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CROP STRAW BURNING PRACTICE-A THREAT TO ARBUSCULAR MYCORRHIZAL BIODIVERSITY

GHAZALA NASIM

Institute of Agriculture Sciences,
University of the Punjab, Quaid-e-Azam Campus, Lahore, 54590, Pakistan
E-mail: ghazalanasim@hotmail.com

Abstract

The burning of crop straw or vegetable remains is a traditional agricultural practice in many countries of the world including Pakistan. Present study reveals that the crop straw /veg remain burning practices in urban Pakistan is a growing threat to the biodiversity of arbuscular mycorrhizal (AM) fungal communities in the region. The study reports that some of the species of AM fungi use these plant portions as their ecological niches and categorically sporulate in decaying sheathing leaf bases/non root portions like scale-leaves of cereal crops and vegetables. This includes species of *Glomus*, *Sclerosystis* and *Acaulospora*. Setting the left over plant materials into fire has lead to complete burning of the biomass into ashes and sterilization of upper 10-15cm of surface soil (Fig. 1). This practice if continues may totally eliminate the threatened species like *Glomus monosporum, Acaulospora bireticulta* and *Sclerocystis pakistanica.*

Fig. 1. Wheat fields around university campus set on post harvest fires.

Introduction

Arbuscular mycorrhizae (AM) are a unique example of symbiosis between two eukaryotes, soil fungi and plants. This association induces important physiological changes in each partner that lead to reciprocal benefits, mainly in nutrient supply (Bajwa *et al*., 1999; Balestrini & Lanfranco 2006; Jalaluddin *et al*., 2008). These fungi have been reported of much wider occurrence than had been considered earlier (Nasim & Iqbal, 1991). Mycorrhizal biodiversity plays a significant role in the ecosystem dynamics and the biogeochemical cycling in forestry or agricultural scenarios. They have been documented to sporulate in the soil or within decaying underground plant portions including roots, sheathing leaf bases, scale-like leaves of modified plant portions like corms and rhizomes (Nasim 1990; 1991; Nasim & Iqbal, 1991; Iqbal & Nasim, 1991; Nasim *et al*., 1991). These have been found forming spores and sporocarps in association with decaying and moribund root pieces and soil aggregates (Nasim, 1992; 2006). Spores of *Glomus mosseae* and *G. fasciculatum* have been isolated from buried *Dalbergia* leaves in the soil of agricultural field (Nasim *et al*., 1996). While in another study the sporocarp formation of *Glomus monosporum* have been observed in the sheathing leaf basis of wheat (Nasim & Zahoor, 1997). Similar observations have been made in the case of rice (Nasim & Bajwa, 2007), maize and sugarcane (Nasim *et al*., 2008) fields in Punjab, Pakistan.

Previous studies have indicated that burning practices meant for rapid recycling of the left over crop straw or other residues affect the AM diversity and density in the upper few cm layer of the soil (Khalid *et al*., 1991; Iqbal *et al*., 1991). However there has been no report of the affect of these activities on the spores/sporocarp populations of AM species which utilize the decaying crop straw as their habitat niches. The present study attempts to document the effect of burning the left over crop straw on the mycorrhizal biodiversity in a wheat field with particular reference to *Glomus monosporum*. We wanted to verify the following hypothesis:

i. Whether or not the number of AM propagules is correlated with the soil depth in the two scenarios.
ii. The %age of viable propagules is correlated to soil depth in two situations.
iii. What are the individual responses of AM propagules of 5 species, including three rare and two commonly occurring ones, to this common practice?

Materials and Methods

Sample collection: The collections on which this study is based are from 3 fields each of wheat, rice and maize in and around new university campus (Fig. 2). Most of our personal collections have been in the form of separate soil samples (10g each) taken randomly from a 100 m^2 area. Samples for study were collected in a way that their aerial portions and root systems (particularly fine roots) were least disturbed. Extra care was taken to sample decaying leaf bases, runners of the grasses and rhizosphere soil. Aerial portions were pressed in the folds of blotting paper at the spot, while roots, runners, decaying leaf bases along with rhizosphere soil were carefully brought back to the laboratory in plastic bags.

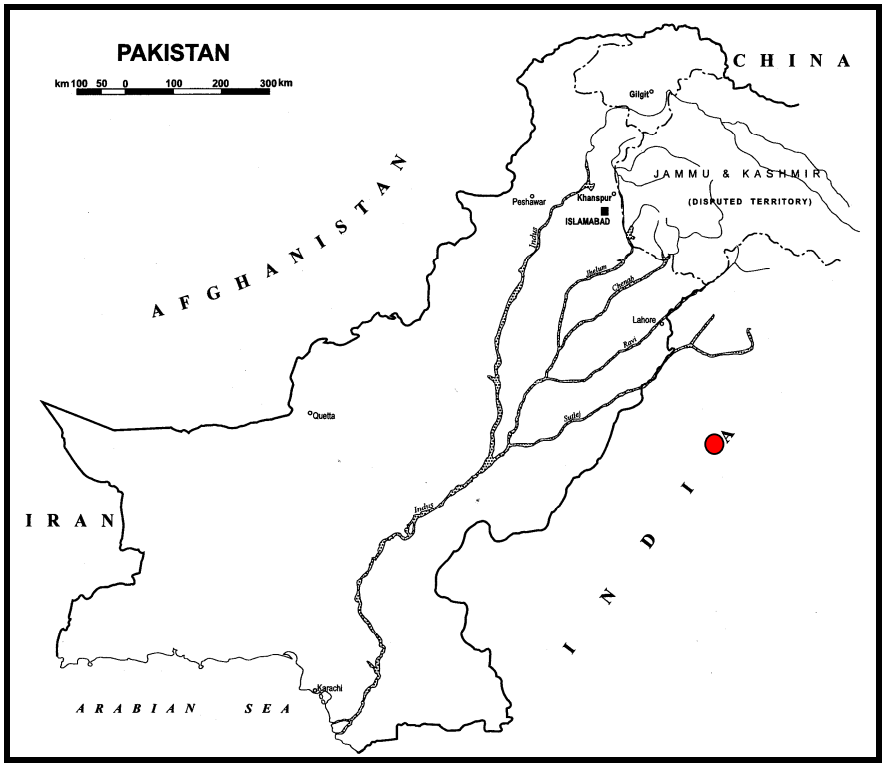


Fig. 2. Map showing the site where the study had been carried out.

Processing of plant materials: The roots were washed thoroughly and fixed overnight or stored in formaline acetic acid alcohol (FAA) in 5:5:90 ratios. Before further processing the roots were rinsed with several changes of tap water to remove FAA. Samples were then transferred into 10% (w/v) potassium hydroxide solution on autoclaved resistant jars (McCartney bottles) and then autoclaved for 15 minutes at 121°C. Samples with delicate roots may require shorter time. After samples have cooled these were rinsed with several changes of tap water followed by deionized water. The roots may be bleached in H_2O_2 (10%) depending upon the presence of dark coloration. After dipping the root system in H_2O_2 for 3-5minutes it has rinsed with several change of tap water. Afterwards the samples were washed with N/10 HCl and washed again. This step of bleaching is very sensitive. A little bit of carelessness may destroy the sample. The roots were then transferred into equal volumes of 85% lactic acid, glycerine and distilled water with 0.05% (w/v) trypan blue or 0.1% (w/v) chlorazol black E for one hour or longer at approximately 90°C. Time may be saved by autoclaving the samples at 121°C for 2-3 minutes. The stained material was then transferred into glycerine for storage or observation. After destaining for overnight in glycerine the roots were mounted in a mixture of glycerine and lactic acid to make semi-permanent slides

Different plant portions were processed separately. Plant parts like roots, leaf bases and rhizome fragments were sorted out. Each of the sample was washed under tap water. Clearing was done in 10% KOH by autoclaving for

2-3 minutes. Samples that remained dark coloured after clearing in KOH were bleached in alkaline Hydrogen peroxide (Koske & Gemma, 1989). These bleached plant portions were washed with 0.01N HCL to neutralise. Staining was done in acidic glycerophenol containing 0.05% trypan blue (Phillips & Hayman, 1970).

Spore extraction from rhizosphere soil: The list of Endogonaceous spore was prepared by examination of the roots of mature plants for the presence of mycorrhizae, direct examination of soil samples and rhizome fragments, for the presence of AM leaf bases of some of the plant species were also processed for examining the role of organic matter in the spread of mycorrhizal fungi. Endogonaceous spore in the rhizosphere were extracted by wet sieving and decanting technique (WSDT) of Gerdemann & Nicolson (1963) and soil paste method (SPM) of Nasim & Iqbal (1991).

For WSDT, 100 g of soil from each sample was suspended in one litre of water ad wet sieved using sieves of 400, 105 ad 63 um pore diameter, placed one above the other in descending order. The contents retained on each sieve were transferred to a beaker of water. The supernatant was filtered and filter paper was examined under a microscope. Total number of spores on the filter paper was counted. Similarly materials collected from each sieve was examined and the total number of spores on all sieves indicated the total number of Endogonaceous spores of different sizes in 100 g of soil samples. To record species diversity slides (semi-permanent) of each morphologically different spore were prepared. Identification was done following the keys of Schenck & Perez (1987) and Morton (1988).

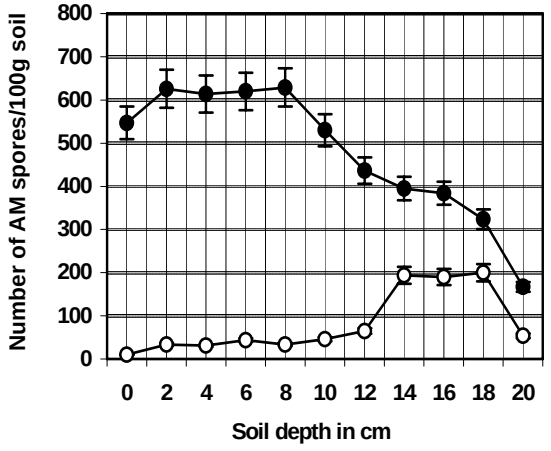
For soil paste method (SPM) of spore extraction, 20 g of soil was spread into a thick paste and spores were directly picked up with a sharpened toothpick or hypodermal needle under a dissecting microscope. After washing several times, spores were mounted in lactophenol. A KOH solution was not used as a mounting medium for Endogonaceae, as it caused extreme swelling of some spore walls with a resulting distortion of spores (Gerdemann & Trappe, 1970). However, 5% KOH is useful for certain purposes e.g., revival of collapsed, thin walled structures such as gametangia and vesicles.

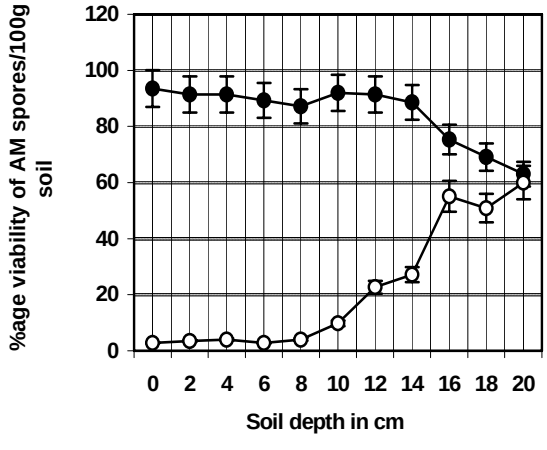
Viability experiments: Spores extracted were used as inoculum for wheat grown in aseptic conditions and the establishment of AM associations was quantified in terms of extent of colonization, formation of vesicles and arbuscules along with external spores if any in mixed propagule inoculum and on individual basis for five major species.

Results

Number of AM propagules and soil depth: As regards the data recorded for the number of AM propagules at different levels along the vertical soil profile, there had been a decrease in values as the depth increased. Though in the case of un-burnt soil, the number of spores and sporocarps at the soil surface has been low but as compared to the subsequent sample values, the difference had been non-significant (Fig. 3). In soil samples from 2, 4, 6 and 8cm depths the spore number remained parallel however in the later samples from an increasing depth the number decreased sharply. The decrease recorded was from above 600 propagules per 100 gram soil to around 200 per 100 gram soil. In comparison to this, the propagule number was significantly low at the surface of burnt soil with a gentle increase in values with an increasing depth. From 12cm to 14cm depth the increase in number was significantly high. The values remained high (around 200 spores per 100g soil) till a depth of 18cm after which the values decreased sharply.

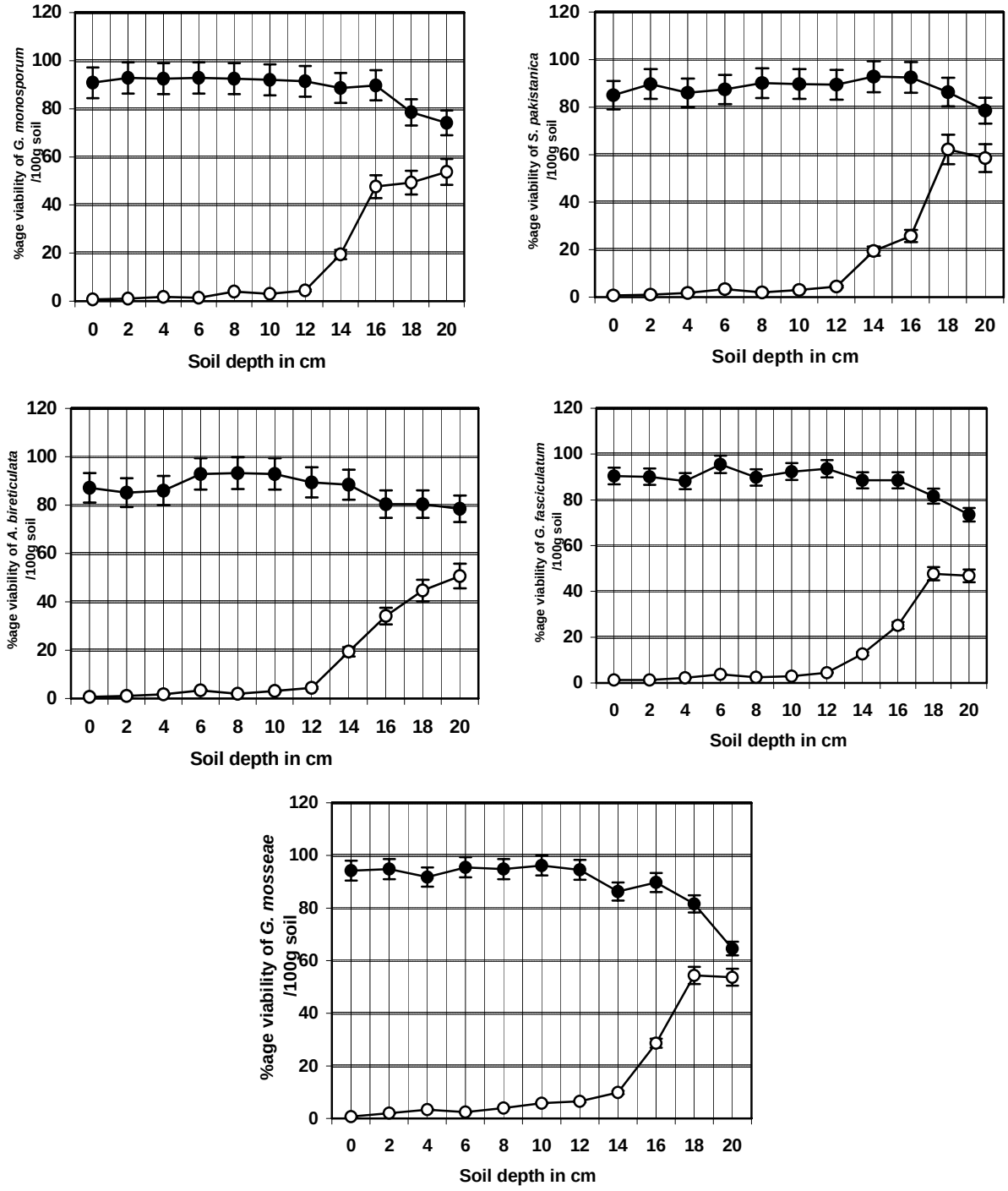
Overall % age of viable propagules: The percentage viability of spores recorded in terms of percent root colonization and formation of arbuscules and vesicles in wheat plants grown in controlled conditions, exhibited a drastic difference between the samples studied from tow scenarios. The viability was extremely poor from the surface of the burnt soil to a depth of 8 cm after which it increased steadily up till 14cm depth. From 14 to 16 cm depth the viability sharply increased from 28 to 55 spores per 100 gram soil. The highest values recorded for this parameter were at a depth of 20cm where it almost coincided with that of sample from the un-burnt situation. At this depth the values for the unburnt soil had been minimum. The maximum percentage of viable propagules had been in the upper 12-14cm layer of soil. From 14cm to 20cm the values decreased sharply (Fig. 4).





Figs. 3-4. The number of AM spores and %age viability in burnt (open circles) and un-burnt (solid circles) wheat field.

Individual responses of AM propagules: The 5 species selected for the individual inoculation and viability percentage data were *Glomus monosporum*, *G. mosseae*, *G. fasciculatum*, *Acaulospora bireticulata* and *Sclerosystis pakistanica*. The selection was primarily based on the previous finding with respect to the mode of sporulation of the said species and their abundance in the wheat field soil. Spore viability for *Glomus monosporum* remained uniform in upper 16cm soil horizon in the unburnt situation after which it decreased. The decline in percentage viability was however non significant when compared with the values at 16cm depth and also for the preceding readings. In the case of fields where straw burning had been practiced the percentage of viable propagules remained at a very low profile to the depth of 12cm. Beyond this depth, it increased to an appreciable value finally becoming very close to the un-burnt field readings. Same trend in the viability percentage of *Sclerocystis pakistanica*, *Acaulospora bireticulata*, *Glomus mosseae* and *G. fasciculatum* was observed, however minor variations in between these AM species could be observed (Figs. 5-9).



Figs. 5-9: %age viability of five species of AM fungi in burn (open circles) and un-burnt (solid circles) wheat field.

Discussion

Arbuscular mycorrhizal biodiversity is an essential component to ensure the establishment and subsequent growth of plant communities in managed and natural ecosystems. These are a group of obligate fungi not being able to grow in exenic culture. These fungi sporulate in association with their host species. About 40 years ago when the spore forming structures of these fungi were first discovered, no one could think of unusual niches they may occupy to form propagules. A number of papers published in 1980s and later have shown that these fungi sporulate in the heating leaf bases of grasses, scale leaves of rhizomes, corms, bulbs and decaying leaf liter. The fungi like *Glomus monosporum* is specifically associated with wheat forming sporocarps in the decaying sheathing leaf basis of the stumps left after crop harvest (Fig. 10). The sporocarps when get mature, the spores, 1 or 2-3 in number, are released into the soil which initiate AM colonization in the next crop thus serving as inoculum for the incoming crop. The burning of the crop straw is one of a number of cultural practices being carried out in crop growing areas of Pakistan. The results of the present study have shown that the propagule number and viability of the remaining ones has tremendously decreased after burning episode. Species like *Glomus monosporum* are the most affected ones utilizing the stumps for their hideout niches. It is therefore suggested that in view of rapily decreasing spore bank in the soil, the practice of setting the left over debris into fire may be prohibited. This would not only let the ecological biodiversity of AM fungi to thrive, but would also save the environment from noxious gaseous emission released as a result of burning of crop straw.

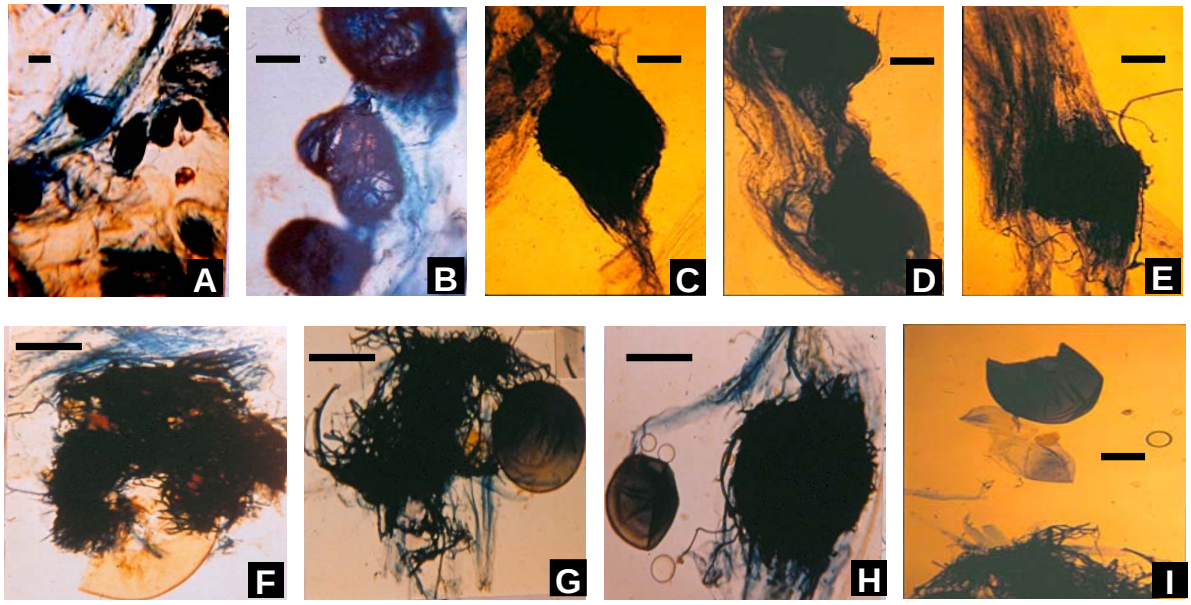


Fig. 10. Stages of sporocarp formation and release of spore by *Glomus monosporum* in sheathing leaf bases of wheat stumps (Bar=50μm).

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