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Assessment of Fungal Population in Rhizospheric Soil of *Triticum aestivum* (wheat) under the Influence of on-field Crop residue Management Practice of 'stubble Burning'

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ABSTRACT: Soil ecology is an extensive area of research. The soil microbiome is a collective terminology used for all the microbial communities residing in the extended rhizospheric area. This includes bacteria, fungi, protozoa, archae and virus. Fungi is an important inhabitant of wheat rhizosphere and is directly involved in sustainability, enzymatic breakdown of organic matter and improvising plant health in a variety of ways. Stubble burning always remains a topic concern for soil ecology. In order to understand the fungal component of soil ecosystem and the impact of alternative human practices on rhizospheric fungi colony forming units (cfu) count remains a good choice. Assessing the number of viable fungal cells in wheat rhizosphere by plate count method helps in interpreting the harmful effects of stubble burning on live cells and their ability to grow. Soil collected from wheat fields at two different intervals from different regions of Madhya Pradesh was subjected to viable plate count method to assess the number of microorganisms present in the soil. The results obtained showed a visible disparity among the samples of before burning and after burning soil. The cfu count was calculated as total fungal count, including the yeast and mold count. The total cfu count was reduced from 5.61 × 10^4cfu/gram of soil to 2.65 × 10^4cfu/gram of soil in the wheat fields of Madhya Pradesh. All the 50 samples collected from the rhizospheric region had different fraction of fungal population depending on soil nutrient characteristics. Cultivating fungus in vitro, is a difficult task to perform as fungal mycelia may damage while sample collection. To minimize this damage, stubble was collected along with roots adhering with rhizospheric soil. The article's goal is to provide a succinct overview of the current situation with stubble burning, loss of fungal biodiversity, and available solutions to improve the sustainability of the agriculture industry.

Keywords: Rhizosphere, *Triticum aestivum,* plate count, cfu count, stubble, soil ecology.

INTRODUCTION

The world's population, which is expanding at an incredible rate. In order to satisfy the daily hunger, the majority of people on the planet rely on the staple food crops, including wheat, rice, and maize. In India, Madhya Pradesh is one of the fifteen agro-climatic zones and is located in the Central Plateau and Hills region (Gangwar and Singh 2011). Even while microbial variety makes up the most astonishing and pervasive life on earth, it is not evenly distributed in the planet's varied ecosystems (Prashar *et al.,* 2013). Nutrient rich niches such as rhizosphere are the regions which allow root growth, respiration and abundance of microbial communities which are primarily made up of bacteria and fungus. (Pérez-Jaramillo *et al.,* 2016; Qin *et al.,* 2019). The microbial life that resides in the soil where plants are grown, coexists in close association with plants. Root exudates play an important role in attracting microbial life as it contains a number of metabolites (Korenblum *et al.,* 2022). Diversity of microorganisms in soil is so vast (Berendson *et al.,* 2012) and difficult to culture that only 1% of soil microorganisms can be cultured in laboratory (Conn, 1918; Schoenborn *et al.,* 2004). The same phenomenon goes for fungi (Van Elsas *et al.,* 2000) which are an inherent part of soil ecosystem specially in organic matter decomposition.

In order to increase production to meet global needs, a continuous cropping system is required (Kumar *et al.,* 2019). This can be accomplished in one of two ways: either by expanding the area under cultivation or by improving the yield per square foot which can be done by enhancing the quality of soil (Etesami *et al.,* 2021).

Fungal diversity in soil: It's importance and benefits. Fungi is a diverse group of organisms, found in soil which helps in decomposition (Bridge and Spooner 2001), as a biocontrol agent, eg. *Trichoderma* (Tyśkiewicz *et al.,* 2022), helps in promoting plant growth and metabolism (Baron and Rigobelo 2022) and increases the ability of endophytic fungus and their host plants in responding towards biotic and abiotic challenges by producing bioactive compounds that are unique to the host plant (Ali *et al.,* 2018) (Fig. 1). Hence, rhizospheric fungi are an essential part of the microbial population in soil and are crucial to the growth and health of plants, and plants in turn substantially regulate rhizospheric fungus (Qiao *et al.*, 2019).

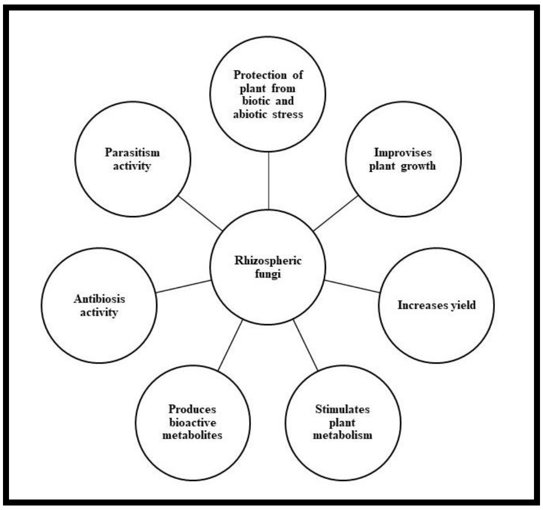

Fig. 1. Multidisciplinary role of soil fungi.

Rhizosphere is the most suitable region of studying the activity between microorganisms and plant system (Li *et al.,* 2021). Plant-fungal mycorrhizal associations involve interactions between plants and fungus in both the rhizosphere, which surrounds the roots, and the mycorrhizosphere, which surrounds the fungal hyphae (Johansson *et al.,* 2004).

Agricultural crop residue. Wheat (*Triticum aestivum* L.) is one of the three largest cereals crops grown along with corn and rice. It is a high reserve of energy for the population around the globe (Çakmakçı *et al.*, 2017). Madhya Pradesh is an agriculture dominant state. Nearly half of the state's total geographic area, 14.9 M Ha (almost 50%) is devoted to agricultural practices. A larger portion of the area (62%) receives moderate to heavy rains during the monsoon season which ensures good fertility. This region is dominated by black and red soil of low to medium fertility (ICAR, n.d.). The term agricultural waste is a vague terminology, covering all aspects of waste arising from the time of sowing uptill post harvesting is done. There can be two major types of harvest waste, field residues and process residues. Field residues are left in the agricultural field itself after the crop has been harvested, for example, stalks, seed pods and stubble. Process residues are leftovers after converting a crop in some useable product, such as sugarcane bagasse (Bhuvaneshwari *et al.,* 2019).

In absence of adequate knowledge and lack of resources, farmer's find it easy to burn crop residues of rice, wheat and sugarcane, the most produced crops, in India. Stubble burning has a huge adverse effect on soil ecology (NPMCR, n.d.). Colony counting has been a traditional method employed to count viable units of fungi (Waksman, 1922). The current study emphasizes on the fungal count deterioration as a result of stubble burning practice for clearing farm lands.

The know-how of fungal communities benefitting the plant rhizosphere has been an immense field of research. Consequently, loss of these communities would have some impact on the plant life. The purpose of this article is to present a concise overview of the existing situation, the difficulties farmers face on a daily basis for land clearing, and potential remedies in order to enhance India's agricultural industry. The practise of sustainable agriculture is strongly endorsed because as it is safer than conventional farming. It is a practical answer to many problems agriculture industry is encountering (Dar *et al.,* 2019).

MATERIALS AND METHOD

A. Sample Collection

Wheat fields' identification for soil sample collection was done during the month of February and March, 2022. The areas selected were Narmadapuram22.7519° N, 77.7289° E, Budni 22.7746° N, 77.6826° E, Powarkheda 22.7065° N, 77.7398° E and Jatkhedi 23.1739° N, 77.4788° E, all in the state of Madhya Pradesh. Soil samples from the rhizospheric regions were collected aseptically with the help of clean shovel digging the top soil for about 10-20 cm. Soil sample collection was done in two phases; the first one was collected from 90-100 day crop period during the month of March 2022 and were designated as Before Burning samples (BB). The second sampling was done immediately within a week period of crop stubble burning (March-April 2022) and were designated as After Burning Samples (AB). 50 samples from before burning period and 50 samples from after burning period were collected making a total of 100 samples. Sample collection was done manually wearing sanitized gloves in sterilized zipper storage bags and kept at 4ºC up till further use.

B. Dilution of Samples

Serial dilution (Jett *et al.,* 1997) is a technique used for reducing the quantity of microorganisms while culturing in laboratory. The preserved soil samples at 4ºC were brought to room temperature for serial dilution. 1 g of sample was weighed and mixed with 10 ml of distilled water. The prepared soil solution was kept on a stirrer for 5-10 minutes for uniform mixing of soil. The soil particles were now allowed to settle down and the above translucent solution was further used for serial dilution. A series of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilutions was subsequently prepared from the starting solution.

C. Media selection

A suitable culture media for fungi was taken which promotes growth of different fungal species as the motive of the study is assessing overall viable fungal cells and not any one in particular. Potato dextrose agar (PDA) medium is a high carbohydrate source media with a pH range range of 5-6 which helps in fungal germination found in soil samples (Basu *et al.,* 2015). Media composition used for the preparation of PDA plates is as follows: Potato infusion (200 g raw potato), dextrose (20 g), agar (20 g) and distilled water (1000 ml).

D. Quantitative Assay

Microbial count is a difficult task to perform. For enumerating the viable number of fungi, colony forming units (cfu count) was calculated on PDA plates. 100 µL of 10^{-2}sample was taken and inoculated in Potato dextrose Agar (PDA) plates. Samples were taken in triplicates and inoculated by the spread plate method. The plates were incubated at 37ºC for 2-6 days depending upon different types of soil samples taken. Total yeast and mold count was taken into consideration for assessing the viable number of microbial population. Colony forming unit (cfu) count was calculated as per the following formula on PDA plates:

cfu/g of soil = (no. of colonies × dilution factor)/inoculum volume

RESULTS AND DISCUSSION

The cfu count was calculated during a period of 3-7 days of incubation depending upon type of soil sample and speed of growth. Stubble burning had drastic effects on the microbial count which became evident by the decrease in number of viable colonies in after burning samples. Among the 50 samples collected, 5 representative results of colony count on PDA plates have been shown in Table 1, which depicts a percentage loss in colony counts of about 67-94 % due to stubble burning. Fungi is an active participant of soil. Counting their numbers in rhizospheric area deciphers involvement of these microorganisms in plant-soil ecosystem. Samples showing vast mycelial growth can be compared to the ones having almost no growth of fungi. (Fig. 2&3). Previous studies related to assessment of fungal population in rhizospheric regions supported our results. Even after a period of 60 days post stubble burning, fungal population was found higher in stubble retained fields and lowest in burnt field. This shows the drastic impact of stubble burning on soil microorganisms (Vieira and Nahas 2005).

A graph showing comparative number of colonies from before burning and after burning samples from the fields showed a severe decrease in number of viable cells (Fig. 4). The total cfu count of all 50 samples which included the number of yeast as well as mold count dropped down from 5.61 × 10^4cfu/gram of soil to 2.65 × 10^4cfu/gram of soil. The results obtained were in accordance withpopulation of fungal cells in plots experiencing stubble burning as compared to those where stubble was removed in the Indo-Gangetic regions. The cfu count went down from 3.42 × 10^4cfu/g soil to 2.33 × 10^4cfu/g soil as reported by Kumar *et al.* (2019). The population density of fungus *Trichoderma* spp. and bacteria *Thiobacilllus* spp. was also reduced in experiments done by Alkooranee *et al.* (2022) in crop fields where crop residue was burnt. Their experiment's findings demonstrated that the burning process has a detrimental impact on the population density of soil microorganisms, which exist 10 cm below the soil's surface.

Even plants prefer a particular microenvironment. They exude carbon molecules into the soil which enhances the growth of particular bacterial and fungal species around their roots. As a result, many plants have a preferred fungus to bacterium ratio (F:B ratio) Hoorman (2011), the F:B ratio for wheat ranges from 0.8 to 1.1.

Soil samples collected prior stubble burning had an undisturbed soil microflora. Fungal mycelia specially get distressed due to various conventional agricultural practices. Samples collected from undisturbed fields exhibited significant fungal growth in vitro (Fig. 5 a-d). The reduction in fungal population from the microbiome indicates a probable decrease in crop yield and crop quality. On the contrary farmers satisfy this microbial depletion by artificial fertilizers. Pest damage is a serious issue for farmers. In the absence of natural biocontrol agents, the conservation and protection of crops frequently depend on the use of pesticides and Madhya Pradesh is one of the leading states in use of pesticides (Balkrishna *et al.*, 2021). A model of sustainable energy management has been studied in Indo-Gangetic plains in India after the threats imposed by conventional crop residue management practices in agriculture. Technologies which aim conservation agriculture were studied in five-year long research done by Jat *et al.* (2020). The strategies include minimum tillage, crop residue retention and efficient crop rotation which projected crop residues as a source of renewable energy instead of a burden to be cleared off from fields. The experiment was targeted on rice-wheat and wheat-maize crop rotation systems. The benefits received from crop retention were tremendous, it improved crop productivity, soil quality and environmental health.

The current research not only elucidates the biodiversity loss in soil in stubble burning areas, rather it also aims to explicate and develop the idea of sustainable agriculture. As explained by Cardoso *et al.* (2013), "soil health" instead of "soil quality" refers to a soil's potential to maintain an ecologically stable ecosystem is now a reference towards sustainable agriculture.

Table 1: Comparative analysis of some samples showing extreme loss of viable fungal cells after stubble burning.

Sr. No.	Sample number	No. of colonies before burning	No. of colonies after burning	% loss(Rounded off)
1.	BB/AB- 17	78	22	71
2.	BB/AB- 31	101	29	71
3.	BB/AB- 39	125	40	68
4.	BB/AB- 47	18	01	94
5.	BB/AB- 50	86	27	67

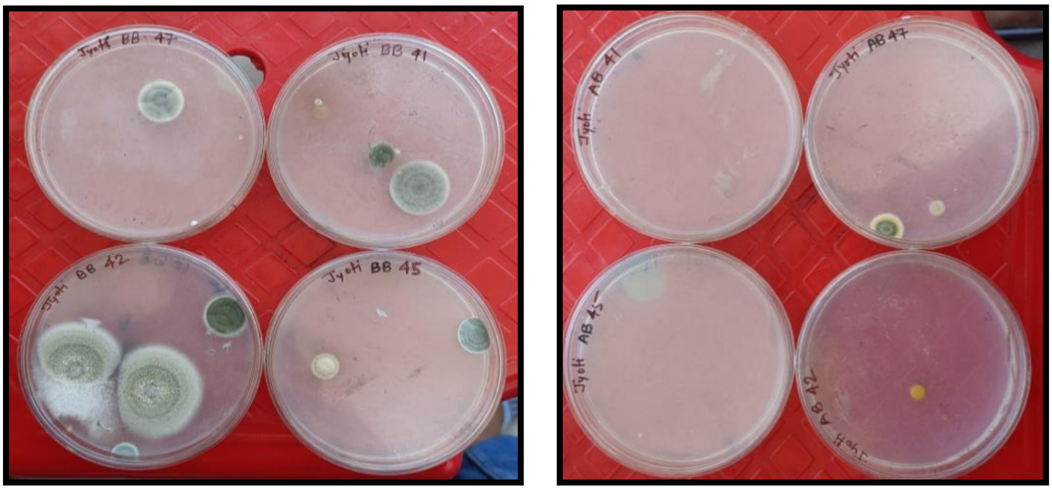

Fig. 2 & 3. Representative images of fungal colonies on Potato Dextrose Agar showing extensive growth in before burning samples (BB) as compared to after burning (AB).

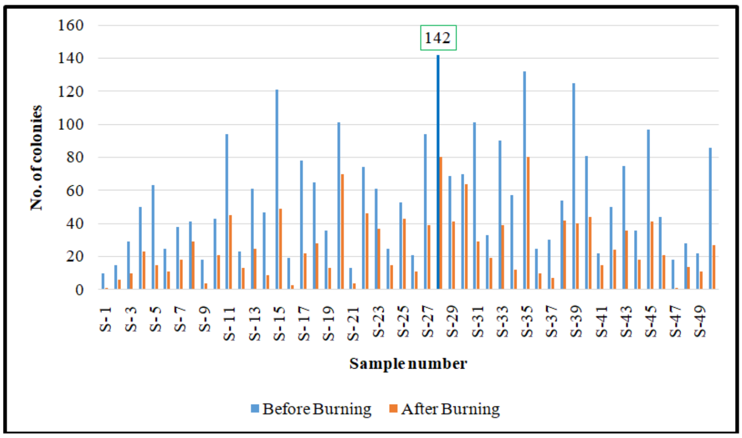

Fig. 4. A comparative graph of number of colonies produced after incubation.

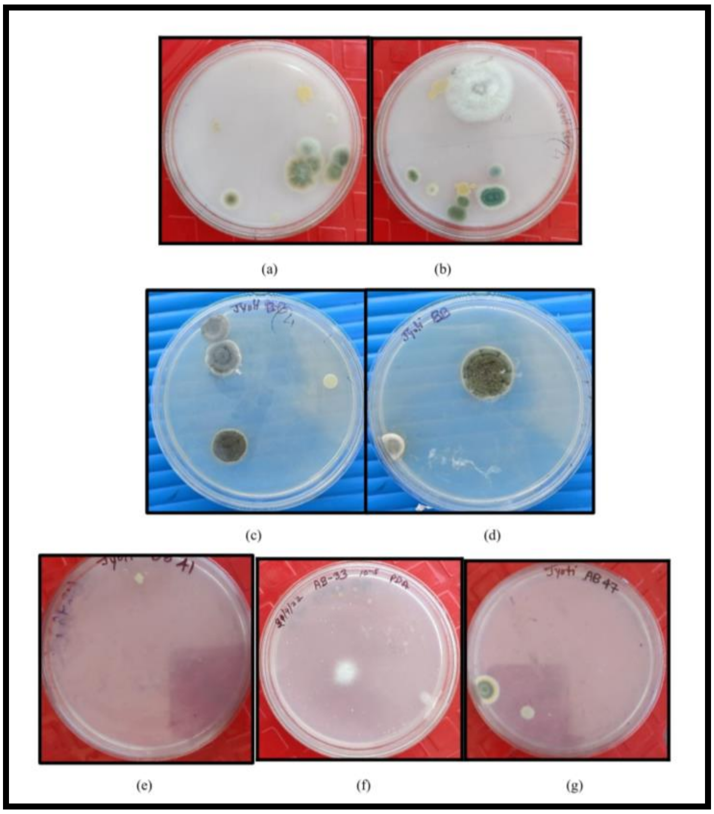

Fig. 5. Fungal colonies counted in undisturbed soils (a), (b), (c) and (d); and in after burning soils (e), (f) and (g).

Soil shouldn't be considered as an inert substrate rather, it's a dynamic cache of multiple assets. Because of this, we prefer to refer to soil health rather than soil quality, which is defined as the ability of the soil to preserve environmental quality, support crop productivity, and enhances biotic and abiotic components. Role and benefits of fungi in and around plant roots have been emphasized by researchers previously (Agnolucci *et al*., 2019; Aishwarya *et al*., 2022; Bollmann-Giolai *et al*., 2022).

CONCLUSIONS

To check and stop a practice like burning crop residue that is environmentally unacceptable, conservation agriculture (CA) must grow and accelerate, and this movement should be led by farmers. The Indian government supports the management of crop residues for environmental and agricultural sustainability. A successful management plan was proposed in accordance with the updated Crop Residue Management Scheme-2020 guidelines. This plan enables appropriate strategies to be used to take advantage of the limited window of time between rice and wheat cultivation in order to minimize burning incidents to zero. The goal should be, to safeguard the environment from the effects of stubble burning, including air, soil, water, and microbes.

FUTURE SCOPE

India's agricultural development necessitates the development of very novel concepts to enhance this sector. Stubble shouldn't be considered as a harvest waste, instead it should be considered as a source of economic benefit.Potential uses of crop leftovers can be many, such as animal bedding, livestock feed, mulching of the soil, production of biogas, production of biomanure and compost, thatching for rural dwellings, mushroom culture, etc. Also, the impact of extreme heat, produced during stubble burning, on soil ecology should be considered as a reason of concern in terms ofloss of soil biodiversity. To meet the needs of growing population agricultural productivity should be increased, but not at a substantial environmental cost.

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Conflict of interest. None.

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