STUDIES ON ARBUSCULAR MYCORRHIZAL SYMBIOSIS AND GLOMALIN - RELATED SOIL PROTEIN DISTRIBUTION DURING DIFFERENT METHODS OF IN-SITU MICROBIAL DECOMPOSITION OF PADDY STRAW UNDER LOW LAND CONDITION

Thesis Submitted to Midnapore City College for the Partial Fulfillment of the Degree of Master of Science (Agriculture) in Genetics and Plant Breeding

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2023 Certificate



This is to certify that the project report entitled Studies of Arbuscular Mycorrhizal Symbiosis and Glomalin related Soil Protein Distribution During Different Methods of *in-situ* Microbial Decomposition of Paddy Straw under low Land Condition submitted by Labani Mandal, Roll No.PG/VUWGP29/GPB-IIS 006, Reg. No.- 250621 of 2013-2014 to the Midnapore City College, Midnapore, West Bengal, India during the year of 2019 in partial fulfillment for the award of the degree of M.Sc. in M.Sc (Agriculture) in Genetics and Plant Breeding is a bona fide record of project work carried out by him/her under my/our supervision. The contents of this report, in full or in parts, have not been submitted to any other Institution or University for the award of any degree.

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CERTIFICATE I

This is to certify that the thesis entitled "Studies of Arbuscular Mycorrhizal Symbiosis and Glomalin related Soil Protein Distribution During Different Methods of *in-situ* Microbial Decomposition of Paddy Straw under low Land Condition" submitted to the Midnapore City College, Paschim Medinipur, West Bengal in partial fulfillment of the requirements for the award of the degree of Master of Science (Agriculture) is a faithful record of bonafide and original research work carried out by Miss Labani Mandal under guidance and supervision of Dr. P. Panneerselvam, Principal Scientist, CPD, ICAR-NRRI, Cuttack, Odisha. It is further certified -that no part of this thesis has been submitted for any other degree or diploma or published in any form and submitted for final evaluation by examiner.

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CERTIFICATE II

This is to certify that the thesis entitled "Studies of Arbuscular Mycorrhizal Symbiosis and Glomalin related Soil Protein Distribution During Different Methods of *in-situ* Microbial Decomposition of Paddy Straw under low Land Condition" submitted to the Midnapore City College, Paschim Medinipur, West Bengal in partial fulfillment of the requirements for the award of the degree of Master of Science (Agriculture) is a faithful record of bonafide and original research work carried out by Miss Labani Mandal under co-guidance of Dr. Anulina Manna, Assistant Professor in Agriculture, Department of Biological Sciences, Midnapore City College, West Bengal, India. It is further certified -that no part of this thesis has been submitted for any other degree or diploma or published in any form and submitted for final evaluation by examiner.

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Declaration

I do hereby declare that the present Master thesis entitled 'Studies on Arbuscular Mycorrhizal Symbiosis and Glomalin related Soil Protein Distribution During Different Methods of *in-situ* Microbial Decomposition of Paddy Straw under low Land Condition' embodies the original research work carried out by me in the Department of Biological Sciences, Midnapore City College, Paschim Medinipur, West Bengal, India under the supervision of Dr. P. Panneerselvam, Principal Scientist, CPD, ICAR-NRRI, Cuttack, Odisha., and Co-Guided by Dr. Anulina Manna, Assistant Professor, Department of Agriculture, Midnapore City college, Paschim Medinipur, West Bengal. No part thereof has been submitted for any degree or diploma in any University.

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Approval Sheet

This project report entitled Studies on Arbuscular Mycorrhizal symbiosis and Glomalin related soil protein distribution during different methods of *in-situ* microbial decomposition of paddy straw under low land condition by Labani Mandal is approved for the degree of Master of Science (Agriculture) in Genetics and Plant Breeding.

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Dedicated to my Parents, Teacher and Friends

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Author

[Labani Mandal]

Abstract

The present study was undertaken at Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha. The main purpose of this study was to understand the changes in soil chemical and microbial properties during the time of in-situ paddy straw decomposition under field condition. Soil chemical and microbial properties were assessed from the ongoing field experiment for *in-situ* decomposition of paddy straw residues under NASF sponsored project at ICAR-NRRI experimental field, Cuttack, at vegetative stage of rice (variety Swarna). The treatment comprises four main treatments viz. MT1 - Conventional rice cultivation; MT2 - Residue retention (simulation like machine cut and spread) (@ 6 t paddy straw /ha); MT3 - Zero tillage (around 30 % of left rice stubbles in the field after harvest), MT4 - Residue incorporation (@ 6 t paddy straw /ha) with six sub-treatments including different type of microbial formulations applications viz. ST1 - No culture; ST2 - Solid inoculum of NRRI decomposing microbial consortium; ST3 - Capsule of NRRI decomposing microbial consortium; ST4 - NRRI Actino consortium; ST5 - IARI capsule based formulation (Reference check). The experimental design was split plot design with three replications. When compared to the other three management systems for rice straw residues, the conventional method of rice cultivation recorded the lowest soil enzyme activities, microbiological properties and mycorrhization. It was revealed that residue incorporation and retention had a greater effect on enzymatic activity as well as soil chemical and microbiological properties. Root colonization, sporulation of arbuscular mycorrhizal fungi and total glomalin content was higher in solid based decomposing microbial consortium applied treatments either in residue retention or incorporation of paddy straw residues. Among different types of decomposing microbial formulations evaluated, either solid or capsule based formulations found significantly improved the soil microbial properties under different methods of insitu paddy straw residues management.

Keywords: Rice, *in-situ* decomposition, arbuscular mycorrhizal fungi, glomalin.

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Chapter 1: Introduction

1. Introduction

Rice is grown in more than a hundred countries, with a total harvested area of approximately 158 million hectares, producing more than 700 million tons annually (470 million tons of milled rice). Nearly 640 million tons of rice are grown in Asia, representing 90% of global production (Bandumula, N., 2018). Rice straw is produced as a byproduct of rice production at harvest. Rice straw is removed with the rice grains during harvest and it ends up being piled or spread out in the field depending if it was harvested manually or using machines. Ratio of straw to paddy ranges from 0.7-1.4 depending on the variety and growth. Globally, roughly 800 to 1,000 million tons per year of rice straw is produced, with about 600 to 800 million tons per year produced in Asia. This continues to rapidly increase due to shorter turnaround time required for intensified rice cropping (Lim et al., 2012). The introduction of game-changing combine harvesters which solves the high labor cost associated with manual straw collection addresses only half the battle. Straw incorporation in soil for fertilization in intensive systems is also not possible with two to three crops per year because the turnaround time is too short for decomposition, resulting to poor soil fertilization properties which ultimately hinders crop establishment . With little options, open-field straw burning has increased dramatically over the last decade. Improved rice straw management and technologies that can help reduce the environmental footprint of and increase revenues from rice production and processing are therefore important for sustainable rice production systems (Munder et al., 2014). In recent years, with continuous increases in rice production, the production of rice straw has also increased. Due to economic development and improving standards of living, crop straw has been transformed from previously living energy and animal feed into agricultural waste (Jin et al., 2020). But in large quantity, the disposal of rice stubbles and straw after harvest of crop remains a major concern in all rice growing areas. In lowland rice ecosystems, where sole cropping is mostly practiced, low energy in situ composting may contribute tremendously towards recycling of solid waste and long-term sustenance of soil fertility . However, agricultural productivity relies on a wide range of ecosystem services provided by the soil biota. Sustainable management practices, such as tillage and residue management, can influence structure and function of the soil microbiota, with direct consequences for the associated ecosystem services (Saikia et al., 2019). Although there is increasing evidence that different tillage regimes alter the soil biological indices, we only have a limited understanding of their temporal changes in a rice (Oryza sativa L.) based cropping system (Saikia et al., 2019).

According to the current scientific consensus supported by linguistic and archaeological data, the domestication of O. sativa first occurred in the Yangtze River basin in China between 13,500 and 8,200 years ago. From that first planting, migration and trade helped spread rice throughout the world, first to much of East Asia, then farther

a field, and finally, as part of the Columbia exchange, to the Americas. Since its vast spread through out the world, a important crop for food and nutrition security and various culinary traditions, rice has become a staple crop (Carney, 1998). A large number of very small family holdings, many of which typically manage less than one hectare of paddy fields per household, are essential to the production of the "grain of life" China and India together produced 769.7 million metric tonnes of paddy rice in 2017, accounting for 848.4 million short tonnes of the total. Other major producers were Indonesia, Bangladesh and Vietnam (Wimberly, J.E., 1983). In 2017, the top fifteen producers accounted for 91 percent of all global production, with the major producers accounting for 72 percent of total production. 95% of the total production is produced in developing nations. When compared to the irrigated ecosystem's average productivity (4.9 t/ha), the rain-fed lowland and flood-prone ecosystem's average productivity is extremely low (2.8 t/ha). In many Asian countries, the productivity of rice in irrigated ecosystems has peaked. The rainfed lowland and flood-prone ecosystem, which makes up more than 30percent of the overall of the total rice area in Asia, is where the future increased demand for rice production has come from (Swain et al., 2005). One of the most significant food crops, rice feeds more than 60% of India's population.

When rice is harvested, rice straw is generated as a byproduct. When rice is harvested, the rice straw is excluded along with the rice grains, and depending on whether it was harvested manually or with machines and equipment, it is either piled or spread out in the field. Depending on the variety and growth, the ratio of straw to paddy ranges from 0.7 to 1.4 (Dobermann and Fairhurst, 2002; l., 2020; Bhattacharyya et al., 2020 . With two to three harvests per year, it is also challenging to incorporate straw into the soil for fertilisation in intensive systems because there is not enough time for decomposition. This leads to inadequate soil fertilization, which ultimately hinders crop establishment (Zhang et al., 2021). Due to a lack of alternatives, open-field straw burning has risen considerably in our country. If we have viable rice straw management technology, that can minimise the environmental footprint in rice cultivation and also increasing profitability in long term (Munder et al., 2014). Rice straw production has grown substantially in recent years, owing to continuous increases in rice production, hence it is very essential to find out an alternate eco-friendly technology for rice straw residues management under field conditions. In general, farmers have been forced to use burning because of a lack of labour, the high cost of removing crop residue from the field and composting, and a lack of the necessary machinery to incorporate crop residues into the soil (Pathak et al., 2012).

Additionally, burning residue is one of the main sources of trace gases and smaller black C particles, both of which are crucial for the formation of ozone through photochemical processes (Andini et al., 2018, ; Zhang et al., 2008). Burning explicitly allows quick and thorough residue removal, particularly for those who use double- or triple-cropping (Singh et al., 2008), but it may cause the loss of significant nutrients (Dobermann and Fairhurst, 2002). Combustion rice straw in the open in the field results in the release of significant amounts of pollutants, including SO2, NOx, toxic gases like

carbon monoxide (CO), dioxins, and furans, volatile organic compounds (VOC), carcinogenic polycyclic aromatic hydrocarbons (PAH), as well as fine inhalable particles(Jenkins et al., 2003). The open burning of residues also helps to release greenhouse gases like nitrous oxide, methane, and carbon dioxide.

There are numerous scientific studies that demonstrate how adding rice straw to paddy soil can improve soil fertility (Dobermann and Fairhurst, 2002; Zhang et al., 2013). But poor management of the incorporation of straw can lead to a decline in production effectiveness and an increase in greenhouse gas emissions (Nayak et al., 2015). It should be noted that farmers frequently refuse to incorporate crop residues because of their slow decomposition. It has been investigated how to accelerate this, for instance, by using fungal inoculums. In order to fertilise the soil, rice farmers frequently incorporate rice straw .

A potential solution to stop open field burning appears to be composting rice stubble. Agricultural waste is nutrient-rich, so composting it increases soil fertility (Romasanta, 2017). Crop waste can be turned into compost to increase crop production by 4–9 %. Vermi-composting , mechanised windrow composting (Gummert, 2020), co-composting livestock and dairy manure with rice straw, etc. are just a few of the composting technologies that have been developed . Because of this, bio-composting is a fruitful but time-consuming task for farmers who practise twice-yearly crop rotation. A few farmers who are aware of the risks associated with crop burning use organic wastes and other similar organic techniques to increase crop yield and lower air pollution from open field burning.

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil organisms that form mutual symbiotic association with over 80% terrestrial plants, providing a direct physical link between soil and plant roots . AMF have been shown to promote plant growth and tolerance by increasing plant access to relatively immobile nutrients, enhancing plant growth hormones, influencing the uptake and distribution of soil components in plant tissues, and improving the conditions of rhizosphere soil micro-environment (Begum et al., 2019; Panneerselvam et al., 2019). Therefore, AMF can serve as a potential biotechnological tool to increase the efficiency of rice growth. The influence of AMF on improving soil quality and structure in decomposition area has been largely neglected, although numerous studies have revealed beneficial role of AMF in influence decomposition of organic materials (Hodge, A., 2001). AMF can contribute to soil aggregate stability directly by a physical effect of a network around soil particles, and indirectly by the hyphal exudation of an iron-containing, heat stable glycoprotein (extracted at 121°C) named glomalin as an aggregate binding agent. Glomalin has been operationally defined as glomalin-related soil protein (GRSP) by extraction and detection conditions from soil, and it is detected in large amounts in diverse ecosystems. The sticky GRSP acts as biological glue, helping to bind soil tiny particles into small aggregates of different sizes. Well-aggregated soil is stable enough to resist wind and water erosion, and has better air and water infiltration rates favorable for plant and microbial growth.

Additionally, GRSP is recalcitrant enough to have a long residence time in soils, and plays a pivotal role in long-term carbon/nitrogen storage and other sequestration. Therefore, the release and accumulation of GRSP in soils can be a very important mechanism for ecological restoration of soils degraded by mining and smelting activities.

In view of above, the present work is proposed to understand the application of decomposing microbial consortium and their effects on microbial, chemical properties, mycorrhizal response and glomalin-related soil protein (GRSP) during in-situ paddy straw decomposition under low land rice cultivation.

Chapter 2: Literature Review

2. Literature Review

2.1. Rice or rice based crop production

The rice or rice based cultivation is an important cropping system for food security, employment, income generation and livelihoods for millions of people in Asia (Singh et al., 2014). Crop residues, renewable biomass generated in large quantities in agriculture, are generally disposed of by burning by the farmers because they interfere with the conventional zero-till seed drills and have no economic benefits in Indo-Gangetic plains of north-west India . The burning of the residues leads to loss of carbon and plant nutrients adversely affects soil health and sustainability, and causes serious air pollution with the emission of greenhouse gases and particulate matter (PM) (PM 2.5, <2.5 µm, and PM 10, <10 µm), which, in turn, alter monsoon patterns (Singh et al., 2014), so there is an urgent need to develop a strategy for sustainable means to recycle crop residues in situ. In-field retention of crop residues can play an important role in replenishing soil nutrient stocks and organic matter, and in making the sustainable (Bi et al., 2009). Soil microorganisms play a key role in the transformation of nutrients, decomposition of organic residues for plant nutrition and improvement in soil structure and fertility. Tillage and crop residue management practices can cause substantial changes in soil physical (moisture and aeration), chemical (nutrient availability and carbon accessibility) and biological conditions below the soil surface (root system development, soil microbial communities and enzymes). Stimulation of microorganisms in the rhizosphere and improved soil fertility, crop health and yield has been observed in crop rotations when legume species are included in cropping systems. Parameters describing the amount, activity and diversity of soil microorganisms are used as biological indicators of soil quality and health. There is no single parameter that could be considered a biological index of soil quality. For example, microbial biomass, metabolic quotient (qCO2) and soil respiration could be pertinent options to characterize the changes in microbial activity as a result of soil-management practices. The microbial biomass carbon (MBC) reflects the soil's ability to store and cycle nutrients (C, N, P and S) and organic matter, and has a high turnover rate relative to the total soil organic matter. The change in microbial activity as a result of changes in environmental factors can be quantified by the metabolic quotient (qCO2) (Anderson, 2003). This quotient is a valuable, relative measure of the efficiency of utilization of C resources by the soil microbial biomass and of the degree of substrate limitation for soil microbes. The basal soil respiration (BSR) of a soil reflects the overall activity or energy spent by the indigenous microbial pool. Some studies have reported decreased soil respiration under conservation tillage practices (MacDonald et al., 2010) have reported an increase in soil respiration under zero tillage (ZT) compared with conventional tillage practices. The improved physical conditions (improved aeration, soil water regime and thermal regime) increase microbial population, thereby increasing soil respiration under ZT. Generally, biological parameters can be correlated or used as

indices to assess changes in the soil environment. Ratios between biological parameters (e.g. metabolic quotient, qCO2) can be used for evaluating the microbial ecophysiology to relate cell's physiological functioning as affected by different environmental factors (Anderson, 2003). The qCO2 may be used to compare microbial communities and to quantify the long-term effect of changes in management practices on soil microbial communities. Bending et al. (2002) reported that soil-management practices had considerable influence on the structure and metabolic diversity of soil microorganisms. In the addition to management practices, Garbeva et al. (2004) found that residue quantity, quality (e.g. cereal versus legume residue), environmental conditions and their complex interactions significantly affected soil microbial functional diversity.

2.2. Environmental factors and management practices

Open-burning of rice straw residues pollutes the air and contributes to global warming through emissions of greenhouse gases (GHGs) (Andini et al., 2018). Although burning of straw residues emits large amounts of CO₂, this component of the smoke is not considered as net GHG emissions and only concludes the annual carbon cycle that has started with photosynthesis (Romasanta et al., 2017). Rice straw burning has advantages in terms of farm operations but disadvantages from an environmental perspective. Openburning of rice straw in the field is of incomplete combustion in nature; hence, a large amount of pollutants are emitted such as SO₂, NOx, (Oanh, N.T.K., 2021) including toxic as carbon monoxide (CO), dioxins and furans, volatile gases such organic compounds (VOC), carcinogenic polycyclic aromatic hydrocarbons (PAH), as well as fine inhalable particles (Romasanta et al., 2017). Burning causes almost complete N loss, P losses of about 25 percent, K losses of 20 percent, and S losses of 5 to 60 percent (Dobermann, A.T.H.F. and Fairhurst, T.H., 2002). The amount of nutrients lost depends on the method used to burn the straw. In areas where harvesting has been mechanized (e.g., Thailand, China, and northern India), all the straw remains in the field and is rapidly burned in situ; therefore, losses of S, P, and K are small. Several attributes could influence straw burning and its emissions. Water content or moisture in plants can either prevent a fire completely or slow down the burning process and eventually terminate the fire. Density and structure of biomass are other characteristics to be considered for combustion properties (Demirbas, A., 2004). Higher density will increase temperatures in the fuel and will extend the burning period. Another critical attribute is fuel size because smaller particles are capable of sustaining flaming combustion, which will in turn support the burning of larger particles. Burning cause's atmospheric pollution and results in nutrient loss, but it is a cost-effective method of straw disposal and also helps reduce pest and disease populations that may occur due to reinfection from inoculum in the straw biomass (Conway, G.R. and Pretty, J.N., 2013).

Returning crop residues to soil can improve soil physical properties by increasing soil moisture content, decreasing bulk density, and increasing total porosity and aggregate stability (Chen et al., 2014). Crop residue returning can increase soil moisture content by reducing surface runoff and direct evaporation, improving soil saturated water conductivity and water infiltration. Microorganism increase soil fertility by incorporating air, minerals and nitrogenous compounds (Pettit, R.E., 2004). They contribute in increasing plant growth by providing essential elements, minerals that plants cannot utilize by their Owen. Microorganisms decompose organic matter to simpler form that can be easily uptake by plants. The way in which the soil is tilled is one factor that determines the losses of crop nutrients and crop-protection chemicals from agricultural land. It influences both the pathways through which percolating water may carry these chemicals and the activity of microbes which may release or immobilize nitrate and phosphate and also break down pesticides or herbicides. Bending et al. (2002), reported that soil-management practices had considerable influence on the structure and metabolic diversity of soil microorganisms. In the addition to management practices, Garbeva et al. (2004), found that residue quantity, quality (e.g. cereal versus legume residue), environmental conditions and their complex interactions significantly affected soil microbial functional diversity.

2.3. Paddy straw production

After China, India is the world's largest producer of paddy. India produces 98 million tonnes of paddy with roughly 130 million tonnes of straw (Côté, R.P., 2000). Of this, about half is used as animal fodder. Every year about 12 million tonnes of rice straw is burned in Punjab. Ratio of straw to paddy ranges from 0.7-1.4 depending on the variety and growth. Globally, roughly 800 to 1,000 million tons per year of rice straw is produced, with about 600 to 800 million tons per year produced in Asia (Tipayarom, D. and Oanh, N.K., 2007). This continues to rapidly increase due to shorter turnaround time required for intensified rice cropping.

2.4. Biochemical composition of paddy straw

Rice straw contain a combination of cellulose, hemicellulose, and lignin, along with appreciable amounts of silica and other minor components (Goodman, B.A., 2020). Cellulose and hemicellulose are both polysaccharides; cellulose is a long straight-chain polymer containing exclusively β -glucose monomers whereas hemicellulose is a shorter cross-linked polymer contains other that sugars, such as xylose, galactose, mannose, rhamnose, and arabinose (You et al., 2015). In contrast, lignin is polymeric aromatic structures that involves oxidative coupling of 4hydroxyphenylpropanoids, primarily *p*-coumaric, coniferyl and synapyl alcohols. In cell walls, these polymers form very stable complex 3-dimensional structures known as lignocellulose, in which cellulose is surrounded by a monolayer of hemicellulose and embedded in a matrix of hemicellulose and lignin (Goodman, B.A., 2020).

2.5. Paddy straw residues management

Straw is either removed from the field, burned in situ, piled or spread in the field, incorporated in the soil, or used as mulch for the following crop. Each of these measures has a different effect on overall nutrient balance and long-term soil fertility (Ladha *et al.*, 2004). Where S-free mineral fertilizers are used, straw may be an important source of S; thus, straw burning should not be practiced. In contrast, burning effectively transforms straw into a mineral K nutrient source, and only a relatively small amount of K is lost in the process (Dobermann, A.T.H.F. and Fairhurst, T.H., 2002). The effect of straw removal on long-term soil fertility is much greater for K than for P. Spreading and incorporation of straw, however, are labour-intensive tasks, and farmers consider burning to be more expedient. Straw is also an important source of micronutrients such as zinc (Zn) and the most important influence on the cumulative silicon (Si) balance in rice (Goswami *et al.*, 2020).

The eco-friendly management of paddy stubble generally encompasses conventional and modern stubble use (Sangwan, V. and Deswal, S., 2021). Various alternatives of *in-situ* like crop residues management by zero-tiller machine, double disc coulters, straw choppers for cultural agriculture adoption and practice, thereby showing reduction in the rice and wheat rotation residue burning. A research, affirmed and highlighted the eco-friendly management of the paddy crop residues for a sustainable environment and development (Bhatt, R., 2017). The research highlights the eco- friendly methods for the management of the residues providing a new dimension for the application of the post harvested residues. There has been the applicability of combustion boilers of rice straw along with the steam turbines for producing heat and electricity (Suramaythangkoor, T. and Gheewala, S.H., 2010). Even the end products i.e. fly ash, and bottom ash is perceived to have economic values, thereby used in cement manufacturing and brick manufacturing, road construction and embankments, etc. The applicability of rice straw in the production of biogas using anaerobic digestion was also perceived in the study (Xia et al., 2018). Apart from this, the rice straw renders applicability in the industry of mushroom also at varying levels. The mushroom paddy straw, Volvariella volvacea is discerned as stress-free mushrooms for cultivating due to the short incubation period (Sangwan, V. and Deswal, S., 2021). According to the studies conducted, the applicability of production of mushroom renders high productivity of about 5-10%. Also, one of the significant substances is rich in carbon, biochar and can be produced using the rice straw (Sangwan, V. and Deswal, S., 2021).

The paddy stubble finds its applicability in the bed material during the winter for the cattle and is a traditional, conventional and regular form of practice in India (Singh, R. and Upadhyay, S.K., 2018). This bedding material formed from the residues of paddy renders an improved ability of milking pertaining to quantity and quality thus contributing udder health and relaxed sleep for the cattle. Moreover, it can be processed for the compost by alternative forms of methods through the applicability of microbial organism and amendments on the farm itself. Owing to its lower nutritional value, rice straw is not used as animal fodder (Sarnklong et al., 2010). Rice straw is ligno-cellulosic and is not readily digested and can cause livestock health issues. Only 7 percent of the total ricestraw stock produced is used as animal fodder, according to a study by whereas 45 percent of wheat straw is used as animal fodder. Just 0.02 to 0.16 percent of the phosphorous in rice straw is inefficient for livestock. The content of silica in rice straw is higher than lignin and is therefore not properly metabolized. Rice straw also differs from other straws in having a high (1-2 percent) content of oxalates (Van Soest, P.J., 2006). Various experiments have demonstrated that soil incorporation is a safer alternative to removal of crop residues. The procedure is to blend the crop stubble into the soil three weeks before cultivation, it not only increases soil fertility and provides essential nutrients, but also increases soil organic carbon by 14-29 percent (Bhardwaj et al., 2019). It offers cost-saving equipment and less labour in the long term. Along with soil benefits such as cooling effect, increased moisture, carbon source and erosion protection (Beard et al., 1994), soil integration has various disadvantages such as crop infestation, nutrient immobilization and phyto-toxin formation. The National Policy for Management of Crop Residue (NPMCR) suggests that methods such as soil incorporation and composting should be promoted in India to not only reduce air pollution due to stubble burning but also increase soil fertility.

2.6. Paddy straw decomposition through microbial intervention

Several biological processes of decomposition take place in the crop residue. The microbes in the soil feed upon the C present in the crop residue and also require N for the process (Turmel et al., 2015). However, more concentration of the C in comparison to the N would result in the soil microbes taking more time in breaking down of the organic material and using of soil N in order to do their work (Six et al., 1998). Studies have proved that utilization of the organic wastes as soil amendment might render good promises for improvement of the soil health and thereby also reduce the waste disposal issue (Park et al., 2011). A study also affirmed that the use of the application of the microbial consortia for the paddy straw is an effective and strategic move for the treatment. Apart from this, it was confirmed that availability of the soil nutrients also increased particularly N, K and organic carbon when the straw is applied with the microbial consortia (Ghosh et al., 2012). Although in the fields, direct association of the residues of agriculture solves the issues pertaining to burning, however, because of the difference in the short time occurring between the rice harvesting and wheat sowing, it is not feasible. Moreover, according to a study long term based observed experiments asserts and highlight that although incorporation of agricultural residue improves the health of the soil significantly, however, the subsequent yields of crops decreases due to the allelochemical and microbial phytotoxins production and immobilization of nitrogen that is available (Khanh et al., 2005). According to a study, the process of Composting is

one of the most well-known and effective technologies for the management of agricultural residues. These residues are of potent source several benefits such as improved soil health and fertility which leads to increased agricultural productivity and soil biodiversity.

In order to undergo composting by the use of microbes, it is essential that the raw material has a ratio (C: N) in the range of 30 and 35 and the content of moisture is discerned to be in between 55 to 65% of the residues for ensuring microbial appropriate conditions in order to decompose and transform into organic matter from the crop residues (Sangwan, V. and Deswal, S., 2021). Many microorganisms have been manifested with activities of cellulose including various fungal and bacterial forms of strains comprising of both the aerobic and anaerobic. A study asserted that *Chaetomium*, Myr othecium, Trichoderma, Fusarium, Aspergillus, Penicillin and Trichonympha, Clostridium, Butyrivibrio fibrisoly ensand Bacteroides succinogenes are some of the bacterial and fungal species accountable to the activity of degrading (Broder, M.W. and Wagner, G.H., 1988). In another study by Shukla et al., (2016), it was asserted that a microbial consortium comprising Aspergillus nidulans, Trichoderma viride, A. awamori and Phanerochaete chrysosporium was rendered in the process of composting of various crop residues with poultry droppings and rock phosphate (%) which produces nitrogen enriched phosp hocompost within two months. *Mesophilic microor* ganisms like bacteria Pseudomonadaceae, Enterobacter iaceae, Streptomycetaceae and Erythrobacteraceae families govern the mesophilic phase of composting which grow in the temperature range of 15 to 35 degree Celsius and thereby utilize the soluble compounds such as sugar, amino acids and lipids (Piotrowska-Cyplik et al., 2017). After metabolic activities these generate exothermic reactions and increase the composting temperature up to 65 to 85 degree Celsius and this phase is called thermophilic phase. A study demonstrated that Pseudonocardiaceae and thermo- monosporaceae and possess hydrolytic enzyme degrading lignin, cellulose, hemicelluloses and proteins. A thermophilic consortium fungi of A. nidulans, Scytalidium hermophilum and Humcola sp was found very useful and beneficial soybean trash and rice straw decomposition. At low temperature, the composting was accelerated by some psychrotrophic microbial consortium comprising Eupenicillium crustaceum, Paceliomyces sp. Bacillus atropheus and Bacillus sp., was employed for biodegrading rice straw (Shukla et al., 2016).

Fungi among the microbial agents forms the most essential form of group and thereby shows colonization on solid substances very quickly (Viji, J. and Neelanarayanan, P., 2015). Fungi hence has a vital function in the bio-degeneration of the organic forms of wastes that are lignocellulosic in nature (Sagarika *et al.*, 2022). Many researchers demonstrated the lignocellulolytic activity of several fungal species. Studies have been carried about *Fusarium sp., Aspergillusterreus, Paecilomycesfusisporous, Micromonospora and Coriolusversicolor*. Some researchers concluded that the lignocellulolytic microorganism is perceived to be strategically associated technology in

order to accelerate the degradation potential of lignocellulose in agriculture wastes (Sangwan, V. and Deswal, S., 2021). It further manifested that *R. oryzae, A. oryzae and A. fumigatus* mixed culture can further be suggested for the paddy straw degradation and quality compost production with a greater population of macronutrients (Rastegari *et al.*, 2020).

2.7. Effect of decomposition of paddy crop residues on soil properties

2.7.1. Soil chemical properties

The application of combination of three lignocellulolytic fungi viz., R. oryzae, A. oryzae and A. fumigatus can be recommended for the degradation of paddy straw which would result in production of good quality compost containing higher amounts of total nitrogen (1.55%), total potassium (1.57%) and total phosphorus (1.48%) content was observed by Viji and Neelanarayanan (2015). observed that soil microbes are one of the important biota of the soil. They studied on culturable bacteria in soil and in-situ analysis with buried slide technique. Total nitrogen content was found to be high 1.12 to 1.44 %. The phosphorus was found in range of 19 to 25.33 kg/ha. Mandal et al., (2004) conducted a field experiment with different straw management options Incorporation leads to build up of SOM, soil N, P and K. Surface retention of residues increases soil NO, by 46% compared to burning. Residue management practices significantly affect the soil moisture, temperature, aggregate formation, bulk density and hydraulic conductivity. Rice straw incorporation coupled with organic manure improves soil physical condition. Kausher et al., (2014) conducted an experiment in which rice straw was inoculated with lignocellulolytic microbial consortium at various pH levels for composting and significantly affected the conductivity, bulk density, total carbon and nitrogen of the soil. Abdulla et al., (2007) using three cellulolytic actinomycetes isolates, of the genera Micromonospora, Streptomyces and Nocardiodes, and used these inocula in combination with different organic amendments for rice straw composting and incorporation into soil. Their results demonstrated that Inoculation of Micromonospora increase the organic matter to 34.9% and nitrates content to 0.59 mg/g, as compared to control reached 20% and 0.21 mg/g, respectively. They concluded that the application of municipal sludge and Micromonospora combination may represent a rapid and ecofriendly approach for disposal of rice straw. examined that rice straw treated with combination of cow dung slurry @ 5% + T. harizianum @ 5 kg Ha⁻¹ + Pleurotus sajorcaju @ 5 kg ha⁻¹ had significant influence in degrading rice straw as evidenced through the activity of Nfixing and P- solubilizing microorganisms in the soil. The highest population of N- fixing and phosphorus solubilizing microorganisms (51.00 x 10°cfu / g and 30 x 10°cfu / g soil) soil at the time of 60 DAT, respectively during summer 2010 and (62.44 x 10°cfu / g soil and 36.77 x 10°cfu/g soil) at the time of 60 DAT, respectively during Kharif 2010 were recorded compared to application of recommended dose NPK. conducted a field experiment to investigate the decomposition and nutrient dynamics from surface placed and incorporated rice residue on two soil types using the nylon mesh bag technique over wheat cropping cycle in rice-wheat system. Nitrogen in surface rice residue increased throughout the decomposition cycle due to microbiological immobilization. The change in P with time was small and weakly defined by the exponential function. Potassium in rice residue decreased at a fast rate during the initial 20 days and >80% was released by 40 days. used annual application of 1500 kg ha⁻¹ of Cajanuscajan, Acacia auriculiformis, Phyllanthus taxodifolius and Samaneasaman in rice soils for maintenance of organic matter and the balancing of nutrient. They found that the average annual nutrient inputs from the leaf litters, in, ranged from 62.7 kg ha⁻¹N, 3.9 kg ha⁻¹ P, 17.9 kg ha⁻¹ K, and 3.5 kg ha⁻¹ S for *Cajanus cajan* to 24.5 kg ha⁻¹ N, 1.5 kg ha⁻¹ P. 8.1 kg ha⁻¹ K and 2.0 kg ha⁻¹ S for Acacia auriculiformis. Nutrient balances, determined by the difference between the inputs (fertilizer and added leaf litters) and outputs (grain and straw) indicated net positive N and P balances of up to 457 and 60 kg/ha respectively, after five seasons of leaf litter applications. In a field experiment with annual applications of low rates (1500 kg/ha dry matter) of leaf litter from Cajanuscajan, Phyllanthustaxodifolius, Acacia auriculiformis and Samanaesaman increased the CL, and Carbon Management Index (CMI). Five seasons of leaf litter application increased total carbonpool by 24-37% and more than doubled CL and soil C lability. Higher rates of fertilizers did not result in increased soil C. Crop residues, leaf litters and green manures with slow breakdown rates are needed to rehabilitate soil C reported by Incorporation of wheat residue (6 Mg ha⁻¹, C/N = 94), rice residue (6 Mg ha⁻¹, CIN = 63) and sesbania green manure (20 or 40 Mg fresh ha⁻¹, C/N = 19) with urea fertilizer N in a rice-wheat cropping system can improve N use efficiency, and SOM. Residue incorporation resulted in reduced recovery efficiency of urea N and green manure N. Wheat residue additions to flooded rice resulted in greater C sequestration in soil than with rice residue or 40 Mg green manure ha⁻¹ studied by Aulakh et al., (2001). Crop residues are the primary source of C inputs and the ways in which these are managed have a significant effect on soil properties. Increase in available K status as a result of FYM application may be attributed to mobilization of K from reserve pool Rao et al., (1999). It is an important component for improving the physical, chemical and biological properties of the soil. It acts as a source of plant nutrients and a water absorbent conducted a field experiment with the application of rice straw compost @ 5 t ha⁻¹ along with half dose of the recommended dose of inorganic fertilizer, increased soil organic C and N from 0.471 and 0.039% to 0.545 and 0.064 % respectively. Carbon and N mineralization rates were also higher than control and soils receiving recommended dose of inorganic fertilizers. Observed that the rice straw c composting with cattle manure through thermochemical pre-treatment altered the soil properties. The moisture content (62.07%), organic matter reduction (16.99%), pH level (7.3), electrical conductivity1058 IS/cm, total organic carbon reduction (17.00%), soluble chemical oxygen demand reduction (83.43%), total Kjeldahl nitrogen (2.06%), carbon-to-nitrogen ratio (16.75%), and germination index (90.33%) was found comparable limits. Their results suggested that the combined application of chemical-biological processes under

thermophilic conditions is a novel method for the rapid composting of rice straw. The application of EM in (compost straw + goat manure + green waste) increases the macro (N, P and K) and micronutrient content compared to compost without EM. Although the Fe in compost with EM is significantly higher than in the compost without EM, for Zn and Cu, there is no significant difference between treatments. The application of EM is suitable to increase the mineralization in the composting process (Jusoh *et al.*, 2013). Vermicompost fertilization resulted in highest available phosphorus, and nitrogen content of wheat soil. It was also found effective in minimizing the alkalinity of soil compared to other treatments as indicated by pH change (Gaind and Nain, 2007).

The long-term incorporation of crop residues in flooded rice soil can increase soil organic matter, total N, and soil biological activity . Continuous incorporation of crop residues after each crop can eventually increase the N-supplying capacity of rice soils . In a study in Vietnam, soil N increased from 0.65% to 0.085% following 9 years of cropping with incorporation of rice straw while straw removal caused a decline in soil N . The benefits of incorporated residues on soil organic matter and soil N supply, however, seldom translate into increased yield or profit for flooded rice . However, Thanh *et al.* (2016) observed that N fertilizer requirement was reduced by about 20% in a long-term study with rice straw incorporation.

In a long-term study in the Mekong Delta in Vietnam observed that the application of 6 Mg ha⁻¹ rice straw compost (fresh weight) increased rice yield where no mineral fertilizer was applied in the wet season. In the same study, they also observed positive effects of rice straw compost on physical soil properties including a lower penetration resistance compared to where no compost was applied. In China, rice yield was greater with rice straw incorporation than removal under conventional tillage where no nitrogen fertilizer was added (Xu *et al.*, 2010). A 3-year study conducted across three rice-growing sites in Asia showed little or no benefit of incorporated rice or wheat straw for the succeeding crop (Thuy *et al.*, 2008). However, at a site in India the incorporation of rice straw 20 days before sowing wheat without N fertilization significantly decreased wheat yield but increased yield of rice that followed after wheat. In contrast, in East China incorporation of rice straw increased wheat yield by about 28% compared to no straw control, but had no significant effects on rice yield .

2.7.2. Soil biological properties

The availability of nutrients is affected by the low quality of rice straw, with a high C:N ratio, resulting in slow decomposition and mineralization of nutrients, particularly short-term availability of N and to some extent P. The C:N ratio of an organic material determines its quality, with high C:N ratio representing low quality and a

slow rate of decomposition, whereas low C:N ratio represents high quality with a faster decomposition. The addition of rice straw to wet soil results in temporary immobilization of nitrogen, making it unavailable and affecting rice yields. Apart from low straw quality, N availability following incorporation of the straw is affected by the accumulation of phenolic compounds that are formed under the straw's anaerobic decomposition. These phenolic compounds tend to bind the N in the soil making it unavailable for plant uptake.

Soil organic C has been shown to be stable under intensive rice cropping, even when straw is removed from the field. Soil organic C was shown not to change in a 50-year, long-term continuous cropping experiment at the International Rice Research (IRRI) in the Philippines where three rice crops were grown annually with the removal of all aboveground biomass even without the addition of N fertilizer . This is in contrast to systems where rice is rotated with an upland crop observed a decline in soil organic C when no residues were added in a rice–wheat cropping system in India. In a 9-year study in Bac Giang Province in Vietnam, soil organic C did not change with straw removal, but the addition of straw increased soil organic C from 1.28% to 1.65% Alberto *et al.* (2015) showed a cumulative effect of continuous straw incorporation in a lowland rice soil, likely due to slower organic matter decomposition. However, the addition of straw increases soil organic C (Bi *et al.* 2009; Yadvinder-Singh *et al.*, 2005), particularly in rainfed upland rice systems or where lowland rice is rotated with an upland crop. In a rice–wheat system, Gangwar *et al.* (2006) observed greater soil organic C and infiltration when 5 t ha⁻¹ rice straw was incorporated in the soil than when it was removed or burned.

2.7.3. Soil enzyme activities

Phosphorous compost blended stubble with N fertilizer resulted in the maximum urease activities in wheat rhizosphere soil. The results revealed that phosphor compost blended rice stubble along with N fertilizer was most effective for rice stubble management in wheat field (Bhattacharjee et al., 2013). Li et al., (2008) examined that the fungus produced laccase, cellobiose dehydrogenase, xylanase and cellulose enzymes during the incubation period. Intracellular lignin peroxidase, manganese peroxidase and laccase were produced during liquid fermentation. Rice straw incorporation coupled with organic manure increase microbial activity than residue removal or burning . Acharya et al., (2010) examined that lignocellulolytic fungi produce a variety of lignocellulolytic enzymes which are responsible for the biodegradation, using wheat straw as a model agro-waste by solid state fermentation. Their result showed that, A. niger, A. oryzae and Sporotrichum sp. produce endocellulase and xylanase insignificant amount. A. niger and Sporotrichum sp. Gave 52.47 Ulg and 69.441 U/g endocellulase activity, and 48.107 U/g and 112.649 U/g xylanase activity, respectively. Goyal et al., (2009) conducted a field experiment to utilization of rice straw through composting. They observed that application of rice straw compost @ 5t ha' along with half dose of the recommended dose

of inorganic fertilizer increased the dehydrogenase activity from 66 to 118 mg TPF/kg soil/ 24h and alkaline phosphatase activity from 370 to 680 mg PNP/kg soil/ h. Gaind and Nain, (2007) conducted a field experiment using organic fertilizers (paddy straw, microbial inoculants and vermicompost) with inorganic fertilizers (urea and superphosphate) for improving the soil biochemical properties. Incorporation of paddy straw in conjunction with 60 kg N and 60 kg P₂O₅ and T. reesei inoculation resulted in maximum dehydrogenase, alkaline phosphatase and highest humus content of soil. Mixed inoculation of A. awamori and T. reesei did not prove effective in improving the soil biochemical properties in comparison to single inoculation of T. reesei. According to Chang et al., (2007) soil enzyme activities such as FDA, dehydrogenase, B-glucosidase, acid and alkaline phosphatases and urease activities increased significantly in compost treated soils than the application of only chemical fertilizers. The incorporation of organic amendments in soil influences soil enzymatic activity because the added material may contain intra- cellular and extracellular enzymes, and may stimulate microbial activity in the soil. Organic amendment had a positive effect on the activity of enzymes, particularly when the amendment was at a high concentration. Developed a composting method for rice straw composting, amended with poultry manure or urea with a co-inoculum of fungi and bacteria in perforated cemented pits. Microbial activity was measured at monthly intervals. Maximum dehydrogenase activity (4095.77 mg TPF $g^{-1} h^{-1}$) was found in P. chrysosporium and C. cellulans inoculated and poultry manure amended paddy straw after 60 days of composting. The lowest alkaline phosphatase activity (918 ug PNP $g^{-1}h^{-1}$ ¹) recorded after 90 days of composting period may be due to change in microbial profile or adsorption of the enzymes in the compost humic matrix. Highest FDA hydrolysis (2.20 Agy 90 g⁻¹ h⁻¹) was found at 60 days of composting in poultry manure amended and coinoculated paddy straw. Reported that the phosphatase activity decreased with depth, and it is positively correlated with organic C and clay content. The pyrophosphatase activity of soil is negatively correlated with the CaCO₃, content of soil. Examined that the highsolids incubations was performed to enrich for microbial communities and enzymes that decompose rice straw under mesophilic (35°C) and thermophilic (55°C) conditions. Thermophilic enrichments yielded a community that was 7.5 times more metabolically active on rice straw than mesophilic enrichments. Extracted xylanase and endoglucanse activities were also 2.6 and 13.4 times greater, respectively. Cellulase activity decreased with time which may be due to the accumulative effect of cellulobiose, which is dimmer of glucose and known to inhibit both endoglucanase and glucosidase . The hydrolytic action of cellulases and hemicellulases is of fundamental importance to obtain fermentable sugars from lignocellulosic biomass . The enzymatic hydrolysis of cellulose into glucose involves the synergistic action of the three different enzymes such as endocellulase (EC 3.2.1.4), exocellulase (EC 3.2.1.91) and B-1, 4-glucosidase (EC 3.2.1.21) (Acharya et al., 2008). Reported that application of NPK fertilizer + FYM has significantly increased the FDA, DHA activity and soil microbial activities. Integrated use of mineral nutrients with organic amendments appreciably increased organic carbon content resulting in enhanced activity of soil enzymes stated by the B-glucosidase activity was significantly higher in the legume than cereal based cropping system also observed that the glucosidase activity significantly and positively correlated with SoC content. Observed that the application of inorganic fertilizers increased the soil bacterial numbers and soil dehydrogenase activity. The incorporation of organic amendments in soil influences soil enzymatic activity because this material may contain intra-cellular and extracellular enzymes, and may stimulate microbial activity in the soil observed by Chang *et al.*, (2007). Reported that the continuous application of integrated nutrient management technique for wheat cropping resulted increased dehydrogenase and phosphatase activity in soil.

2.8. Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) symbiosis, which may be found in almost all naturalistic habitats, perform activities such as nutrition uptake, stress modulation, growth stimulation, soil structure, and fertility management (Mitra et al., 2023). AMF are soil beneficial fungi that form a symbiotic relationship with plants and many agricultural crops such as rice (Liu et al., 2021; Sarkodee-Addo et al., 2020; Gosling et al., 2006). They are classified as a member of subkingdom Mucoromyceta and the phylum Glomeromycota being Glomus, Funneliformis, Rhizophagus, Gigaspora, Claroideoglomus, Sclerocystis and Acaulospora are the predominant AMF species in soil (Mitra et al., 2021a; Wang et al., 2019; Sadhana, 2014). AMF has an important role in sustainable agricultural systems. Even though they are symbiotic organisms, they also contain some structures similar to those of organisms that have been detected in spores and mycelium. They are obligate biotrophs and their life cycle completion is dependent on the colonization of the host plant, therefore it is difficult to culture AMF in absence of the host plant (Berruti et al., 2016). However, AMF grown in sterile culture with plant root explants is a very appropriate method for molecular studies especially for understanding the mechanisms of AMF symbiosis and its role in plant development (Diagne et al., 2020; Chabaud et al., 2006; Harrier, 2001).

The effects of AMF on plant growth have been widely studied in many species including relevant crops. It's important to note that the degree of host plant benefits varies depending on the AMF species adopted, and macro and micronutrient uptake may be influenced by both the fungal partner and the host plant (Etesami and Jeong, 2021; Thirkell et al., 2020; Ingraffia et al., 2019; Kim et al., 2017). This plant growth promotion is because AMF associated with the plant facilitates the nutrient uptake by the hyphae from the soil increasing the absorbing capability of surface host root (Bonfante and Genre, 2008). Several nutrients, such as P, do not diffuse easily through the soil; therefore, the roots quickly deplete these nutrients from their surrounding zone. AMF hyphae can spread in the depletion zone increasing the total absorption surface of

inoculated plants and thus improving plant access to nutrients and nutrient uptake (Diagne et al., 2020). AMF contributes to soil carbon storage via modifying the quality and quantity of organic matter in the soil, as well as influencing the root's kinetic characteristics, which improves its nutrient uptake capacity . AMF also protects plant from parasitic fungus, other parasites, and undesired plant species like weeds, as well as resisting environmental challenges and assisting plants in dealing with biotic and abiotic pressures (Schouteden et al., 2015; Purin et al., 2008). The processes of adaptation of AMF to abiotic stressors are generally associated to enhanced hydromineral feeding, ion sensitivity, gene regulation, osmolyte synthesis, phytohormones, and antioxidant synthesis (Mitra el al., 2021a; Wu et al., 2017; Lenoir et al., 2016). AMF are involved in pathogen resistance, including competition for colonization sites and the enhancement of the plant's defense system, when it comes to ecological stressors. Interesting reviews concerned with the interaction between AMF and weeds or invasive plants were published recently (Li et al., 2016; Menzel et al., 2017; El Omari and El Ghachtouli, 2021). AMF biofertilizer and crop protection role is based on their ability to obtain nutrients from the soil in a more efficient manner, as well as their protective characteristics (Berruti et al., 2016). The impact of the use of AMF on agriculture can be visualized on the quality and quantity of agricultural products impacting in life since their use improves nutrition of the plant, tolerance to water and heavy metal stress, resistance to low temperature, and resistance against pathogens increasing synthesis of primary or secondary metabolites (Mitra et al., 2022; Samuel and Veeramani, 2021; Kaur and Suseela, 2020; Diagne et al., 2020).

2.9. Background of AMF

For the last 400 million years, AMF has had a connection with plants, assisting higher plants in the colonisation of arid regions and most ecosystems (Stürmer et al., 2020). The symbiosis of AMF improves the plant rhizosphere microenvironment, increases the absorption of mineral elements by the plant, improves stress and disease resistance and promotes plant growth (Li et al., 2019). The plant provides AMF with the necessary carbon source and energy in exchange, generating a huge extra root hypha network in the soil (Andrino et al., 2021; Thirkell et al., 2020). AMF improves the nutritional condition of host plants by expanding the range of plant root absorption and increasing the plant's intake of N, P, C and water (Liu et al., 2020). It is known that the content of P in soil is low and there are many soils P deficient and therefore poor to support effective crop production which is the major reason for extensive application of chemical fertilizers (Syers et al., 2008). Inorganic P, which is the form absorbed by the plants, is adsorbed to some cations in the soil causing low solubility and mobility of it so that when inorganic P is absorbed by roots, replacement from bulk soil is extremely slow, and depletion zones develop that reduce uptake by the epidermis and root hairs via a direct pathway.

2.10. AMF diversity and symbiosis in rice plant ecosystem

Rhizosphere soil is essential for determining AMF richness in the roots of the plant host and is also linked to a wide range of plants from various taxonomic groupings. Plant diversity patterns are known to be influenced by mycorrhizal fungus in a range of habitats around the world . AMF diversity has been achieved by a morphological determination of spore diversity; however, there are few morphologically and proteomic-based distinguishable characters present in the spores of AMF for this . Even within the same species, genetically diverse AM fungal isolates have varied impacts on their host plants (Lee et al., 2013). In addition, their hidden lifestyle inside the roots and soil can be a reason for the difficulty to determine their diversity since they cannot be cultured in artificial media. Despite the problems of determining diversity will reflect back into the natural ecosystem, resulting in changes in plant biological diversity and production (Sarkodee-Addo et al., 2020).

To start the communication between AMF and plant, the later secretes signaling molecules called strigolactones, compounds that are carotenoid derivatives, in the root exudates (Mitra et al., 2021b). These compounds increase the metabolism of AMF in a pre-symbiotic phase resulting in hyphal branching, which improves root contact and enhances symbiosis. Besides, this allows that AMF can colonize the plant roots establishing a mycelial network in the soil (Gupta et al., 2019). Then the hyphae proliferate in the soil forming appressoria, which facilitates the penetration of the epidermal cells starting the branching in the outer cortex. These branching structures are known as arbuscules, and they function as nutrient exchangers, transferring carbohydrates from plant tissues to the fungus while also supplying nutrients and water from the AMF to the plants . Vesicles are generated in older roots to store nutrients and act as propagules. Extraradical mycelium is made up of spores and sporocarps, as well as mycelium that acts as propagules to complete the life cycle (Mitra et al., 2021b; Gupta et al., 2019; Gupta, 2017).

Rice is the most widely consumed main and fundamental food on the planet. Oryza sativa (Asian rice) and O. glaberrima (African rice) are the two primary crop species of cultivated rice (Linares, 2002). O. sativa is a high-yielding crop widely farmed around the world, but O. glaberrima has a lower grain yield but is more stress tolerant and is only cultivated in Africa. Sustainable rice production through improving the yield and lowering the environmental cost of AMF, can be an emerging alternative and even improve rice resistance to abiotic stresses (Mbodj et al., 2018). The beneficial effects of AMF on rice plants can be seen in Fig. 2.

The root system of rice plants is complex and is formed by different root types; therefore it is important to know rice root system architecture to control AMF symbiosis. In the case of rice cultivation, the most limiting factor for the presence of AMF in the soil is the anaerobic irrigated cultivation since AMF are aerobic microbes. Commonly the rice plants are grown under a delayed-flood cultural system; however in some places where the water is scarce and to save water, rice can be cultivated under rain-fed conditions or alternating wet and dry cultivation. It has been observed that AMF works in both flooded and non-flooded rice, resulting in increased yield (Panneerselvam et al., 2017) colonizing rice plants in different rice producing field locations . AMF can contribute up to 80% of the P absorption by rice plants, encouraging plant growth. AMF can also effectively transfer N and improve plant resilience to abiotic stressors such as drought. Drought affects rice yields to some extent since almost half of the world's rice land lacks sufficient water to maintain flooded conditions. AMF-associated root system architecture benefits the rice adaptation to drought stress. Furthermore, AMF symbiosis in rice induced the osmoprotectant and antioxidant molecules accumulation while reducing the accumulation of hydrogen peroxide and oxidative damage on lipids. Although the beneficial effects of AMF on rice growth and resistance to abiotic stresses have been reported by in vitro assays, there are few reports of the beneficial effects in vivo .

2.11. Arbuscular mycorrhizal fungi relation to the decomposition and glomalin

Arbuscular mycorrhizal fungi may contribute to decomposition of complex compounds in soils (Herman et al., 2012). Decomposition by mycorrhizal fungi has important consequences for how soil carbon © stocks respond to global change. If mycorrhizal fungi decompose significant quantities of soil C, the effects of global change on both plant and microbial communities could control the loss of soil C stocks. Reaching a predictive understanding of soil C feedbacks to atmospheric C pools requires a synthesis of knowledge about the ability of mycorrhizal fungi to act as decomposers as well as an evaluation of how global change regimes could regulate the extent to which this occurs in nature (Talbot et al., 2008). Glomalin is produced by living hyphae of obligate biotrophic AMF and the concentration depends on soil properties, climate, fungi involved, the host plants and their productivity (Rillig et al., 2001).

Chapter 3: Aims and Objective

3. Aims and Objective

There is not much systematic information available on changes of soil functional properties and Arbuscular mycorrhizal activities due to application of decomposing microbial consortium during in situ decomposition of paddy straw under wet land condition.

To understand how the introduced decomposing microbial consortium alters the soil microbial, chemical properties, AMF response and glomalin-related soil protein distribution in wet land rice cultivation during *in-situ* paddy straw decomposition in

different way of approaches like residue retention, residue incorporation, zero tillage and conventional methods.

Objective(s):

1. To study the changes of microbial and physicochemical properties at different stages of rice under field condition

2. To study Arbuscular mycorrhizal colonization, sporulation and glomalin-related soil protein at different stages of rice under field condition

Chapter 4: Materials and Methods

4. Materials and Methods

The present study was undertaken at Microbioogy, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha (20°25' N latitude, 85°55' E longitude with an altitude of 24m above mean sea level).

4.1. Soil sample collection and preparation

The main purpose of this experiment is to understand the changes in soil chemical & microbial properties and AMF diversity during the time of *in-situ* paddy straw decomposition under field condition. Soil samples were collected from ongoing field experiment for *in-situ* decomposition of paddy straw residues under NASF sponsored project at ICAR-NRRI experimental field, Cuttack, at vegetative and reproductive stage of rice (*Var.* Swarna) (Fig. 1).

The treatment comprises four main treatments

MT1 - Conventional rice cultivation;

MT2 - Residue retention (simulation like machine cut and spread) (@ 6 t paddy straw /ha);

MT3 - Zero tillage (around 30 % of left rice stubbles in the field after harvest),

MT4 - Residue incorporation (@ 6 t paddy straw /ha)

And six sub-treatments

ST1 - No culture;

- ST2 NRRI decomposing microbial consortium (Solid formulation)
- ST3 NRRI decomposing microbial consortium (Capsule formulation)
- ST4 NRRI Actino consortium;
- **ST5** IARI capsule based formulation (Reference check).

The experimental design was split plot design with three replications. As per the experimental design, the experimental field has been divided into 72 equal sub plots (4 main treatments x 6 sub treatments x 03 replications) and soil samples were collected from all the plots (Fig. 2).



Figure 1. View of field experiment



Figure 2. Collection of soil sample from field

The chemicals and media components used were of analytical grade (AR) obtained from Merck limited, India, Sigma-Aldrich Inc. USA and Hi Media, India. The materials used and methods employed are presented under the following sub-heading.

4.2. Isolation of cultivable microbes

4.2.1. Isolation of cultivable bacteria, fungi and actinobacteria

The collected soil sample were processed and microorganisms were isolated by using serial dilution method and spread plate technique on nutrient agar, potato dextrose agar (PDA), and actinomycetes isolation agar medium for bacteria, fungi and actinobacteria, yeast and diazotrophs respectively. The inoculated plates were incubated at $30 \pm 2^{\circ}$ C for 24, 72, 96, 120 and 72 hours respectively. In serial dilution technique, 10 g of processed samples were added to 90ml of sterile distilled water taken in a 250ml Erlenmeyer flask and the solution was stirred at 150 rpm for an hour. Serial dilutions were prepared by sequentially transferring 1 ml soil samples into test tubes containing 9 ml of sterile distilled water and then selected diluents of 100µl samples were transferred onto selective media. The plates were examined regularly. The single colony of bacterium, fungus, actinobacterium were transferred onto fresh respective selective media to obtain pure culture.

4.3. Isolation of phosphate solubilizer

For the isolation of phosphate solubilizing microbes, 1g soil was suspended in 90ml of distilled water. An aliquot $(100\mu l)$ from decimal dilutions was inoculated on Pikovskaya's medium containing insoluble tri-calcium phosphate by spread plate technique and incubated at 27-30°C for 7 days. Colonies showing phosphate solubilizing zone around them were considered as PSM. Single colonies appearing on Pikovskaya's agar (HiMedia, India) plates were transferred in liquid broth of Pikovskaya's and on agar slants for further study.

4.4. Biochemical estimation of soil enzymatic activities

4.4.1. Fluorescein diacetate activity (FDA)

Fluorescein diacetate (FDA) hydrolysis activity measurement was made following the method of as modified by Adam and Duncan (2001). The details of the method are as follows: two gram of field moist soil samples and the control were placed in a 50ml conical flask. Then 15ml of 60mM potassium phosphate buffer (pH 7.6) was added to each flask. 0.2ml stock solution of FDA (1000 μ g mL⁻¹) as substrate was added to start the reaction. Then the flasks were stopper and contents were shaken well and the flasks were incubated at 30°C for 20 minutes. 15ml of chloroform/methanol (2:1 v/v) solution was immediately added after incubation to terminate the reaction. Then the contents of flasks were transferred to 50ml centrifuge tubes and the tubes were centrifuged at 2000

rpm for 3 minutes. Then the supernatant was filtered through Whatman No. 41 filter paper into 50ml conical flasks. Then the intensity of yellow-green colour of the filtrates were measured at 490nm in a spectrophotometer. The concentration of fluorescein released during the assay was calculated using the calibrating graph produced from 0 to 5 μ g fluorescein mL⁻¹ standard which were prepared from a 20 μ g fluorescein ml⁻¹ working standard solution. All data were statistically analysed.

4.4.2. Dehydrogenase activity (DHA)

Dehydrogenase activity was estimated by the method of using triphenyl tetrazolium chloride (TTC) as substrate. 3g of field moist soil samples were mixed with 0.2 g of CaCO3, 1ml of 3% (w/v) 2,3,5-triphenyltetrazolium chloride (TTC) and 2.5 ml of distilled water and incubated at 37'C for 24 hours. Then after 24 hours the stopper was removed and 10 ml of methanol was added. Dehydrogenase enzyme converts TTC into 2,3,5-triphenylformazan (TPF). The TPF formed was extracted with methanol, the extracts were filtered and absorption was measured at 485nm with a spectrophotometer.

4.4.3. Microbial biomass carbon (MBC)

Microbial biomass carbon was determined using the chloroform fumigation extraction method. MBC with chloroform fumigation extraction method (CFE-MBC), 10g of moist soil samples were taken and kept in oven at 105°C for 24 hours and moisture content was calculated. 50 ml beaker was taken and 3 g of soil was put in the beaker (2 sets). One set was un-fumigated while the other was fumigated in vacuum desiccator. Vacuum was created inside the desiccator until the chloroform boiled. The desiccators were kept in dark for 24hours. Both fumigated and un-fumigated samples were transferred to 250 ml conical flask and 25 ml of 0.5 M K₂SO₄ was added. Total organic carbon (TOC) content in the soil extracts was measured with the dichromate digestion method. The CFE-MBC was calculated as 2.64 times the difference in extractable organic C between the fumigated and un-fumigated soils (Vance, et al., 1987).

4.4.4. β-glucosidases activity

β-Glucosidase activity was measured by the standard method given by Eivazi and Tabatabai, (1988). 1g of field moist soil was added in 50 ml Erlenmeyer flask in which 0.25 ml toluene, 4ml of modified universal buffer (pH 6.0) and 1.0 ml of 0.025M *p*nitrophenyl-β-D-glucosidase (*p*NG). After swirling the flasks for 1 minute, the flasks were incubated at 37°C for 1 hour. Then 1 ml of 0.5M CaCl₂ and 2 ml of 0.1M THAM (pH 12) was added. A control was prepared in a similar way without adding p-nitrophenyl β-D-glucosidase. After swirling for few second, 1 ml of pNG was added to the control. All samples were filtered through Whatman No. 41 filter paper. Intensity of the yellow colour developed was recorded in spectrophotometer at 420nm. A standard curve was prepared with p-nitophenol (0-50 µg ml⁻¹) and amount of p-nitrophenol liberated was calculated with standard curve. β -Glucosidase acitivity was calculated in terms of μg pnitrophenol (PNP) g⁻¹ h⁻¹.

4.4.5. Acid and alkaline phosphatase activity

The activity of acid- and alkaline-phosphatase of soil samples were estimated by the method of Tabatabai and Bremner, (1969) using p-nitrophenyl as substrate and expressed in 1 g of pNP released per gram of soil per hour. 1 g of field moist soil sample was taken in a 50 ml Erlenmeyer flask in which 0.2 ml toluene followed by 4 ml of MUB (pH 6.5 for acid phosphatase assay and pH 11 for alkaline phosphatase assay) and 1 ml of 0.05M p-nitrophenyl phosphate solution was added. After swirling for 1 minute, the flasks were incubated at 37°C for 1 hour. Then 1 ml of 0.5M CaCl₂ and 4 ml of 0.5M NaOH was added to the flasks. A control was also prepared in similar way without adding p-nitrophenyl phosphate. After swirling the flasks for few seconds, 1 ml of p-nitrophenyl phosphate was added to the control. All samples were filtered through Whatman No. 41 filter paper. Then the intensity of yellow colour developed was measured through spectrophotometer at 389 nm. A standard curve was prepared with p-nitrophenol (0-50 µg ml⁻¹) and amount of p-nitrophenol liberated was calculated with standard curve. Alkaline phosphatase activity was calculated in terms of µg p-nitrophenol (PNP) g⁻¹ h⁻¹.

4.5. Isolation of mycorrhizal spores

100 g dried rhizospheric soil sample was weighed and mixed with 500 mL water in a beaker. The suspension was kept undisturbed for 5 minutes for the sedimentation of heavier particles. After 5 minutes, the suspension was carefully decanted through the sieves stack that was arranged in a descending order of 710, 250, 75, and 45 μ m mesh size. Each level of the sieve was washed with water until it appeared that all possible material had passed through. The retained material on the sieve was re-suspended in water and the process was repeated for two or three times until a clear solution was visible. The AM fungal spores in the bottom sieves, viz., 75 and 45 μ m were transferred into the 15 mL polypropylene tube with distilled water.

4.5.1. Observation of spores

The spores collected through sieving technique were washed into glass Petri dishes and examined under a sterio binocular microscope (Stemi, Carl Zeiss Microscopy, LLC, Thornwood, NY, USA). Healthy spores were picked by using a disposable Pasteur pipette, and spores were mounted with cover slip on slides for identification purpose.

4.6. mycorrhizal colonization

Freshly collected secondary and tertiary root samples were gently washed to remove rocks that was attached to the root surfaces, submerged in 10% KOH solution and autoclaved for 15 minutes at 121 °C. The KOH solution was decanted and the treated roots were rinsed with tap water for 3–4 times until no brown colour appeared in the rinsed water. The treated root samples were further immersed in 2% HCl solution for 5 minutes. Without rinsing with water, HCl was decanted and the root samples were stained with 0.05% trypan blue in lactoglycerol (400 mL lactic acid + 400 mL glycerol + 100 mL water) and autoclaved for 15 minutes at 121 °C. After autoclaving, the stained solution was decanted and the roots were destained with lactoglycerol solution to remove the excess of stains and used for microscopic observations (Mitra et al., 2017). The slide was prepared by keeping 10 segments of stained root on a clean glass slide and observed under compound microscope (OLYMPUS microscope, Olympus®, Tokyo, Japan). The AM infection was detected by the presence of spore or hyphae in the root cortex.

Percentage of AM root colonization was calculated using the formula:

Percentage of colonization = Number of root segments colonized \div Total number of root segments \times 100

4.7. Estimation of glomalin content

Total glomalin content of soil sample was estimated by pressure cooker method as described by Wright and Upadhyaya (1996). One gram of soil was taken in an autoclavable centrifuge tube and 8 mL of 50 mM sodium citrate was added. The mixture was then autoclaved for 60 minutes at 121 °C. After autoclaving, the tubes were centrifuged at 7,000 rpm for 15 minutes to sediment soil particles. The supernatant containing the extract was collected and stored at 4 °C. This process was repeated for two more times and the extracts were pooled and stored. The protein content in the extract was quantified using Bradford assay protocol using bovine serum albumin as the standard. The glomalin content was read by spectrophotometer at 595 nm and expressed as milligram per gram of soil.

4.8. Statistical analysis

Statistical analysis was done by using STAR- Statistical Tool for Agricultural Research. Standard errors were calculated for all mean values. Differences were considered significant at the $p \le 0.05$ level.

Chapter 5: Results

5. Results

5.1. Population of culturable heterotropic bacteria, fungi and actinobacteria

The data of the microbial population which includes bacteria, fungi, actinobacteria, phosphate solubilizing bacteria (PSB), are presented from (Fig. 3 - 6, 7) respectively and standard methods were followed for evaluation. The bacterial population was comparatively higher in residue incorporation (MT4) with solid based formulations (ST2) at both vegetative and reproductive stage. In vegetative stage residue incorporation treatment recorded higher population (7.921 Log cfu ml⁻¹) in solid based formulations treated plots followed by NRRI capsule-based inoculum (ST3) (7.829 Log cfu ml⁻¹). In reproductive stage residue incorporation treatment recorded higher populations treated plots followed by NRRI capsule-based inoculum (ST3) (7.829 Log cfu ml⁻¹).

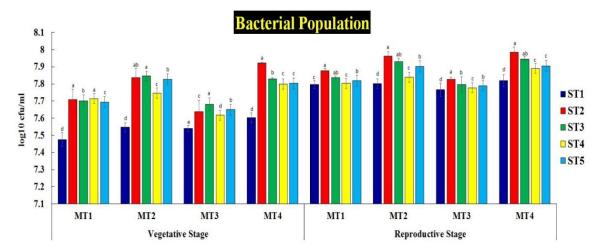


Figure 3. Bacterial population in different rice straw residues management systems under wet land rice cultivation at vegetative and reproductive stage of growth

Fungal population was comparatively higher in residue incorporation (MT4) with solid based formulations (ST2) *i.e.* (7.921 Log cfu ml⁻¹) applied treatment in vegetative stage followed by NRRI capsule-based inoculum (ST3) *i.e.* (7.829 Log cfu ml⁻¹) and solid based formulations (ST2) *i.e.* (7.921 Log cfu ml⁻¹) in reproductive stage followed by NRRI capsule-based inoculum (ST3) *i.e.* (7.829 Log cfu ml⁻¹).

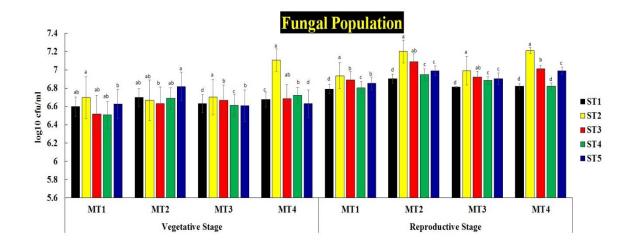
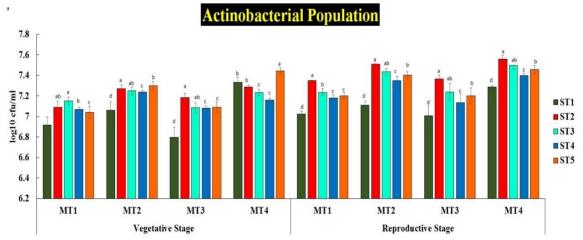
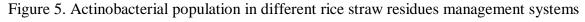


Figure 4. Fungal population in different rice straw residues management systems under wet land rice cultivation at vegetative and reproductive stage of growth

Residue retention and residue incorporation showed higher actinobacteria population as compared to other methods. At vegetative stage residue retention treatment recorded higher population (7.442 Log cfu ml⁻¹) in solid based formulations treated plots followed by NRRI capsule-based inoculum (ST3) (7.232 Log cfu ml⁻¹). At reproductive stage residue incorporation treatment recorded higher population (7.56 Log cfu ml⁻¹) in solid based formulations treated plots followed by NRRI capsule-based plots followed by NRRI capsule-based inoculum (ST3) (7.232 Log cfu ml⁻¹).





under wet land rice cultivation at vegetative and reproductive stage of growth Phosphate solubilizing bacteria (PSB) showed higher population in residue incorporation with soild based formulation i.e. (4.630 Log cfu ml⁻¹) at vegetative stage and with NRRI capsule-based inoculum (ST3) i.e. (5.964 Log cfu ml⁻¹) at reproductive stage.

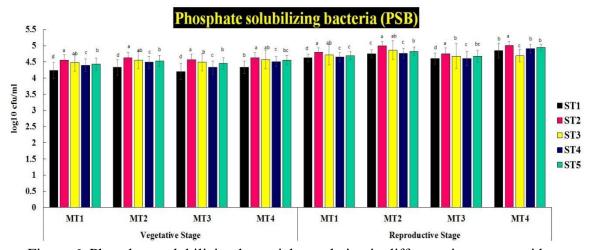


Figure 6. Phosphate solubilizing bacterial population in different rice straw residues management systems under wet land rice cultivation at vegetative and reproductive stage of growth

Vegetative stage

Reproductive stage

Bacterial population

(10-6)



population (10⁻⁶)

Fungal

Actinobacterial population (10⁻⁶)

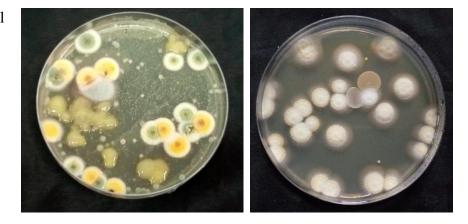


Figure 7. Bacterial, fungal, and actinobacterial population during *in-situ* decomposition of paddy straw at vegetative and reproductive stage of rice crop under low land condition

5.2. Biochemical estimation of soil enzymatic activities

5.2.1. Fluorescein diacetate activity (FDA) activity

FDA activity of samples from different treatments is shown in Fig 8. At vegetative stage the results showed that there was a significant variation in FDA, which was recorded in the range of $13.11 - 24.15 \,\mu g$ fluorescein g⁻¹ h⁻¹. Residue incorporation with NRRI capsule-based treatment showed the higher FDA activity compared to other paddy straw residues management system. At reproductive the results showed that there was a significant variation in FDA, which was recorded in the range of $15.43 - 26.75 \,\mu g$ fluorescein g⁻¹ h⁻¹. Residue incorporation with solid based inoculum treatment showed the higher FDA activity compared to other paddy straw residues management system. NRRI capsule and solid based inoculums recorded higher activity of FDA compared to check. Microbial intervention found to increase 18-21% higher FDA activity at vegetative and 26-28% FDA at reproductive stage in residue incorporation compared to the conventional treatment.

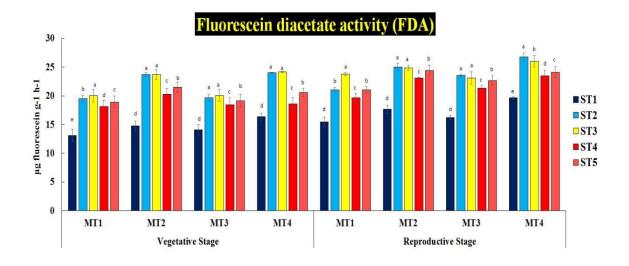


Figure 8. Effects of *in-situ* decomposition of paddy straw residue management practices on FDA activity in soil

5.2.2. Dehydrogenase activity

Dehydrogenase activity is presented in Fig. 11 and results indicated that there was lot of variation in the range of $42.60 - 63.18 \ \mu g$ TPF g⁻¹ d⁻¹ and $47.33 - 70.12 \ \mu g$ TPF g⁻¹ d⁻¹ both at vegetative and reproductive stage respectively. At vegetative stage residue retention with solid based inoculum showed highest dehydrogenase activity under residue retention (63.18 TPF g⁻¹ d⁻¹) followed by residue incorporation (63.13 TPF g⁻¹ d⁻¹) as compared to other type of formulations and at reproductive stage residue incorporation with solid based inoculum showed (70.12 TPF g⁻¹ d⁻¹) followed by residue retention (69.43 TPF g⁻¹ d⁻¹) as compared to other type of formulations. With solid based inoculum the dehydrogenase activity was higher in residue retention at vegetative stage and residue incorporation at reproductive stage compared to conventional treatment.

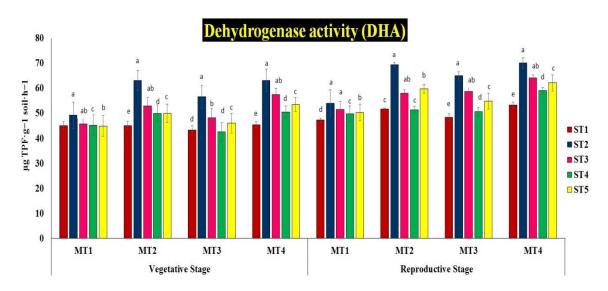


Figure 9. Effects of *in-situ* decomposition of paddy straw residue management practices on DHA activity in soil

5.2.3. Microbial biomass carbon

The microbial biomass carbon (MBC) data is presented in Fig 10. There was variation in the MBC fraction ranging from $616.06 - 734.74 \ \mu g \ g^{-1}$ soil h⁻¹at vegetative stage and $645.01 - 792.92 \ \mu g \ g^{-1}$ soil h⁻¹ at reproductive stage. Among different treatments, the residue incorporation with solid based inoculum recorded higher MBC compared to other treatments followed by residue retention with solid based inoculum. The MBC lower in content in the un inoculated control as well as in check compared other type of microbial formulations application.

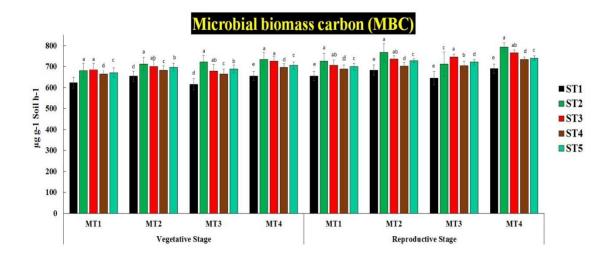


Figure 10. Effects of *in-situ* decomposition of paddy straw residue management practices on MBC activity in soil

5.2.4. β- glucosidase activity

Data related to the β - glucosidase activity is presented in Fig 11 and ranged from 62.33 to 112.48 µg pNP g⁻¹ h⁻¹ at vegetative stage and 67.84 to 132.04 µg pNP g⁻¹ h⁻¹ at reproductive stage indicating variation among treatments. Residue incorporation showed significantly higher activity compared to other treatments. Solid and NRRI capsule inoculum showed higher activity of β - glucosidase compared with un inoculated control and check. Application of solid based inoculum with residue incorporation resulted in higher β - glucosidase activity which was 33.50- 46.00% higher compared to conventional treatment both at vegetative and reproductive stage respectively.

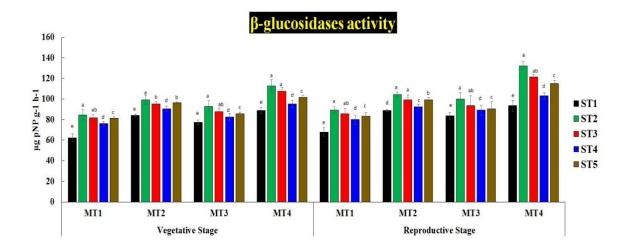


Figure 11. Effects of *in-situ* decomposition of paddy straw residue management practices on β - glucosidase activity in soil

5.2.5. Acid and alkaline phosphatase

The acid phosphatase and alkaline phosphatase activities are given in Fig 12 and Fig 13. Acid phosphatase was recorded in the range of $41.53 - 148.31 \ \mu g \ pNP \ g^{-1}h^{-1}$ at vegetative stage and $38.01 - 50.64 \ \mu g \ pNP \ g^{-1}h^{-1}$ at reproductive stage in different treatments. Similarly, the alkaline phosphatase varied from $19.17 - 29.77 \ \mu g \ pNP \ g^{-1}h^{-1}$ at vegetative stage and $17.85 - 25.91 \ \mu g \ pNP \ g^{-1}h^{-1}$ at reproductive stage. In general, uninoculated control recorded significantly lower activity as compared different microbial formulations applied treatments.

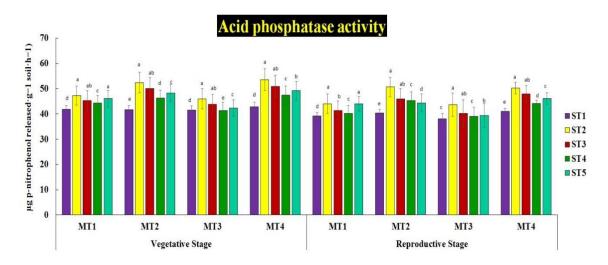


Figure 12. Effects of *in-situ* decomposition of paddy straw residue management practices on acid phosphatase activity in soil

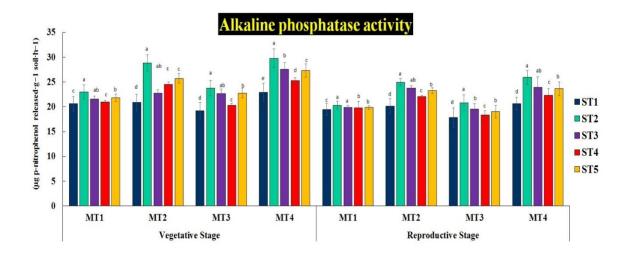


Figure 13. Effects of *in-situ* decomposition of paddy straw residue management practices on alkaline phosphatase activity in soil

5.3. AM fungal rice root colonization under *in-situ* decomposition of paddy straw residue

The root colonization percentage varied with different paddy straw residue management practices and microbial intervention. Fig 14 & 15. represented the percentage of AMF colonization and image of AMF colonization in different in-situ decomposition of paddy straw residue management practices and application microbial formulations.

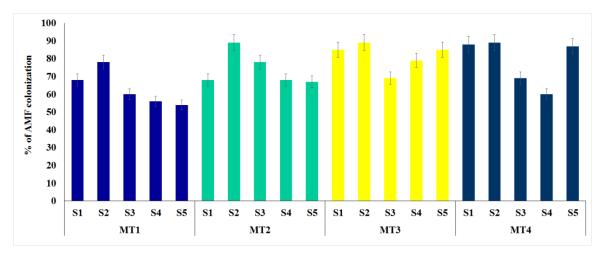
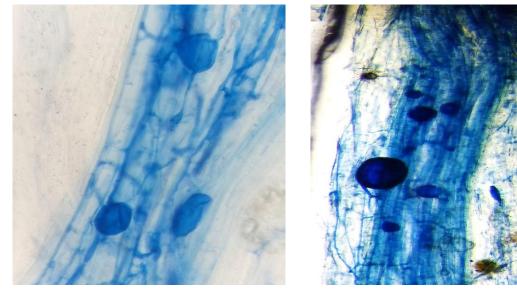


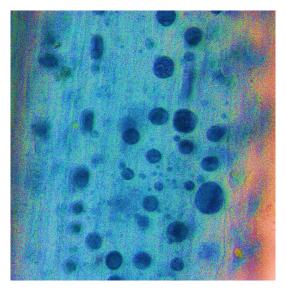
Figure 14. Percentage of AMF colonization in rice variety [MT1 - Conventional rice cultivation; MT2 - Residue retention (simulation like machine cut and spread) (@ 6 t paddy straw /ha); MT3 - Zero tillage (around 30 % of left rice stubbles in the field after harvest), MT4 -

Residue incorporation (@ 6 t paddy straw /ha) ; ST1 - No culture; ST2 - NRRI decomposing microbial consortium (Solid formulation); ST3 - NRRI decomposing microbial consortium (Capsule formulation); ST4 - NRRI Actino consortium; ST5 - IARI capsule based formulation (Reference check)]

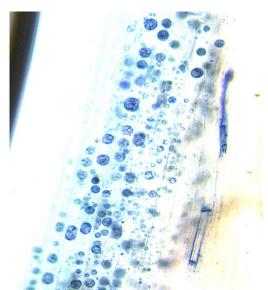


Conventional rice cultivation

Residue retention



Zero tillage

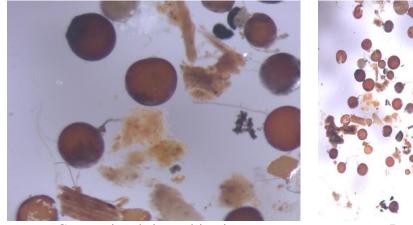


Residue incorporation

Figure 15. AM fungal colonization in different system of *in-situ* decomposition of paddy straw residues

With various methods of managing paddy straw residue and microbial intervention, the AMF sporulation changed. Figures 16 and 17 showed the sporulation of AMF and an

illustration of AMF sporulation in various in-situ decomposition techniques for paddy straw residue management.



Conventional rice cultivation

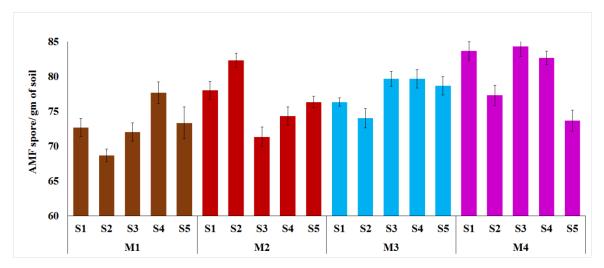


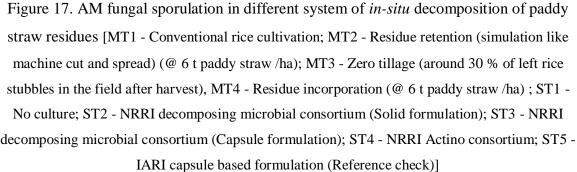


Zero tillage

Residue incorporation

Figure 16. Sporulation image of AMF in different system of *in-situ* decomposition of paddy straw residues





5.4. Soil glomalin content from *in-situ* decomposition of paddy straw residue management field

Glomalin is the glycoprotein produced by AM fungi to bind soil particles and improve soil aggregate stability. Quantified glomalin helps in selecting the AM species for their intended use in agriculture. Hence, glomalin was estimated from soil samples collected in this study (Fig. 18). The glomalin content in soil varied from system to treatment, however, highest glomalin content was observed in MT3 - ST3 & ST5 (1.3910 mg/mL).

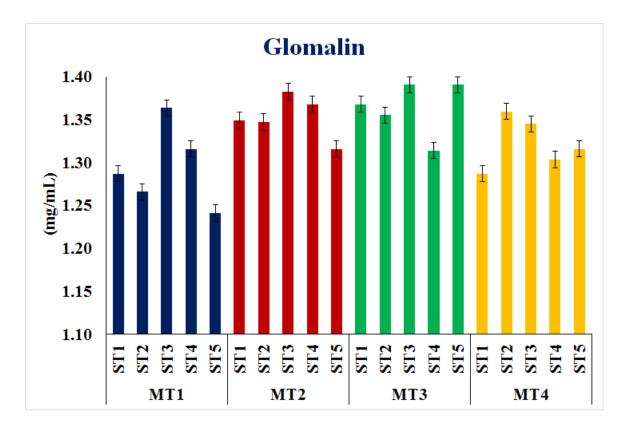


Figure 18. Glomalin concentration in the collected soil samples [MT1 - Conventional rice cultivation; MT2 - Residue retention (simulation like machine cut and spread) (@ 6 t paddy straw /ha); MT3 - Zero tillage (around 30 % of left rice stubbles in the field after harvest), MT4 - Residue incorporation (@ 6 t paddy straw /ha); ST1 - No culture; ST2 - NRRI decomposing microbial consortium (Solid formulation); ST3 - NRRI decomposing microbial consortium (Capsule formulation); ST4 - NRRI Actino consortium; ST5 - IARI capsule based formulation (Reference check)]

Chapter 6: Discussion

6. Discussion

Rice straw is composed of cellulose, hemicellulose, lignin and silica. One of the best solutions to the problem of paddy straw burning is to efficient recycling at field level (Shukla et al., 2016). Rice straw decomposition occurs naturally at field condition by slow decomposition process (Devêvre, O.C. and Horwáth, W.R., 2000). Inadequate decomposition of paddy straw in the field, which results in methane production on flooded fields (Naser et al., 2007). According to Fang et al. (2018), microbial technology has been shown to accelerate the degradation of organic materials. Decomposition is a microbiological process that breaks down the substrate into a more stable product (Mohammad et al., 2013). Different microorganisms may play distinct roles in the degradation process (Sagarika et al., 2022). Several bacteria and fungi are involved in lignocellulose degradation, with bacteria/fungi acting as cellulose degrading microorganisms (Singh and Nain, 2014; Sagarika et al., 2022). To produce value-added products, certain bacteria could hydrolyze the celluloses and hemicelluloses in rice straw using both solid state and submerged fermentation (Mussatto, S.I. and Teixeira, J.A., 2010). Microorganisms can produce enzymes and metabolites that aid in the decomposition of organic waste and the quality of soil humus. In the current study, silicate solubilizing and cellulase producing bacteria were found to be common in residue retention, incorporation, and zero tillage management practises, indicating that paddy straw amendments, in conjunction with a suitable decomposing microbial consortium, may trigger other beneficial microbial populations in rice soil.

Tillage and residue management practises will have an effect on the structure and function of the soil microbiota (Saikia et al., 2020), which can have a direct impact on the ecological services provided (Saikia et al., 2019). Tillage, straw management, and crop management changes all have an impact on soil microorganisms. The incorporation and retention of paddy straw residues has a significant impact on soil chemical and biological properties. In the current study, the majority of the chemical and microbial properties were higher in the residues incorporation, retention, and zero till treatments when compared to the conventional method of rice cultivation, indicating that paddy straw amendments in wet land conditions of rice cultivation combined with microbial inoculants improves both microbial and chemical properties. Paddy straw (@ 6.0 t ha) application in wet land conditions may serve as good organic amendments; additionally, application in combination with a decomposing microbial consortium may hasten the decomposition process, resulting in increased soil nutrients and fertility in soil, which in turn improves soil microbial and chemical properties.

A number of studies have shown that incorporating paddy straw residues into fields improves soil nutrient content (Gaind, S. and Nain, L., 2007)Cautioned that excessive residue removal could result in a loss of soil attributes such as C, and they

suggested using cover crops (CC) in conjunction with residue assimilation and retention. The carbon content of soil is largely dependent on the addition of organic amendments, which supply C substrate and thus increase biological activity. The organic matter composition of the soil, which includes microbial biomass carbon, is linked to the activity of soil microorganisms, their biomass, and enzyme activities (Kandeler et al., 2006). Microbiological characteristics, such as soil enzyme activity, are used to assess the impact of various treatments on the rate of decomposition and soil quality. Soil enzymes are one of the most important components of soil, and different management approaches affect them (Schinner et al., 2012). Plant growth, dynamics, and nature all have an impact on microbial activity (Canellas, L.P. and Olivares, F.L., 2014). The effect of microbes on insitu degradation of paddy straw on soil enzymes such as fluorescein diacetate, dehydrogenase, beta-glucosidase, acid and alkaline phosphatase in the current study revealed increased activities in the treatment that received residue incorporation. Several studies have found that, when compared to the conventional method, residue inclusion increases enzymatic activity (Domínguez et al., 2021) and microbial population (Li et al., 2020) through faster decomposition (Balota et al., 2004; Balota et al., 2014; Blagodatsky et al., 2010). Overall, this finding clearly indicates that the various methods of in-situ paddy straw residue management (incorporation, retention, zero tillage) along with decomposing microbial consortium significantly improve soil chemical, biological properties and factors affecting mycorrhizal activity, but the other consequences, such as AMF interaction and influences, need to be investigated further.

Chapter 7: Conclusions

7. Conclusions

The objective of this dissertation was to better understand how soil chemical and microbial properties changed as paddy straw decomposed in-situ under field conditions. The following is a summary of the research's findings on the effects of various paddy straw management practices on the microbial and chemical composition of soil under wet land rice cultivations.

- As compared to conventional rice farming, residue incorporation followed by residue retention and zero tillage significantly increased the microbial population in vegetative and reproductive stage, which includes bacteria, fungi, actinobacteria, and phosphate solubilizing bacteria (PSB). Among different microbial inoculants intervention, application of either solid or capsule based NRRI decomposing microbial consortium application significantly increased microbial populations with irrespective of different methods of straw residues management as compared un-inoculated control
- At vegetative stage the FDA activity was recorded in the range of 13.11 24.15 μg fluorescein g⁻¹ h⁻¹ in different methods of paddy straw residues management plots. However, residue incorporation showed highest FDA activity at vegetative stage compared to other treatments, similar trend was noticed at reproductive stage. Application of decomposing microbial consortium could increase 15.43% 26.75 % higher FDA activity at vegetative and reproductive stage, respectively in residue incorporation compared to the conventional method.
- Application of either solid or capsule based decomposing microbial consortium increased DHA by 44.97 63.18% in residue incorporation or retention fields compared to conventional treatment in both vegetative and reproductive stage. Whereas application of NRRI decomposing consortium along with paddy straw residues recorded two-fold increase in DHA activities (47.33 69.43µg TPF g⁻¹ d⁻¹) as compared to uninoculated control.
- Application of NRRI decomposing microbial consortium in paddy straw residue incorporation found to increase 112.48 - 132.04 β- glucosidase, acid and alkaline phosphatse activities at vegetative and reproductive stages compared to conventional method of rice cultivation.
- The amount of glomalin in the soil varied depending on the different methods of residues management, but the highest concentration was found in MT3-ST3 & ST5 (1.3910 mg/mL). The root colonisation percentage varied depends on the diverse paddy straw residue management practices and microbial intervention,

Different in-situ paddy straw residues management viz. conventional (CS), residue incorporation (RI), residue retention (RS) and zero tillage (ZT) along with different formulations of decomposing microbial consortium found significantly influences the

microbial and chemical properties at vegetative and reproductive stage of rice cultivation under low land condition. Among the different rice straw residues management system, the residue incorporation or retention along with NRRI decomposing microbial consortium either solid or capsule based formulations found significantly improved soil chemical and microbiological properties. Further the application of solid based microbial consortium or capsules based formulations increased the population of AMF and glomalin particularly under residue incorporation and retention practices compared to zero tillage. **Chapter 8: Future Scope**

8. Future Scope

From the present investigation it was evident that microbial activity under in-situ decomposition had a bigger role in shaping the overall soil microbial community. Further the increase in AMF colonization. Hence, the diversity of arbuscular mycorrhizal fungi (AMF) and other soil microbial communities should be examined in relation to various techniques for in-situ microbial breakdown of paddy straw. Further it is important to examine their functional contributions to the improvement of soil structure, nutrient cycling, and responses of organic matter decomposition. Moreover, the effects of these symbiotic interactions and the distribution of soil proteins on the availability of nutrients, plant development, sequestration of carbon in the soil, and general soil health needs to be investigated. Furthermore, long-term field-scale studies are required to assess the impact of in-situ microbial decomposition on arbuscular mycorrhizal diversity and glomalin-related soil proteins under different crop management practices.

APPENDIX I

1. Nutrient Agar Media (HiMedia, India)

Peptone - 5g Beef Extract - 3g NaCl - 5% Dist. water - 1000ml pH - 6.8 Agar - 1.8%

2. Potato Dextrose Agar

Potato infusion - 200.000g Dextrose - 20.000g Agar -15.000g Dist. water - 1000ml Final pH (at 25°C) 5.6±0.2

3. Actinomycetes Isolation agar

Sodium chloride - 2.0g L – Asparagine - 0.1g Sodium propionate - 4.0g Dipotassium phosphate - 0.5g Magnesium sulphate - 0.1g Ferrous sulphate - 0.001g Agar - 15.0g Distilled water - 1000ml pH 8.1±0.2

4. NBRIP Broth

Glucose - 10.0g

Tricalcium phosphate - 5.0g

Magnesium chloride hexahydrate - 5.0g

Magnesium sulphate heptahydrate - 0.25g

Potassium chloride - 0.2g

Ammonium sulphate - 0.1g

Distilled water - 1000ml

5. Pikavoskaya Media

Yeast extract - 0.500g

Dextrose - 10.000g

Calcium phosphate - 5.000g

Ammonium sulphate -0.500g

Potassium chloride - 0.200g

Magnesium sulphate - 0.100g

Manganese sulphate - 0.0001g

Ferrous sulphate - 0.0001g

Agar - 15.000g Dist. water - 1000ml

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