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Impact of burning of postharvest debris on soil arbuscular mycorrhizal flora in agricultural field

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Post harvest burning is a serious problem in India and south east Asia. The crop residue burning adds greenhouse gases and pollutants, which are serious threat to environment. Beside these, burning has an impact on soil microflora. Effect of crop residue burning on Arbuscular Mycorrhizal Fungal (AMF) flora in agricultural soil was studied in two sites, Beraberia than Singdiha from two districts of Purba and Paschim Medinipur, W.B., India. Burning affected the mycorrhizal spore density significantly (p>0.01), especially the smaller spores, mostly belonging to *Glomus*. The larger spores were studied and identified. Total 15 species with larger spores were noticed in both fields, six species were found only in Singdiha, in both burned and unburned sites. *Acaulospora nicolsonii* and *Glomus scintillans* are common in both in burned and unburned soil of both sites. *Acaulospora excavata* and MS8, *Claroideoglomus claroideum* was noticed only in unburned soil. *Racocetra* spp. were noticed only in burned sites.

Keywords: Arbuscular Mycorrhizae, Ecosystem, Post harvest burning, Sustainable agricultural practices.

INTRODUCTION

Post-harvest burning, also known as crop residue burning, is the practice of setting fire on leftover residue in the field after crop harvest. This is a prevalent agricultural practice worldwide to clear and prepare the field for the next crop quickly and economically, but it has a significant effect on the environment and human health. Farming is machine dependent because of the lack of labour and livestock management. The machine harvested remains are more than manual harvest remains in the field and are a real problem for successive cropping, and the easiest solution is burning. Although crop residues are a good source of renewable resources, sustainable management is lacking. In India, crop residue burning is a serious threat to human health, increasing gaseous pollutants and particulate matter in the air, especially in winter (Jain *et al.* 2014). After 2015, the condition worsened (Bhuvaneshwari*et al*. 2019), posing a threat to air quality in Southeast Asia (Singh and Kaskaoutis, 2014; Yang *et al.* 2008).

Despite court orders, the trend is to add greenhouse gases. Burning, though it offers an instant increase in soil nutrients, decreases soil water retention ability and soil sustainability in the long run, negatively affecting soil structure and microbial flora and function (Kumar *et al.* 2019), which may affect productivity in the near future.

Arbuscular mycorrhizal fungi (AMF) are a group of fungi belonging to the subphylum Glomeromycotina (Spatafora *et al.*2016), which symbioses with over 85% of terrestrial plants, including agricultural crops (Mathur *et al.*2018), and can act as bio-protectors of plants (Dey and Ghosh, 2022). AMF are mainly treated as biofertilizers that enhance the uptake of phosphorus (Grümberg *et al.*2014) and other less mobile nutrients (Garg and Singh, 2017). In addition to nutrients, AMF are capable of taking up water from low hydraulic gradients and help plants endure drought (Auge *et al.*2014). Additionally, AMF help host plants cope with other

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abiotic stresses, such as temperature (Bainard *et al.*2014), salinity (Hashem *et al.*2018), and heavy metal pollution (Miransari, 2017). The use of agrochemicals in agricultural fields hampers the diversity and function of AM (Kuila *et al.*2022). Burning may also affect the soil sustainability and crop production. This study was conducted to assess the effects of crop residue burning on the diversity and population of Arbuscular Mycorrhizae in agricultural fields.

MATERIAL AND METHODS

Study Site

Two sites were selected for sampling: Singdiha village located in Dantan-1 Block, Paschim Medinipur district of West Bengal (21°87'71.59ºN-87°29¹50.96 º E). Beraberia village is located in the Panskura subdivision of the Purba Medinipur district in West Bengal (22°33'93.79ºN – 87°78'90.37ºE) (Fig.1). In Singdiha, the temperature ranges from 17 to 21°C in the winter months and from 24 to 41°C in the summer months. The average rainfall is 1550 mm, which occurs mainly during the monsoon from mid-June to August. Soil is sandy loam. The average temperature in Beraberia is 19 °C in winter and 38 °C in summer. The average rainfall is 1600 mm. Monsoon rains can last from mid-June to late-August. Soil is clay loam. This area has four distinct seasons: winter, spring, summer, and monsoon.

Soil Sampling

Soil samples up to a depth of 10 cm were collected from both burned and unburned parts of the same agricultural fields of three different agricultural lands from each village. Soil samples were collected from different sites in clean plastic bags with tags. Each soil sample was spread on clean paper for drying. Large lumps were broken using a wooden roller, and debris was removed from the soil by sieving. Air-dried soil samples were stored in tagged plastic bags at 4°C for spore density estimation.

Spore isolation, study of spore density and identification

Firstly, the separation of Mycorrhizal spores (MS) from soil samples was done by using the wet sieving and decanting method (Gerdemann and Nicolson, 1963) using sieves of the following sizes: 300µm, 180-300µm, 90-180µm, 53-90µm. Residues of sieves were collected separately on filter paper and observed in a petri dish under a stereo-zoom microscope at 40x. Spores of the respective sieves and total spores per 100g of soil sample were counted. Single spores were collected using a capillary tube and mounted on a glass slide with Melzer's Reagent / lactophenol cotton blue and Poly Vinyl-Lacto Glycerol (PVLG). Then, spores were observed under a compound microscope (10x and 40x), to study their size, shape, color, and wall structure. As small spores are abundant and common to both sites, larger spores, (90 µm onwards) were taken for detailed character study. Spores were studied and identification of the spores were carried out by comparing them to the descriptions in Schenck and Perez (1990), and International Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (https://invam.ku.edu/species-descriptions).

RESULTS AND DISCUSSION

The mycorrhizal spore density varied between the two sites in both normal and burned soils (Fig.2 and 3). In normal soil, the density of large mycorrhizal spores was higher in Beraberia, and smaller mycorrhizal spores were higher in Singdiha. The total mycorrhizal spore density was significantly ($p>0.01$) higher (22.25%) in Singdiha than in Beraberia. Large-sized spores (90 to >300µm) were less abundant at both sites, although Beraberia contained little more (Fig.3). Burning affected the mycorrhizal spore density significantly ($p>0.01$), but more so in Beraberia than in Singdiha, especially the smaller spores. The impact on smaller spores is evident in both sites, but more severe in Beraberia than in Singdiha.

Among the 15 species with larger spores, six species were found only in Singdiha, in both burned and unburned sites; *Acaulospora nicolsonii* and *Glomus scintillans* are common in both sites and in both soil type (Table1). *Acaulospora excavata* and MS8 were present only in unburned soil. *Dentisculata nigra* was noticed in only in both sites ofBeraberia. *Claroideoglomus claroideum* was noticed only

Table 1: Number of different AMF spores of >90 µm present in burned and unburned sites of Singdiha and Beraberia per 100 g soil

AMF Species	Spore population/ 100 g soil			
	Singdiha		Beraberia	
	Unburned soil	Burned soil	Unburned soil	Burned soil
Entrophospora sp.	21	32 (52.38%)	-	-
Dentiscutata nigra	-	-	31	9 (70.96%)
Acaulospora excavata	55	-	-	-
Acaulospora foveata	62	30 (51.61%)	-	-
MS5	46	21 (54.34%)		
Claroideoglomus claroideum	-	-	22	-
Racocetra fulgida	-	-	-	11
MS8	37	-	-	-
MS9	42	14 (66.66%)	-	-
Acaulospora nicolsonii	51	20 (60.78%)	24	8 (66.66%)
Glomus scintilllans	54	23 (57.40%)	27	7 (74.07%)
MS12	47	16 (65.95%)	-	-
Racocetra gregaria	-	21	-	8
Ambispora leptoticha	-	-	-	6
Gigaspora albida	48	22 (54.16%)	-	-

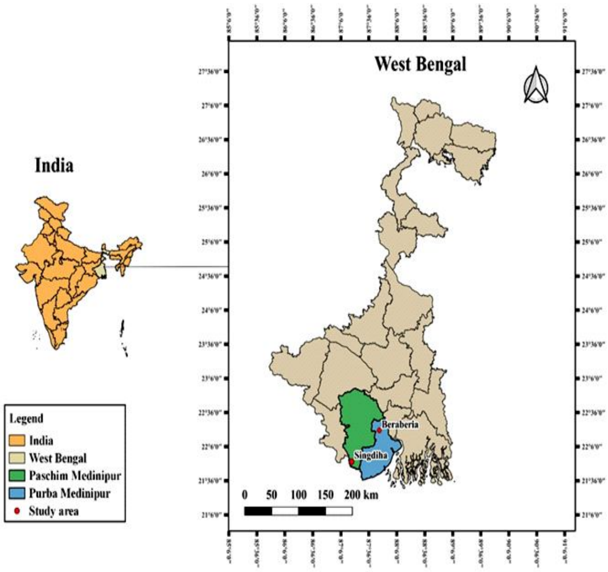

Fig 1: Location of the Study sites

inunburned soil ofBeraberia. *Racocetra fulgida* and *Ambispora leptoticha* was noticed only inburned soil. *Racocetra gregaria* was noticed only inburned soil of both sites. The predominant larger spores in soil collected from sieve size more than 90ìm are mostly identified (Table1) and characteristics are noted as below (Fig. 4).

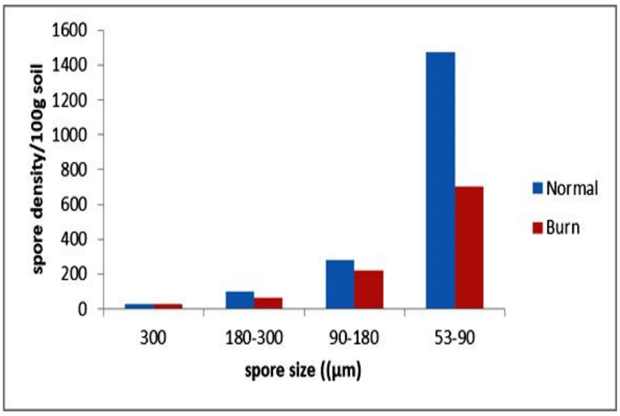

Fig 2: Mycorrhizal Spore Density of Singdiha's Agricultural Land

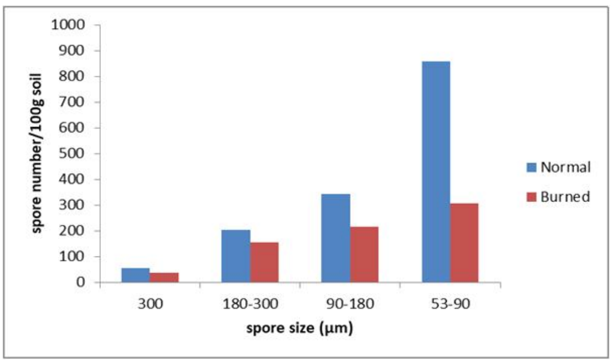

Fig 3: Mycorrhizal Spore Density of Beraberia's Agricultural Land

***Entrophospora* sp.**

The spore was present in both burned and unburned agricultural soil in Singdiha. Normally black colour was seen under the stereomicroscope but in the PVLG, deep brown in colour, elongated shape and 125-180μm in size. The number of spore walls was three, outer wall, middle wall, and inner wall was 2.5μm, 0.5μm, 1μm thick respectively. Mother's vesicle thin-walled, frequent and walls of the vesicular stalk spread to accommodate spore. The outer wall is with two scars on both ends.

***Dentiscutata nigra* (J.F.Redhead) Sievard., F.A. Souza & Oehl**

This spore was found in both burned and unburned agricultural soil in Beraberia. Normally yellow in colour under the stereomicroscope, but in the PVLG, pale yellow to brownish in colour, globose in shape and 255μm-310μm in size. The number of spore walls was two, the outer wall was black to brown, pitted with pores, 2μm thick and the inner wall observed yellow, transparent of several laminas but continuous, it was 1μm thick.

***Acaulospora excavata* Ingleby & C.Walker**

The spore was present in Singdiha unburned soil. Under the stereomicroscope, this spore was black in colour, under the compound microscope deep yellow to brownish colour in PVLG, oval shaped and 270-317μm in size. The number of spore walls was three, outer layer was 2.7μm thick with pit and middle layer was 2.5μm thick and inner layer was 4.2μm thick.

***Acaulospora foveata* Trappe & Janos**

This type of spore was found in both normal and burned soil in Singdiha. The normally brown colour was observed under the stereomicroscope but in PVLG deep yellow to brownish in colour, oval shape and 408-467μm in size. The number of spore walls was two, the outer wall reddish brown and the inner wall yellow in colour. The outer layer was 13μm thick and inner layer was 3μm thick.

MS5

The spore was present in both burned and unburned agricultural soil of Singdiha. Normally it was observed light brown coloured under the stereomicroscope. In the compound microscope, this spore colour was deep yellow to reddish brown in colour, round in shape, 405μm in diameter. The number of spore wall was two, outer layer was 11μm and inner layer was 3.2μm thick.

***Claroideoglomu sclarodium* N. C. Schenck & G.S. Sm.**

This spore was found in unburned soil of Beraberia. Normally light yellow in colour in reflected light, in the PVLG was seen as yellow to light brown in colour, subglobose in shape and 90x155μm in size (approx.). The number of spore walls was four, outer wall was laminated and usually thicker than the inner wall, outer spore wall was smooth but frequent with soil particles. The spore walls were progressively 1.5μm, 0.8μm, 3.7μm, 0.6μm thicker from outside to inside.

***Racocetra fulgida* (Koske & C. Walker) Oehl, F.A. Souza & Sieverd**

The spore was present in burned soil in Beraberia. Normally it was seen as white spore in stereo microscope, but in the compound microscope, was seen as hyaline to light green or pale yellow in colour, globose to subglobose in shape, 175μm-195μm in size. The number of spore walls is two; the outer wall is rigid, smooth, 0.8μm thick, and the inner wall 4.7μm thick.

MS8

The spore is unidentified. It was found in unburned soil in Singdiha. Normally it was observed as off-white or cream coloured spore under the stereomicroscope. In PVLG, it was seen light greenish to yellowish in colour, oval shaped, 186μm to 201μm in diameter. The number of spore walls was two, the colour of outer wall was off-white, 0.3μm thick, and the inner wall was pale yellowish colour, 0.8μm thick.

MS9

The spore is unidentified. It was found in both burned and unburned soil in Singdiha. Normally it was seen as light greenish colour. In PVLG it was seen as green in colour, 235μm in size, and globose shape. The number of spore walls two, the outer wall was 1ìm thick and the inner wall was 0.3μm thick.

Acaulospora nicolsonii **C.Walker, L.E. Reed & F.E. Sanders**

The spore was found in both burned and unburned soil in Singdiha and Beraberia. Normally it was seen as green in colour. In PVLG it was seen as yellow colour, round in shape and 175μm-190μm in diameter. The number of spore walls is three, with the outer wall thicker than the other two walls.

Glomus scintillans **S.L. Rose & Trappe**

The spore was present in burned and unburned soil in both Singdiha and Beraberia. Normally it was seen as brown coloured spore. Under the

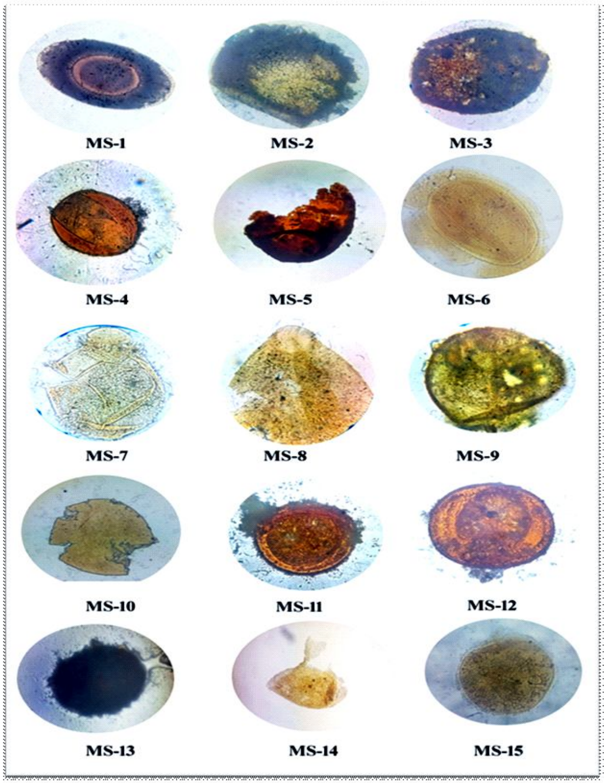

Fig. 4: Spores of arbuscular mycorrhizal fungi in burned and unburned agriculture soil. MS1 – *Entrophospora* sp.; MS2- *Dentiscutata nigra*; MS3- *Acaulospora excavata* ; MS4- *Acaulosporafoveata*; MS5- Not Identified (Burned, Singdiha) ; MS6- *Claroideoglomus clarodium*; MS7- *Racocetrafulgida*; MS8- Not Identified (Unburned, Beraberia) ; MS9-Not Identified (Unburned, Singdiha) ;MS11- *Glomus scintillans*; MS12- Not Identified (Burned, Singdiha); MS13- *Racocetra gregaria*; MS14- *Ambisporaleptoticha*; MS15- *Gigaspora albida*

compound microscope, it was seen as deep brown to reddish brown in colour, oval to round in shape, and 175μm-194μm in size. The number of spore wall was observed three, the outer wall (1.5μm) was thicker than the other two walls (0.7μm, 0.5μm), almost smooth.

MS12

The spore was not identified. This spore was found in Singdiha burned and unburned soil. Normally it was seen as brown colour. In PVLG this spore was seen as yellowish to brown colour, oval in shape, and 405-415μm in size. The number of spore walls two, the outer wall (1μm) thicker than the inner wall (.5μm).

Racocetra gregaria **N.C. Schenck & T.H. Nicolson) Oehl, F.A. Souza & Sieverd**

The spore was found in burned soil in Singdiha and Beraberia. Normally it was seen as brown colour. In PVLG it was seen reddish-brown colour. The shape of this spore was globose to subglobose, and 380μm-520μm in size. The number of spore walls was two; the outer wall was 6.2μm thick and inner wall 5.7ìm thick outer-wall double pores in nature with large pores overlaying a system of smaller ones.

Ambispora leptoticha **(N.C. Schenck & G.S. Sm) C. Walker**

This spore was present in Beraberia burned soil. Under the stereomicroscope, it was seen as 'off-white' in colour and under the compound microscope, this spore was seen as white to pale yellow colour. The shape of spore is irregular and 92μm-155μm in size. The number of spore walls is two, spore walls with adhering debris on the outer surface especially at the hyphal attachment. Spore walls with an indistinct alveolate reticulum of shallow ridges. Outer wall was 2.8μm thick and inner wall was 4.5μm thick.

Gigaspora albida **N.C. Schenck & G.S. Sm**

This spore was found in Singdiha burned and unburned agricultural soil. Normally it was seen as light greenish colour. In PVLG it was seen as greenish to light yellowish in colour, oval in shape and 172µm-201µm in size. The number of spore walls was three, the outer wall was smooth, thin, and 1.5µm thick, the middle wall was 2.5µm thick, the inner wall was 3µm thick, and surface ornamentation of the hyaline knob was present.

Burning caused significant changes in both the species population and diversity in agricultural field sites, although some species were able to resist heat to some degree. Reports on agricultural land are not available. Forestland showed various results. In a neotropical forest type, short-term consequences of the slash-and-burn process showed reduced mycorrhizal colonization and propagules, and alteration of species richness and composition (Aguilar-Fernández *et al.* 2009). The effect of megafire in the Brazilian Cerrado ecosystem reported the recovery of AMF community conditions as per the mycorrhizal parameters evaluated, and spore density and root mycorrhizal colonization rates were similar in burned and unburned areas. The presence of AMF genera did not differ between burned and unburned areas, with *Acaulospora, Claroideoglomus, Diversispora, Glomus, Funneliformis*, *Sclerocystis,* and *Gigaspora* being present (de Moura *et al.*2022).

AMF have a positive impact on soil health by producing organic acids, phosphatases, and glomalin, which stabilize soil particles, protect soil from erosion, improve carbon sequestration, and chelate heavy metals. AMF together with beneficial microbes form a 'mycorrhizosphere' and influence microbial composition and activity. All of these AM activities contribute to soil fertility and ultimately soil sustainability (Fall *et al.* 2022; Kuila *et al.*2022). Spore density increases 52.38% in burned soil compared to unburned soil for *Entrophospora* sp. in Singdiha agricultural land. In Singdiha agricultural land, spore density decreased in burned soil compared to unburned soil for other AMF species *Acaulospora foveata*, MS5, MS9, *Acaulospora nicolsonii*, *Glomus scintillans*, MS12 and *Gigaspora albida*. Besides, In Beraberia agricultural land, spore density decreases in burned soil compared to unburned soil for *Dentiscutata nigra*, *Acaulospora nicolsonii* and *Glomus scintillans*.

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DECLARATIONS

Conflict of interest: Authors declare no conflict of interest.

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