kyllinga brevifolia* (Rota.)

Scientific Name - *Imperata cylindrica* L.P. (Beauv)
Local Name - Thet Kal
English Name - Cogongrass
Family - Poaceae

Imperata cylindrica is a perennial, colony-forming grass which can grow up to 6 ft. (1.8 m) tall. Foliage, leaves have an off-center, whitish midrib and finely serrated margins. Leaves are up to 6 ft. (1.8 m) long, 0.5-0.75 in. (1.3-1.9 cm) wide, stiff and have a sharp, pointed apex. Rhizomes are whitish, branched, scaly and sharp at the tips. Flower heads are 2-8 in. (5.1-20.3 cm) long, silvery-white and cylindrical. Fruit *Imperata cylindrica* is best identified in the spring by the large fuzzy panicle of flowers and seeds, giving the plant a cottony or silky look.

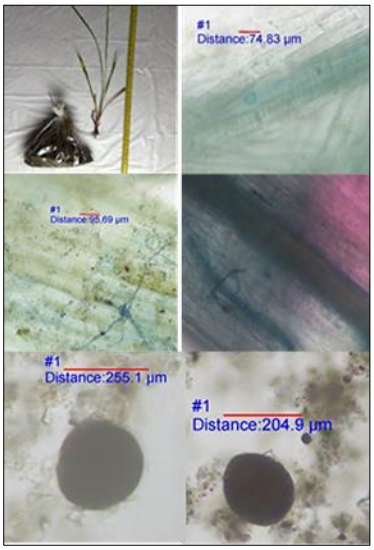

Fig 13: Imperator cylindrica (L.) P. Beauv.

Isolation of spore in ten grasses roots

In the present study, all of the collected species of plants were found to have AM fungi infection. In august, collection of spores in *Leptochloa chinensis* L. (25.71) showed the highest spore number. In June, *Scirpus grossus* L. was the lowest spore number (5.14) in all of the seven months. The data is shown in table (1).

Table 1: Mycorrhizal colonization and spores population (Average) of ten weeds in YAU Campus (June to December, 2018)

No.	Scientific Name	Stage	Collected sites	Soil type	Soil pH	Colonize-Lion (%)	Spore population (50 gm)	Vesicle
1.	*Cyanodon dactylon* L.	Ve	**Sable** 16°80'11" N 96°16'31" E	Silty Clay Loam	4.49	32.99	23.14	+
2.	*Eleusine indica* L.	Ve	Sable 16°80'11" N 96°16'31" E	Silty Clay Loam	4.49	53.42	18.57	+
3.	*Echinochloa colona* L.	Flo	Sable 16°80'11" N 96°16'31" E	Silty Clay Loam	5.61	92.62	19.42	+
4.	*Echinochloa crux gall* L.	ye	Sable 16°80'11" N 96°16'31" E	Silty Clay Loam	5.61	64.45	20.71	+
5.	*Sporobolus diander* L.	Flo	Sable 16°80'11" N 96°16'31" E	Silty Clay Loam	4.49	30.86	18.14	+
6.	*Saccharum spontaneum* L.	ye	Sable 16°80'11" N 96°16'31" E	Silty Clay Loam	4.49	78.99	15.57	+
7.	*Leptochloa chinensis* L.	ye	Sable 16°80'11" N 96°16'31" E	Silty Clay Loam	4.49	72.29	25.71	+
8.	*Scirpus grossus* L.	flo	Sable 16°80'11" N 96°16'31" E	Silty Clay Loam	5.61	37.88	5.14	+
9.	*Kyllinga brevifolia* (Rottb)	flo	Sable 16°80'11" N 96°16'31" E	Silty Clay Loam	5.51	60.93	18	+
10.	*Imperator cylindrica* (L.) P. Beauv.	flo	Sable 16°80'11" N 96°16'31" E	Silty Clay Loam	4.78	71.70	19	+

Estimation of mycorrhizal colonization in ten grasses roots: According to the result of mycorrhizal colonization in *Echinochloa colona* (L.) Gaertn. (92.62%) showed the highest mycorrhizal colonization. The second was *Saccharum spontaneum* L. (78.88%). Among all these months, the lowest colonization in grass root was *Cynodon dactylon* (L.) Pers (30.6 6%). This result indicated that the plant species might be considered as good host for arbuscular mycorrhizal fungi. The variation of root infection intensity might be due to the host plant physiology and host- plant specificity.

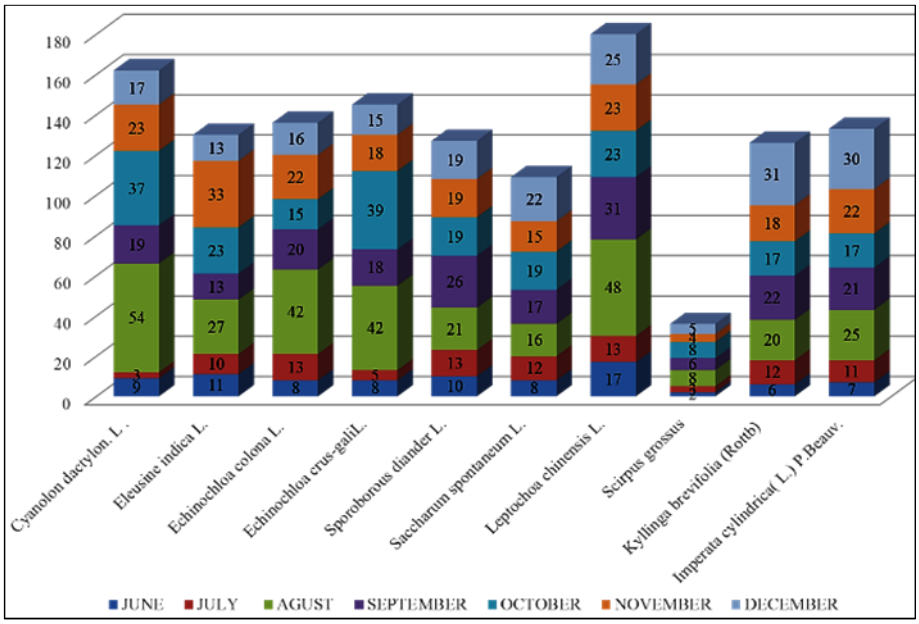

Fig 14: Mycorrhizal spore population of ten weeds in YAU campus (June to December 2018)

Discussion and Conclusion

The results of the study were the demonstrations that association of Arbuscular Mycorrhizal in plants performance with natural environment. The present paper, study the rhizosphere soils of ten species weeds from Yezin Agricultural University Campus and observed mycorrhizal spore and colonization percent in roots. The mycorrhizal colonization was calculated by gridline intersection method (INVAM). The collections of spores were counted from the rhizosphere and attachments of roots were counted by using wet-sieving and spore decanting method (Gerdemann and Nicolson 1963) [19]. Nearly, all the selected plant was observed on mycorrhiza, its widespread distribution and its association in roots were found in most plant.

According to the finding, all of ten species were observed the spore and colonization. The collected spore sizes were different. In this experiment, the average highest colonization percent have been found in *Echino colona* L. (92.62%) and the lowest one is *Sporobolus diander* L. (30.86%). The average highest number of spores was found in *Leptochloa chinensis* L. (25.71) and the lowest one was *Scirpus spontaneum* L. (5.14). The largest spore size was observed in *Echinochloa crus-galli* L. (443.0µm) and the smallest one is *Saccharum spontaneum* L. (49.08 µm). Vesicles were found in all species. Zaw Myo Tun (2009) [62] stated that variations in presence of root colonization were not related to spore population numbers. Powell (1977) [42] showed that the different species might be considered as good hosts for arbuscular mycorrhiza fungi. Zaw Myo Tun (2009) [62] and Powell (1977) [42] were agreed with these statements of the experiment result.

The result supports the concepts the plant host-mycorrhizal soil environment interaction is extremely complex as well as variable. Mycorrhizal inoculation tended to increase macro and micronutrient and increase growth of plant. Thus, uses of mycorrhiza could economic the fertilizer applied in plant production providing a sustainable and environmentally safer substitute. As a result, it is becoming critical to recover not only the vegetation but also these biological and physico-chemical soil qualities. In future, the research on going to multiply the mycorrhiza and use as mycorrhizal biofertilizer to treat the maize cultivation in field experiment only and combine with other organic fertilizers.

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