AMF Spore Density in Three Agricultural Sites in Two Districts of West Bengal

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Abstract-*Arbuscular Mycorrhizal Fungi (AMF) are cosmopolitan in distribution though their density become lowers due to conversion of land to degraded kind and huge application of chemical fertilizer with varied applications of chemical pesticides, insecticides and foliar spray of chemicals unscientifically in agricultural lands. Another fact is that, high tillage and creation of water logged condition rapidly diminishes the growth of the AM fungi in and around the cultivated land. Forest is a natural land as well as repository that protects its biodiversity obviously the soil microorganisms and therefore keep the number of AM fungal spores constant during winter in compare to the land of degraded and cultivated one which have altered the conditions in the same area in same season. The present study is therefore a study of AM fungal spore density in different cultivated lands in Kanaipal potato cultivation land of Jhargram Distrct and Hatidhara rice field of Paschim Medinipur District respectively. Some AMF species under 4 different genera have been isolated from the soil samples collected from the areas in West Bengal, India. Here Glomus was a dominant genus over the other 4 genera studied in the same sites.*

Keywords*-*AM Fungal spore density, Hatidhara, Kanaipal, Management.

I. INTRODUCTION

Arbuscular Mycorrhizal Fungi (AMF) are the most common type of beneficial fungi and most ubiquitous in their distribution over the globe (1, 2, 36, 38). They are associated with almost all land plants in an ecosystem (3-5). Over the last decades, there has been growing appreciation of the importance of VAM fungi on terrestrial ecosystems (6-8) as they showed natural infection over 80% of vascular plants. Arbuscular Mycorrhizal (VAM) fungi play a major role in soil fertility, nutrient acquisition and transport(7) especially uptake of phosphorus from the soil and thereby enhancing plant vigour (8), enhance growth, yield (27), provide greater resistance to plant diseases (9) and increase tolerance to drought, salinity and unnatural stress imposed by several factors (10). The soil rhizosphere is a habitat of complex interactions between plants and microbes in which environmental factors such as soil physico-chemical parameters as well as fertilizers or cultivation practices may have large effect on microbial communities. The rhizosphere, where VAM fungi and soil organisms coexist side by side and constantly change the non linear environ because of several altered factors. The activity of rhizosphere-inhabiting microorganisms exerts a significant effect on plant health and give support better to work more. Depending on the type of interaction between two different symbionts and the environment, the degree of spore production by VAM fungi varies. In general variation of spore density in rhizosphere soil varies with the variation of seasons. Seasonal influence affects the spore density and even colonization in different host plants under field conditions, depending on the efficacy of indigenous fungi. Therefore, there is a need to take a close look at the nature of natural processes that help to increase the yield of agricultural crops and quality of the same with more efficient use of nutrient inputs, reduced rate of applicable pesticides and insecticides under nursery condition followed by field along with horticultural crops. Arbuscular mycorrhizal fungi, therefore provides essential elements to the nutrient deficient soils especially phosphorus and can provide support for management of nutrients and maintenance of better crop growth. Because of the public concerns about the side effects of agrochemicals, more attention is now being given to research areas concerning biological balance in soil, microbial diversity or microbial dynamics in soil, health support and better adaptability through the change of the soil greedily from microhabitat to macro-habitat. Therefore, the study of spore interactions in the myco-rhizosphere is an interest topic of current concern. Despite the importance of mycorrhiza in agriculture and floriculture, little work has been done regarding their distribution and diversity in the rhizosphere soil associated with the pot or poly bag culture for varied crops have been taken in to consideration. It will be helpful to the researchers working in this field and may be a first time study as preliminary one to give better understanding between two different agriculturally raised ecosystems side by side. Hope that future study and research will take the creamy part to isolate AM fungal spores from agricultural soils to make bio-fertilizer in a global basis and solve a genuine problem to produce eco-friendly developed crops in different field of biology in near future.

II. STUDY AREA

Study area was taken in West Bengal which comprised of cultivated land at Kanaipal under Jhargram District and Hatidhara of Paschim Medinipur District. Three study samples in each study site were taken in to account. At Hatidhara, three rhizosphere samples and at Kanaipal 6 rhizosphere soil samples from two stations were collected. In each station at Kanaipal, 3 rhizosphere soil samples were collected during January 2017. At Hatidhara, soil samples of rice field was collected whereas at Kanaipal, potato cultivated land near Kansai river was selected and rhizosphere soil samples were collected. Soil samples were marked as near River and away from the Kansai River were collected. The rice field was selected from which rice was harvested and the crops was used as 'aman' rice (June to November). No rabi crops including boro rice (December to February) was there but nearby land filled by potato plants. Other hand, Kanaipal field was agriculturally developed and the field was amended by chemical fertilizers. Dissimilarity is that Hatidhara is free from any type of cultivar during soil collection whereas the Kanaipal is filled with potato plants at mid time of production. Another potato cultivation field was selected which was away from Kansai river basin.

III. MATERIAL AND METHODS

The present study was carried out in three eco-climatic fields like Hatidhara of West Midnapore, agicultural crop land used by potato near Kansai as Kanaipal and other one was Kanaipal farthest from River Kansai under Binpur-I community Development Block of Jhargram District. The Kanaipal site of the forest was more or less aside the Jhitka-Lalgarh Forest (11-13, 16) and Hatidhara near Chandra Forest Range of Paschim Medinipur. Kansai river flows towards Dherua of Paschim Medinipur District in West Bengal from Kanaipal and lower part flows from Hatidhara to lower part of Midnapore. Samples were collected at winter season starting from December 2016 to January 2017 with a gap interval of 15 days.. The winter temperature goes from 9˚C to 13˚C. Rhizosphere soils of 2 fields and 3 stations each were collected from randomly designed selected sites. Three rhizosphere soil samples were collected from each site. These rhizosphere soils collected at the middle and end of each month were pooled together to form a composite sample and stored in polyethylene bags at 4˚C for further analysis. From each composite sample, three replicates were taken for further analysis. The physico-chemical characteristics of soils such as soil moisture content (%) and soil reaction (pH) were done using dry weight method and pH meter. Arbuscular Mycorrhizal Fungal (AMF) spore density was calculated from the rhizosphere soil samples using 100 grams soil samples for each sample. Three replicas were used and then mean was taken to determine the number month wise. SD value was calculated using software. From the month wise data mean was calculated to draw a final conclusion following multiplication by 10 as the soil was 100 gm. Wet sieving and decanting technique was used (15) and direct count was used for quantification using the "stereomicroscope". Results were expressed as mean of three replicates for each sample. The abundance of spores determined for each sample was expressed as the number of AM fungal spores per 10 grams of soil for all the samples studied after that it was multiplied by 10 to get 100 g soil sample because a large number get so many number of spores might be problematic during counting. Intact spores and sporocarps were mounted in lacto-phenol glycerol and identified according to their spore morphology by using taxonomic key (17, 18). The qualitative estimation was expressed as percentage frequency occurrence of AM fungal species. Other literatures used were literature 19-35 published time to time. Authors delivered lectures on the same topics also put forth for reference to support the same (36-37).

III. RESULTS AND DISCUSSION

The data on rhizosphere soil analysis showed that the soil pH was mainly acidic in nature (6.8) and range varied from 6.6 to 7.1 (Basic) i.e. high variation thereby, indicating a major variation from one site to another as there were different management for agricultural land use practice. These soils obviously affect the plant growth (Table 1). Moisture content varied from 7-26% in different types of study soil. The AM fungal spores isolated from the present soils during the seasonal survey exhibited the association of many species under 4 genera. No Sclerocystis species were observed. Among them isolated genus Glomus represented higher density over other species under 4 genera, whereas genus, Acaulospora represented second dominated species. Other genera found there were Gigaspora and Scutellospora in the same study sites. The present study showed that there is a wide range of variation in spore number at different study sites under different management regimes in a season. Highest AMF spore density was observed at Hatidhara rhizosphere soil followed by Kanaipal potato field (river side) than at Kanaipal potato field which was away from Kansai river i.e. 653±112.64, 453±47.84, and 375±77.31 respectively per 100 g rhizosphere soil (Table 2).

Table 1.

Moisture content and soil p^H of study soil samples of Hatidhara and Kanaipal, West Bengal, India

Name of the study sites (Abbreviations used for study sites name)	Range of soil moisture Content (%)	Mean Soil p^H During Winter
Hatidhara (HC)	7% to 16%	6.6
Kanaipal Potato cultivated land (Nearer to Kansai River) (KPCLNK)	12% to 24%	7.1
Kanaipal Potato cultivated land (KPCLDK) (Distantly located from Kansai River)	13%-26%	6.6

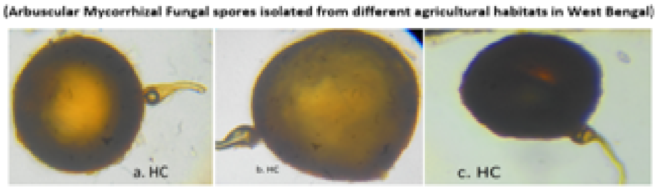
Table 2.

Arbuscular Mycorrhizal Fungal (AMF) spore number in Rhizosphere soil of cultivated land

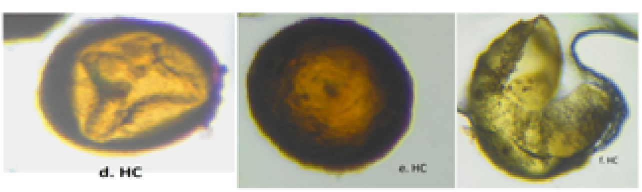
Type	Sample No.	100 BSS Mess Sieve	<100 BSS Mess Sieve	VAM Spore in 100 gm Rhizosphere soil	
				Spore no. in samples	Mean spore number
Hatidhara	1.	380	220	600	653 ±112.64
	2.	200	350	550	
	3.	410	400	810	
Kanaipal (KAL) River side	1.	110	300	410	453±47.84
	2.	180	250	430	
	3.	160	360	520	
Kanaipal Away from River (KAL)	1.	073	193	266	375±77.31
	2.	130	300	430	
	3.	120	310	430	

N.B.: *Per 100 gram rhizosphere soil therefore contains 653±112.64, 453±47.84, and 375±77.31 number of AMF spores during winter at Hatidhara, Kanaipal (river side) and Kanaipal (away from river) respectively. This indicates aman rice cultivated field shows maximum spore density than station at river side of Kanaipal than station away from river at Kanaipal.*

PHOTO PLATES OF AMF SPORES
(Arbuscular Mycorrhizal Fungal spores isolated from different agricultural habitats in West Bengal)



a-b: **Gigaspora margarita**; c-**Scutellospora** sp. ; HC-Hatidhara cultivable land (Winter Collection)



d-**Gigaspora** sp. e-**Gigaspora** sp. f. **Gigaspora margarita**

PHOTO PLATES OF AMF SPORES
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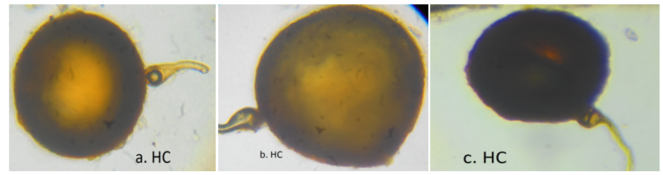

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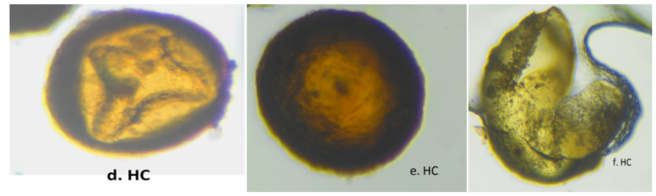
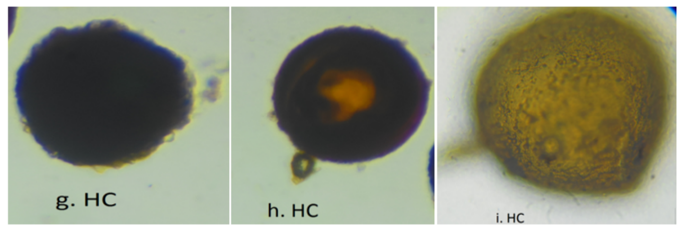
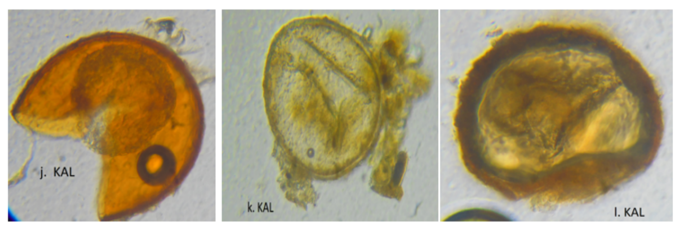


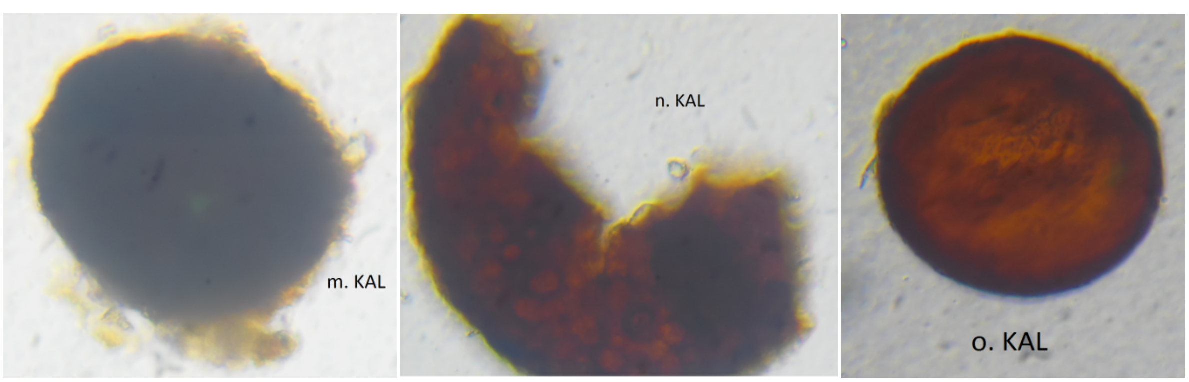
d-**Gigaspora** sp. e-**Gigaspora** sp. f. **Gigaspora margarita**



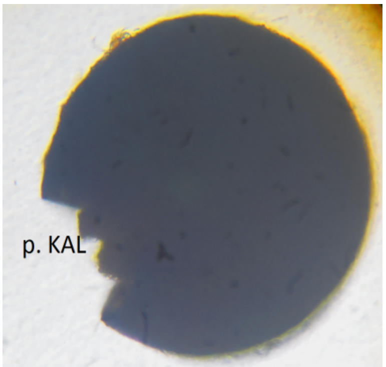
g-Sporocarp of **Glomus** sp. ; h-**Gigaspora** sp. from lateral view; i- **Gigaspora** sp.



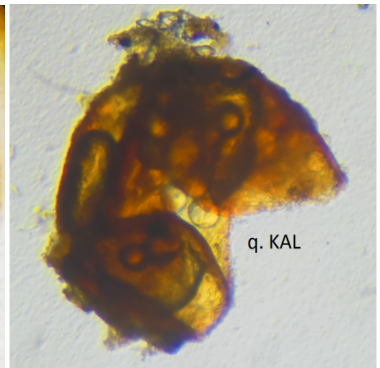
j.-**Glomus aggregatum** k.-**Acaulospora** sp. l.-**Glomus** sp.; KAL-Kanaipal



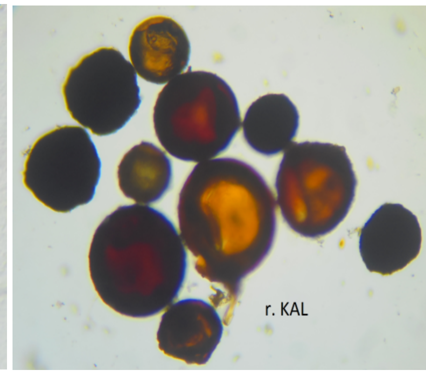
m.- **Acaulospora** sp. n.- **Acaulospora** sp. o.- **Gigaspora** sp.



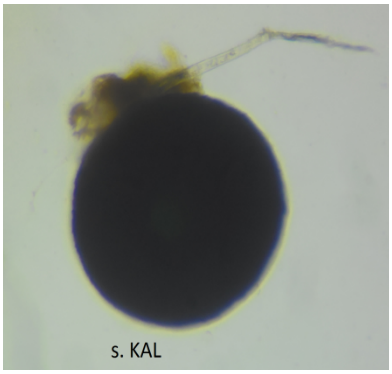
p.-**Scutellospora nigra**



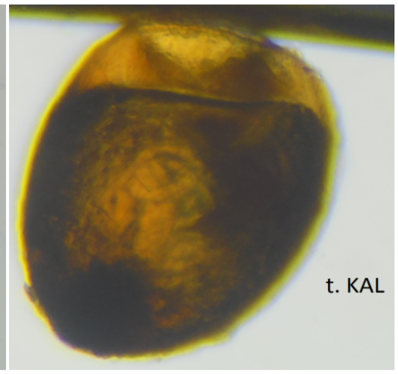
q.-**Glomus** sp.



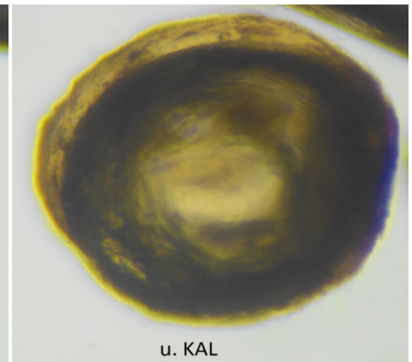
r.- AM spores,



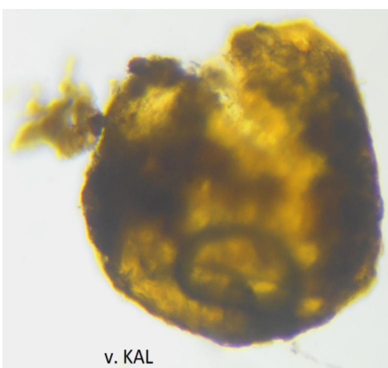
s.- **Scutellospora** sp.



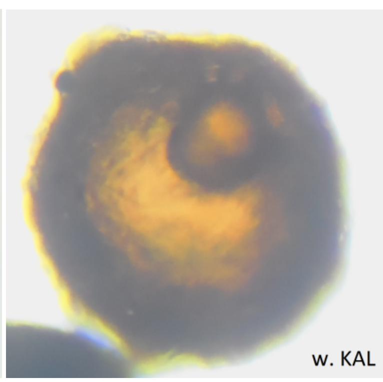
t.- **Scutellospora** sp.



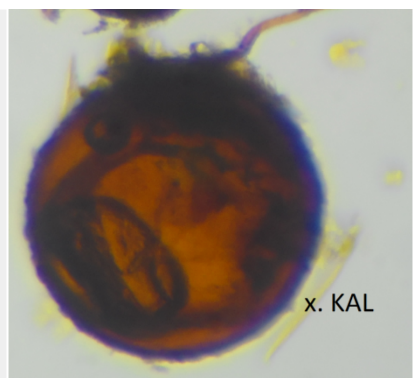
u.- **Scutellospora** sp.



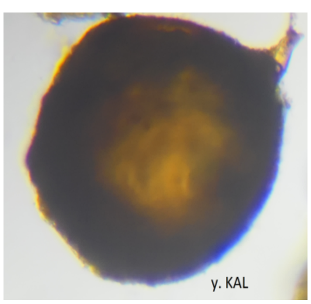
v. **Scutellospora** sp.



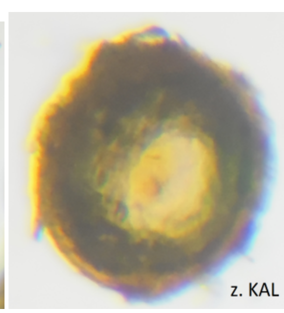
w.- **Scutellospora** sp.



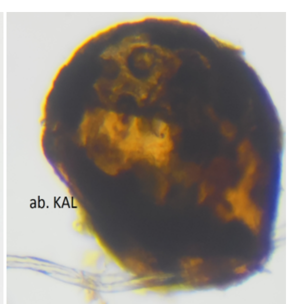
x.- **Scutellospora** sp.



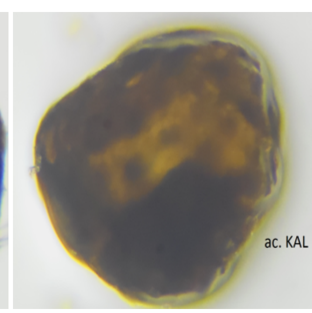
y. -**Glomus** sp.



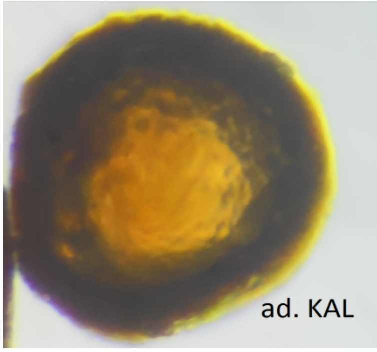
z.-**Glomus** sp.



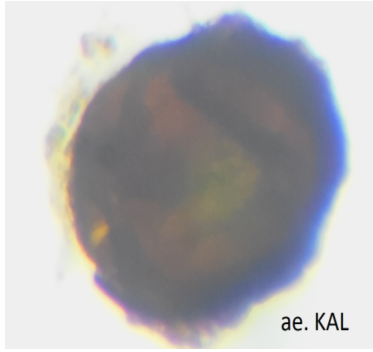
ab.-**Glomus** sp.



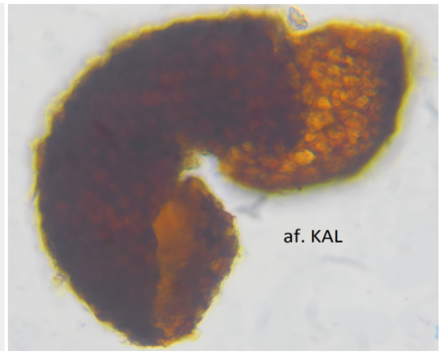
ac.- **Glomus** sp.



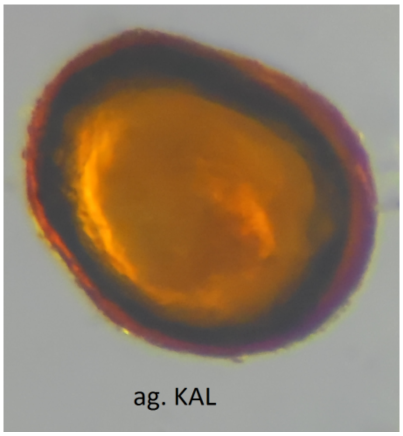
ad.- **Glomus** sp.



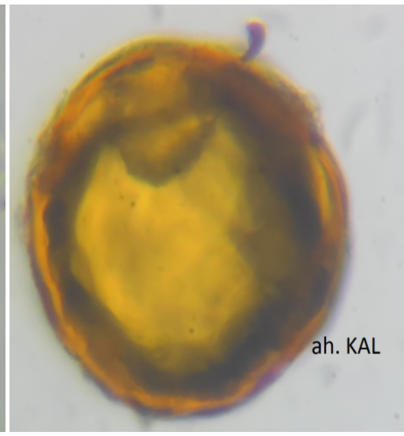
ae.-Sporopcarp



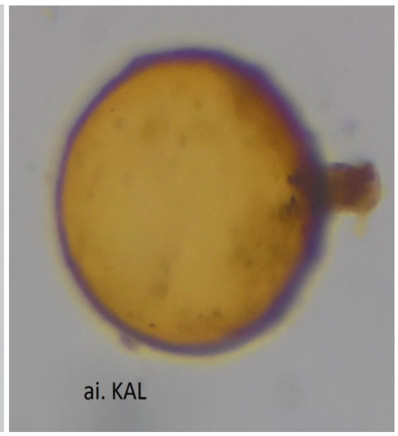
af.-**Acaulospora** sp.



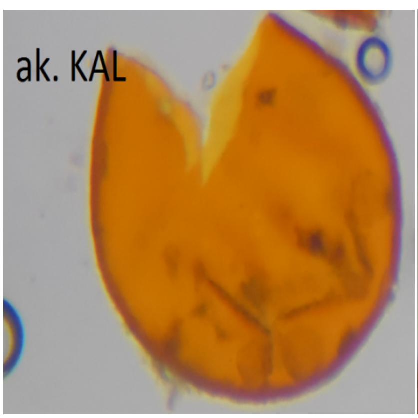
ag.-**Glomus** sp.



ah.- **Glomus** sp.

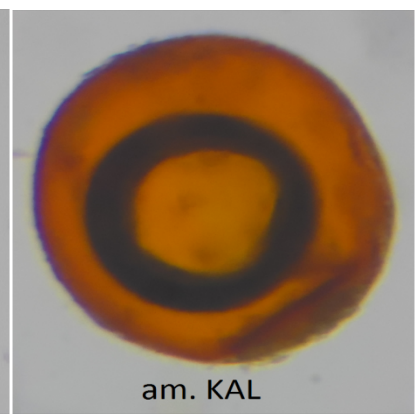


ai.-**Glomus** sp.



ak.-**Gigaspora** sp.

al.-**Gigaspora** sp.



am.-**Gigaspora** sp. , KAL-Kanaipal

IV. CONCLUSIONS

The present study showed important AM fungal spores (AMFS) bin rhizosphere soils in cultivated land of Hatidhara (HC) of Medinipur Sadar under Paschim Medinipur Distriact and in rhizosphere soils of Kanaipal agricultural land (KAL) area under Binpur-I Block of Jhargram District (Formerly Paschim Medinipur District) during winter. The other two types of soil showed less to lesser number of AMFSs as the soil was amended by chemical fertilizers as well as rapid applications of chemical insecticides and pesticides successively during cultivation. They used 10:26 (2 quintal per bigha), urea (40 kg urea per bigha) at Kanaipal potato field. In a gap of one year they used organic manure and farm yard manure to get better productivity. But, after the production potato it is concluded that the potato is damaged by rot disease during and after harvest, which indicates the huge application of urea and other chemical fertilizers. Total dry biomass of potato tuber was less to lesser in compare to the production without or less use of chemical fertilizers and huge applications of bio-fertilizers at other study sites. In compare to the soil of forest, it is evident that forest soil is a good repository of AMF species which exhibit rapid function of the natural ecosystem and get good yield at the end but agricultural soil has less to lesser number of soil get insufficient result. Agro-ecosystem of the same area has less to lesser number of AMF spores which could be the cause for less productivity of agricultural soil. Higher spore density means higher fungal interactions which could be a boon to develop the level of nutrient richness. Not only that it indicates better fungal root contact during monsoon which is a prerequisite for increased benefits of AM symbiosis and better adaptation in the present soils. Seasonal studies round the year or successive years under different management regimes should be incorporated in our research to know the standard of infectivity in different microclimatic sites of agri-horticultural ecosystem even degraded land and cultivated rice field. Pot experiment and specific studies should be conducted immediately to isolate the AMF spore to prepare the VAM bio-fertilizer in near future for rapid application to restore the ecosystem and save the soil micro-flora not instantly but in a long term basis through slowly process.

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