

Colonization of Arbuscular Mycorrhizal fungi (AMF) in Cowpea (VIGNA SPS) from Three Different Jhum Lands in Mizoram

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ABSTRACT

The study was conducted to assess the association of Arbuscular Mycorrhizal Fungi (AMF) in cowpea from three different jhum fallows. The jhum fallows are of three different years i.e., 1-3 years, 4-6 years and 7-10 years. Root samples were taken from cowpea to study colonization of AMF and spores were recovered from the rhizosphere region of the roots. It was observed that the length of jhum fallows as well as human exploitation affects the soil properties and AMF colonization of roots.

Keywords: Jhum lands, Cowpea, AMF Colonization.

I. INTRODUCTION

A mycorrhiza is a symbiotic association between a fungus and the roots of a vascular plant. In this association, the fungus colonizes the host plant's roots, either intracellular as in arbuscular mycorrhizal fungi or extracellularly as in ecto mycorrhizal fungi. They are an important component of soil life and soil chemistry (Sadhana, 2014).

Cowpea is an economically important staple food crop in many semiarid regions of the tropics and subtropics, grown mainly by subsistence farmers under low input of agrochemicals or irrigation water (Timko and Singh, 2008). Cowpea being a protein-rich grain is a suitable complement to starchy tuber crops and staple cereals. It serves as livestock fodder, improves the soil via nitrogen fixation, and benefits households by bringing in cash and diversifying income sources. Vital household income is also generated through the sale of cowpea leaves and stems for animal feed during the dry season. Plant growth is affected by low phosphorous availability in many soils as a result of P fixation by Fe, Ca and Al, which leads to formation of

inorganic phosphates that are insoluble in soil (Ibijbijen *et al.*, 1996). However, AMF have been found to increase nodulation and atmospheric nitrogen fixation potential in legumes such as cowpea (Turk *et al.*, 2008). This is because AMF improves phosphorous uptake by the plant, which in turn would avail more energy for nitrogen fixation by rhizobia. Mycorrhizal colonized roots are highly unlikely to be colonized by other microbes, and their susceptibility to soil-borne pathogens such as phytopathogenic fungi or nematodes is lowered (Selveraj and Chellappan, 2006).

Shifting cultivation is one of the main forms of crop husbandry in Mizoram and other North Eastern Region of India and is commonly known as jhuming. This system often involves clearing a piece of land by slashing and burning of woods followed by wood harvesting or farming. Once the land becomes unproductive, it is left to be reclaimed by regeneration of natural vegetation, or sometimes converted to different long-term cyclical farming practices. The present study aims to investigate the impact of length

of jhum fallows on soil properties and colonization of AMF in cowpea in Mizoram.

II. MATERIAL AND MATHODS

Study sites description:

The study sites i.e., jhumlands were situated at Muallungthu village in Aizawl District, Mizoram. It is a village with about 250 houses, 10kms south of Aizawl. It lies at Latitude 23°36'41"N and Longitude 92°43'10"E. The topography is steep with a number of vegetations were grown. The selected sites were different jhum fallow sites of three different ages, i.e., (i) 1-3 years (ii) 4-6 years (iii) 7-10 years

Sampling Method

Soil sampling was done every month during May - July 2017. The fine roots of the cowpea were taken for observing and quantifying mycorrhizal colonization. Three different jhumlands were taken as study sites as follows

Y1 =1-3 years jhum site,

Y2 = 4-6 years jhum site and

Y3 =7-10 years jhum site.

Soil analysis: Sampling was conducted every month from May to July 2107 by taking about 200mg of soil was taken to study the physic chemical properties of the soil. Soil moisture was determined by drying 10 g fresh soil at 105 °C for 24 h in a hot-air oven. Soil pH was determined using a digital pH meter. Organic carbon was analyzed by colorimetric method (Walkely and Black, 1934). The temperature of soil was taken by using soil thermometer. Available phosphorus was estimated by employing Dickman and Bray's (1940) method using UV-VIS spectrophotometer.

AMF Assessment:

Root colonization:

For the analysis of mycorrhizal colonization in the plants, the roots were samples were washed free of

soil and cut into 1cm long bits, cleared in 2.5% KOH at 90°C for 20-30 minutes, rinsed in water, acidified with 5N HCl and stained in lactophenol containing 0.05% trypan blue (Phillips and Hayman, 1970). 50 segments approx. stained root samples were mounted on slides and examined for AM colonization under a compound microscope at 10x10 magnification. Percent root colonization was calculated (Dhar and Mridha, 2012). Percent root colonization was determined using the following formula:

$$\% \text{ Root colonization} = (\text{No. of positive segments} \div \text{No. of segments observed}) \times 100$$

Statistical Analysis: The individual soil parameters were analysed using mean, standard deviation and standard error using Microsoft excel. Pearson's correlation coefficient was analysed between mycorrhizal colonization and various soil parameters using SPSS.

III. RESULTS

The physical and chemical characteristics of soil in different sites are presented in Table 1. The soil is acidic in all the jhum fallows and lies between pH 4.43 - 5.45. Soil moisture content shows an increase of 23.29% to 27.83%, 25.85% to 28.73% and 24.78% to 25.59% in Y1, Y2 and Y3 respectively from 2016 to 2017. There was increasing soil organic carbon in Y2 (1.96% to 2.43%) and Y3 (1.75% to 1.91%) while decrease in Y1 (1.64% to 1.63%). Phosphorus was found to increase from 3.42 to 3.45 kg/ha in Y1, 2.51 to 2.54kg/ha in Y2 and 3.46 to 3.51kg/ha in Y3 while soil temperature decreases from 2016- 2017 ie., 27.5 to 25.7°C, 32.1 to 25.4°C and 32.8 to 27.8°C in Y1, Y2 and Y3 respectively (Table 1).

Among the three different jhum fallows, Y1 has the least acidic soil which was having the most frequent cycle of jhumming period i.e., 1-3years which is followed by continuation of human exploitation and intermittent farming. It also has the least organic

carbon content as well as available phosphorus compared to the Y2 and Y3.

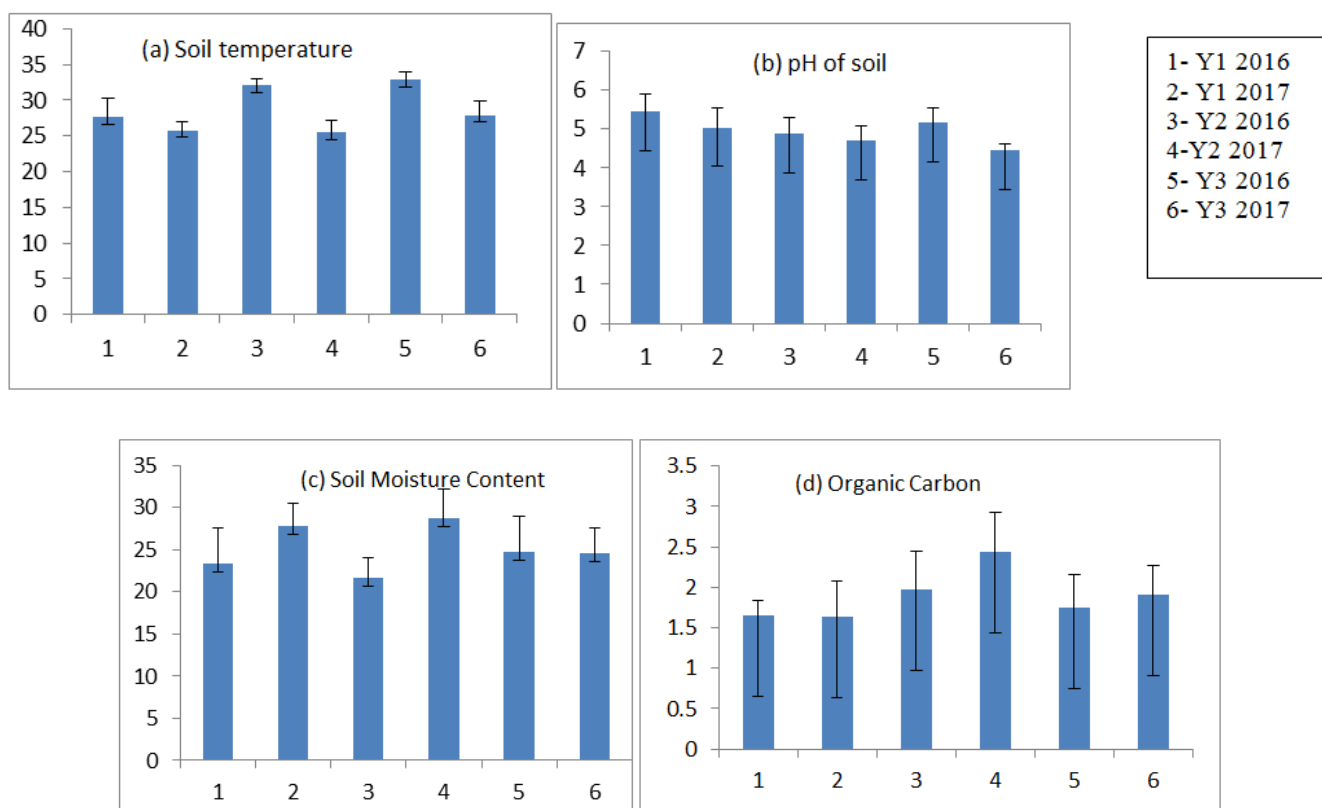
2016 and 2017 and the least colonization was 45%(SD±2.645) and 46.33%(SD±3.05) in Y3 in the year 2016 and 2017 respectively.

The highest colonization was found to be 56.67%(SD±7.023) and 61.5%(SD±2.17) in Y2 in both

Table 1. Selected properties of soil from three different ages of jhum fallows

Sites	2016			2017		
	Y1	Y2	Y3	Y1	Y2	Y3
pH	5.45±0.429	4.86±0.429	5.16±0.368	5.04±0.503	4.69±0.381	4.43±0.185
Moisture	23.29±4.231	25.85±2.645	24.78±2.403	27.85±3.303	28.73±4.105	25.59±2.62
OC	1.64±0.195	1.96±0.47	1.75±0.408	1.63±0.44	2.43±0.489	1.91±0.362
P	3.42±0.101	2.51±0.32	3.46±0.31	3.45±0.12	2.54±0.33	3.51±0.44
Temp	27.5±2.762	32.1±1.201	32.8±0.833	25.7±1.74	25.4±1.039	27.8±2.027
Colony	55.67±6.027	56.67±7.023	45±2.645	57.33±4.509	61.5±2.17	46.33±3.05

Where, Moisture- Soil Moisture Content(%), OC- Organic Carbon(%), P- Phosphorus(kg/ha), Temp- Temperature(⁰C), Colony-AMF Colonization(%).



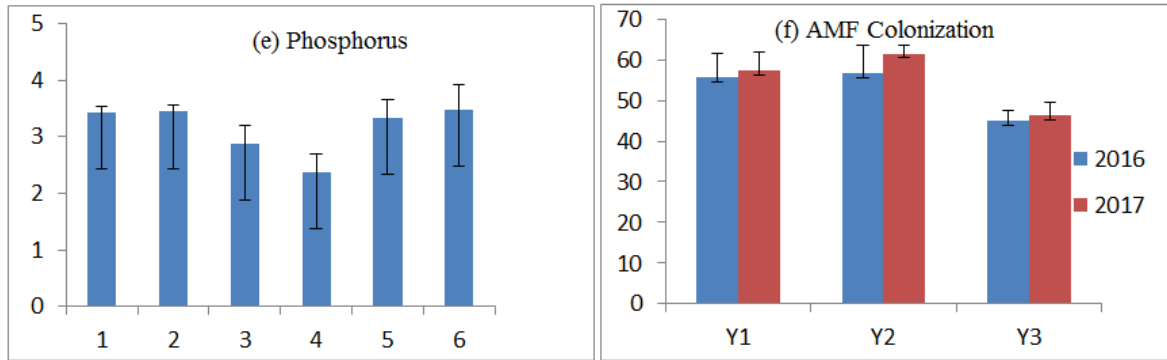


Figure 1(a-f). Bar chart showing mean values and standard deviation of different soil parameter and AMF colonization.

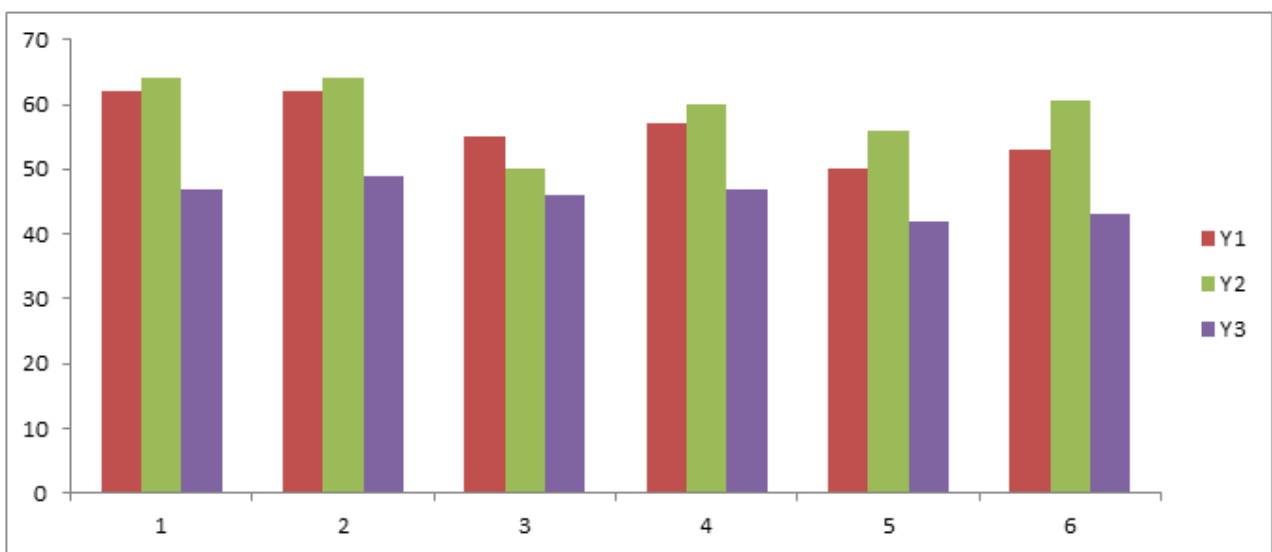
Table 2. Pearson correlation between different soil parameters and colonization of roots in cowpea.

	pH	soil moisture	soil carbon	phosphorus	soil temperature
colonization	.199	.719**	.294	-.344	-.316
pH		.309	-.395	.309	-.406*
soil moisture			.395	-.040	-.078
soil carbon				.090	.205
phosphorus					-.097

N=18

*. Correlation is significant at the 0.05 level (1-tailed).

**. Correlation is significant at the 0.01 level (1-tailed).



Where, 1- May 2016, 2- June 2016, 3- July 2016, 4- May 2017, 5- June 2017, 6- July 2017.

Figure 2. Percentage of colonization of AMF in roots of cowpea in 2016 and 2017.

Figure 2 shows the percentage of AMF colonization in cowpea decreases from May to July in both years. Y2 has the highest colonization percentage in both the years. There was an increase in colonization percentage from 2016 to 2017. Pearson Correlation (Table 2) shows a high significant correlation(0.719**) between soil moisture content and AMF root colonization while negative correlation was observed for AMF colonization with phosphorus(-0.344) and soil temperature(-0.316).

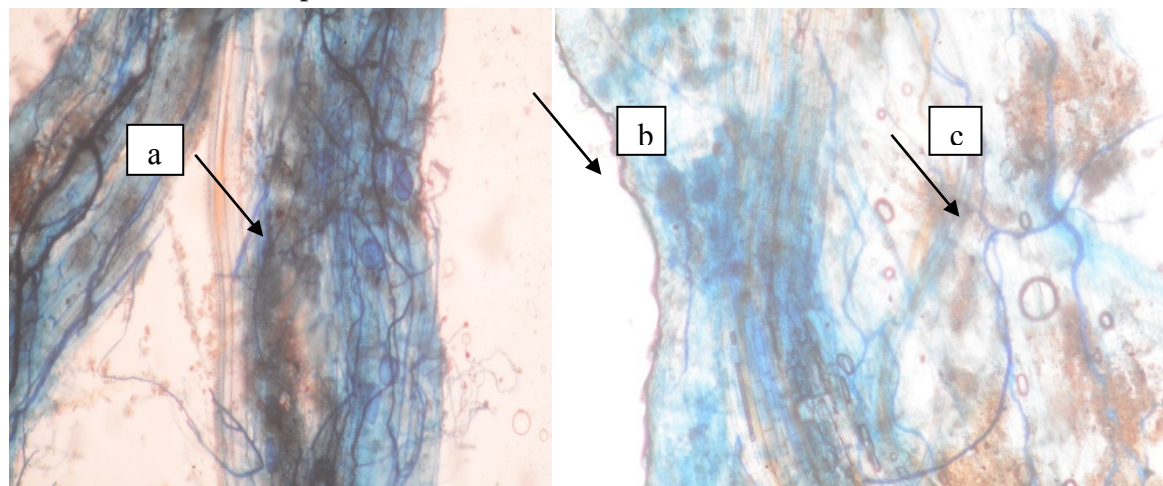


Photo plate showing a.vesicles, b.arbuscles and c.hyphae in the roots of cowpea.

IV. DISCUSSION

The soil moisture was found to be higher in 2017 than the previous year in all the jhum lands which might be due to increase rainfall. There is also simultaneous decline in the soil temperature from 2016 to 2017 which may be attributed to increase in rainfall and soil moisture. The moisture content was also found to have a significant correlation with AMF colonization in the roots from Pearson's correlation (Table 2). Singh (2001) has also reported that soil moisture plays a significant role on mycorrhizal development and colonization.

The soil of the three different study sites were found to be acidic which might be attributed to deposition of above ground biomass through litter fall and in situ-deposition of grasses which resulted in subsequent enrichment with the bases . Soil fertility built up was also considerably affected by short duration fallow cycles (3-4 years) (Venkatesh et al., 2001). A positive correlation was found between soil pH and AMF

colonization which was also found by Chase P and Singh O.P (2014).

Irregular crop-fallow rotation in intermittent agriculture as in Y1 and Y3 resulted in repeated soil disturbances and thus reduced the soil resilience and so restoration capacity as well. In aggradation-degradation cycle of ecosystem health, lessening of jhum cycle to 3-4 years as well as intermittent agricultural production system are certainly acting as a precursor in escalating the pace of degradation over aggradation processes. This may be the reasons why Y1 and Y3 had lower organic carbon content than Y2. It has been reported that the organic carbon and availability of other major nutrient (N, P and K) contents were decreased by 46-73% in just over a span of 3-4 years from fresh burning. However, with the increase in post burning fallow periods from 3-4 years onward, soils exhibited consistent increase in fertility built up, more particularly in 8-10 years old by 27% increase in organic carbon and 30-70% increase in macronutrient contents over 3-4 years cycle

(Funakawa *et al.*, 1997, Kang *et al* 1984,Christanty 1986) .

Y3 has a longest jhum cycle of 7-10years, and was expected to be restored of its fertility. However it was found that it had the lowest colonization of AMF. This may be due to continuation of human exploitation which alter the productivity of the soil. Reduction in the rate of host root infection, formation and colonization of VAM fungi have been reported from disturbed soils (Bellgard 1993; Jasper *et al.* 1987; Mc Gonigle *et al.* 1990).

Y2 has the highest colonization among all the study which might be because the jhum fallow is left undisturbed. This help the land to replenish itself with soil minerals. The phosphorus content in the soil is also the lowest compared to the other jhum fallows. Pearson's correlation shows a negative correlation between Phosphorus and AMF colonization which indicates that the higher phosphorus content have lower AMF colonization. This is in agreement with reports that plants grown in soils with lower available P had a higher AMF colonization rates (Sang Joon Kim *et al.*, 2017). Increases in available soil P levels and related reduced root colonization have also been reported by several workers (Jensen and Jakobsen, 1980; Douds and Schenck, 1990; Miller *et al.*, 1995; Kahiluoto *et al.*, 2001).

V. CONCLUSION

Among the study sites of jhum lands, Y2 (4-6 years jhum) has the most favorable type of soil which promote the highest colonization of AMF in the roots of cowpea. The fallow periods as well as human exploitation affect the soil properties which inturn affect AMF colonization of roots in cowpea.

VI. ACKNOWLEDGEMENT

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VII. REFERENCES

- [1]. Bellgard, S.E. (1993). Soil disturbance and infection of *Trifolium repens* root by vesicular-arbuscular mycorrhizal (VAM) fungi. *Mycorrhiza* 3: 25-29.
- [2]. Christanty, L.(1986). Shifting Cultivation and Tropical Soils: Patterns, Problems, and Possible Improvements. Traditional agriculture in south east Asia: A human ecology perspective, West view press (Boulder, Colarado), 226-240
- [3]. Dickman S.R and Bray R.H.(1940). Calorimetric estimation of phosphate. *Industrial Engineering and Chemical Electrolyte Design*.12. 665-668.
- [4]. Douds, D.D. and Schenck, N.C.(1990). Relationship of colonization and sporulation by VA mycorrhizal fungi to plant nutrient and carbohydrate contents. *New Phytol.* 116, 621-627.
- [5]. Funakawa, S., Tanaka, S., Kaewkhongkha, T., Hattori, T. and Yonebayashi,K.(1997). Soil ecological study on dynamics of K, Mg and Ca and soil acidity in shifting cultivation in Northern Thailand. *Soil Science and Plant Nutrition.* 43 (3), 1997, 695-70
- [6]. Ibijbijen, J., Urquiaga, S., Ismaili, M., Alves, B. J. R., and Boddey, R. M. (1996). Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition and nitrogen fixation of three varieties of common beans (*Phaseolus vulgaris*). *New Phytologist*, 134, 353-360.
- [7]. Jasper, D.A., A.D. Robson and L.K. Abbott. (1987). Effect of surface mining on the

- infectivity of vesicular-arbuscular mycorrhizal fungi. *Australian Journal of Botany* 35: 641-652.
- [8]. Jensen, A. and Jakobsen, I., (1980). The occurrence of vesicular-arbuscular mycorrhiza in barley and wheat grown in some Danish soils with different fertilizer treatments. *Plant Soil* 55, 403-414.
- [9]. Kahiluoto, H., Ketoja, E., Vestberg, M. and Saarela, I. (2001). Promotion of AM utilization through reduced P fertilization II Field studies. *Plant Soil* 231, 65-79.
- [10]. Kang, B.T., Wilson, G.F., and Lawson, T.L. (1984). Alley Cropping: A Stable Alternative to Shifting Cultivation. *International Institute of Tropical Agriculture*, Ibadan, Syria.
- [11]. McGonigle T.P., Miller M.H., Evans D.G., Fairchild G.L., and Swan J.A. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol.* 1990;115:495-501.
- [12]. Miller, M.H., McGonigle, T.P. and Addy, H.D. (1995). Functional ecology of vesicular-arbuscular mycorrhizas as influenced by phosphate fertilization and tillage in an agricultural ecosystem. *Crit. Rev. Biotechnol.* 15, 241-255.
- [13]. Chase P and Singh O.P. (2014). Soil Nutrients and Fertility in Three Traditional Land Use Systems of Khonoma, Nagaland, India. *Resources and Environment* 2014, 4(4): 181-189
- [14]. Philips, J. M. and Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.*, 55:158-161.
- [15]. Sadana, B. (2014). Arbuscular Mycorrhizal Fungi (AMF) as a Biofertilizer- a Review. *International journal of current Microbiology and Applied Science.* pp. 384-400.
- [16]. Sang Joon Kim, Ju kyeong, Eun-hwa Lee, Hyeok Park and Ahn-Heun Eom (2017). Effects of Arbuscular Mycorrhizal Fungi and Soil Conditions on Crop Plants Growth. *Mycobiology.* 2017 Mar;45: 20-24
- [17]. Selveraj, T., & Chellappan, P. (2006). Arbuscular mycorrhizae: a diverse personality. *Journal of Central European Agriculture*, 7(2), 349-358.
- [18]. Singh, S. (2001), Role of mycorrhiza in field inoculation, fungal succession, and effect of climatic and edaphic factors on tree plantations, *Mycorrhiza News*, 12(4):2-12.
- [19]. Timko, M. P. and Singh, B.B. (2008). Cowpea, a multifunctional legume. In: P. H. Moore and R. Ming (Eds). pp. 227-258. *Genomics of Tropical Crop Plants.* Springer Verlag, Germany.
- [20]. Turk, M., Assaf, T., Hamed, K., & Al-Tawahi, A. (2008). Significance of Mycorrhizae. *World Journal of Agricultural Sciences*, 2, 16-20.
- [21]. Venkatesh, M.S., Mishra, A.K., Satapathy, K.K. and Patiram (2001). Effect of burning on soil properties under Bun cultivation in Meghalaya. *Journal of Hill Research.* 14(1), 2001, 21-25.
- [22]. Walkley, A. and Black, I.A. (1934). An examination of Degtjareff method for determining soil organic matter, and proposed modification of the chromic acid titration method. *Soil Science* 37:29-38.