Spore abundance and morphology of Arbuscular mycorrhizal fungal under Conservation Agriculture

Abstract

Soil is full of microbial diversity and microbes present in soil interact diversely with other living form present in soil e.g., soil microbes and plants. These interactions range from symbiosis to parasitic. Among these, the most predominating mutualistic symbiotic relation is mycorrhiza (associated with ~80% of terrestrial plant species), formed between arbuscular mycorrhizal (AM) fungi and vascular plants. An experiment was conducted under three cropping system rice-maize-cowpea (RMCp), rice-wheat-green gram (RWGg) and rice-cauliflower-bororice cropping (RCfBr) to study influence of three different tillage intensities i.e., CT (conventional tillage), RT (reduced tillage), and ZT (zero tillage) with five different levels of fertilizer and residue application, R1 (0%Residue & 100%RDF), R2(100%Residue & 50%RDF), R3 (100%Residue & 75%RDF), R4 (50%Residue & 100%RDF), R5 (50%Residue & 75%RDF), on AMF spore load and diversity.The experimental design followed was split plot with tillage was main plot treatment and residue as sub plot treatment. Result showed that AMF spores and diversity are significantly influenced of degree of soil disturbance under different tillage practices and cropping system in practice, however residue application imparted non-significant influence on AMF spore load and diversity. A significant lower spore count was observed under CT as degree of soil disturbance is excessive high under CT compared to RT and ZT. With respect to spore diversity, round(shape), brown (color) and free spores were predominating spore type. In reference to spore shape abundance sequence was round>spherical> oval while for spore color it was brown>yellow>hyaline and >90 % spore was free while attached spores were <5%. In case of AMF spore count influence of cropping system was more prominent compared to other factors (i.e., tillage and residue). In RMCp and RWGg, significant increase inspore count was observed compared to RCfBr cropping system. On second year of experimentation, in RCfBr cropping system a significant decline in spore number was detected due to inclusion of cauliflower which act as non-host of AMF. Hence to attain benefits associated with AMF to arable crop we must reduce level of soil disturbance and avoid crucifer crops in cropping system.

Keywords: Conservation tillage, spore count, spore diversity, cropping system etc.

INTRODUCTION

History of agriculture started with sustenance farming, where with limited resources or inputs we could produce only scanty food to meet farmers' need. But rapid increase in human population after World War II made the world to suffer from acute food shortages especially in the developing countries (Battisti and Naylor, 2009). To combat food shortage Green Revolution" received warm welcome and tripled the global food production.Such intensive agricultural practices though increased food production but over the years started to show its bad face and depleted soil health and environmental quality to a greater extent. Continuing such practices is a great threat to human and to entire earth as resulted to loss of crop diversity, extinction of local crop varieties, disappearance of biodiversity (beneficial insects andmicro-organisms), waterlogging, salinization of irrigated land, contamination of groundwater and emergence of GHGs from agriculture field (Choudhary et al., 2018b).One of the `faultiest practice in conventional agriculture is tilling of soil which was began in Mesopotamia era in order to soften soil, prepare seedbed to ensure uniform seed germination and mobilizing nutrients for plant uptake, managing weed (as multiple tillage operations are required to control perennial weeds). India belongs to tropical (southern part) and sub-tropical region (northern part of India) thus warming and consequent CO_2 emission from soil is much more than temperate part of the globe. This is natural phenomenon which hardly can be regulated by anthropogenic management. This was not so problematic prior to pre-independence era where farming was sustenance type. But the problem started when we began feeding increasing population with limited arable resource that too with low productivity under intensive farming practices.These intensive farming practices have long lasting legacy even when practiced for short term especially on soil carbon and microbial count. The damage done by such agriculture practices can be only addressed by regenerating type agricultural practice e.g., Conservation Agriculture. Practices under conservation agriculture (minimization of soil disturbance, crop diversification and residue retention) are believed to improve biological properties of soil along with physical and chemical properties. In 19th century industrial revolution made availability of range of tilling equipment and mechanized conventional farming. Other faulty practices that come to this list are burning of crop residues, monocropping, keeping soil uncovered. To overcome these shortcomings a set of resource-saving crop management practices emerged and popularized as "conservation agriculture". This ensures acceptable profit along with conserving natural resources (soil, air, water and environment). CA is set of agriculture technologies that include minimum soil disturbance, permanent crop cover and diversification of crops along with weed management. Hence capable of reverting ill effects of conventional farming such as decline in soil organic matter, water loss, physical degradation of soil, reducing fuel use, runoff and water loss, CO2 and GHGs emission. The concept of conservation agriculture (CA) evolved as a response to concerns of sustainability of agriculture globally, has steadily increased worldwide to cover about ~8% of the world arable land (124.8 M ha) (FAO, 2012). The major CA practicing countries are USA, Brazil, Argentina, Canada and Australia. In India, CA adoption is still in the initial phases. Over the past few years, adoption of zero tillage and CA has expanded to cover about 1.5 million hectares (Jat et al., 2012). The major CA based technologies being adopted is zero-till (ZT) wheat in the ricewheat (RW) system of the Indo-Gangetic plains (IGP). The barriers to CA adoption are: low popularization of principles, procedure and adoption techniques of CA, unavailability of appropriate equipment and machines (e.g., seed drill, happy seeders to reduce mechanical soil disturbance, and of suitable herbicides and alternative management strategies to facilitate weed and vegetation management and unavailability of residue after burning and meeting livestock requirements in developing countries like India (Kassam et al., 2015).

Microorganisms are essential tool in majority of the soil ecosystem services (Aislabieet al., 2013) such as efficient utilization of solar energy and recycling of organic molecules. They play crucial role in the biogeochemical cycling of soil nutrients (Bhattacharyya and Jha, 2012). Exploration of diverse ways of recycling organic energy usually accumulated in plants and animal residues through direct utilization of organic molecules by plants is an area need to focus in an ideal agricultural system. One such microbe belonging to fungal group is Arbuscular mycorrhizal fungi (AMF), microbiota forming symbiotic associations with the roots of higher plants (Finlay, R. D., 2008).AMF are obligatory saprophytic fungi and need a living host for their survival. The hyphae of thesesymbioticfungiprovideanincreasedsurface areaforinteractionswithothermicroorganisms as well as nutrient absorption, for making nutrient availability to crops. But intensive farming particularly tillage operation and high fertilizer application impose threat to AMF performance and marginalizetherolesofmycorrhizosphereorganisms (Chavarria et al., 2018). AM fungi improve soil structure and plant nutrition, particularly phosphorous uptake, and protect plants from pathogens and abiotic stresses. Extraradical hyphae play a main role in soil stable aggregate formation by exudation of specific glycoprotein belonging to group glomalin related soil protein

(GRSP) which maintains a specific peculiarity of not being effortlessly decomposable on coming in contact of soil protease. Thus, this group of protein is considered as highly recalcitrant and capable of regulating global warming by checking release of GHGs (like CO2, NOx). This glycoprotein is exclusively metabolized by a fungal group belonging to phylum Glomeromycota (an obligate biotroph) thus named as glomalin (Singh et al., 2022)

In sustainable, low-input cropping systems the natural roles of microorganisms in maintaining soil fertility and biocontrol of plant pathogens may be more important than in conventional agriculture where their significance has been marginalised by high inputsofagrochemicals.Betterunderstandingoftheinteractionsbetweenarbuscularmycorrhizalfungiandot hermicroorganismsis necessary for the development of sustainable management of soil fertility and crop production. Mycorrhizal Fungal (AMF) association with crop plants under low input CA is one which imparts sustainability in crop production system. However, little is known how CA practices influence Arbuscular Mycorrhizal Fungal (AMF) communities in rice-based cropping system in the lower Gangetic Plains of West Bengal. In this backdrop, this study was carried out during 2018-19 and 2019-20 to assess the effect of tillage, cropping systems, residue and chemical fertilization on AMF spore abundance and diversity.

MATERIAL AND METHODS

Description of experimental site: The experiment was conducted at BCKV, Mohanpur, Nadia, West Bengal (Latitude 22.96°N, Longitude- 88.50°E, and altitude 9.75 m above mean sea level). This place belongs to Lower Gangetic Plains of West Bengal having sub-tropical humid climate with 1500 mm average rainfall and 32°C of average temperature (Max=36.3°C, Min=12.5° C). Around 77 % of annual rainfall receives during the southwest monsoon season. The region belongs to alluvial soil zone and was neutral in soil pH.Experimental design opted was split plot where all the cropping system were divided into 3 plots(main plot) for imposing different tillage operations (Conventional, Zero and Reduced tillage). All the main plots were subdivided 5 sub plots for 5 different sub-treatments i.e., residue and fertilizer application. The subplot treatments were: R1 (0% Residue (@ 0 t/ha) + 100% RDF), R2 (100% Residue (@12.5 t/ha) + 50 % RDF) + 100% RDF), R3 (100% Residue (@12.5 t/ha) + 75 % RDF), R4 (50% Residue (@ 6.25 t/ha) + 100 % RDF), R5 (50% Residue (@ 6.25 t/ha) + 75 % RDF).

Collection and storage of soil samples: Soil samples were collected from rhizosphere region of the soil (depth: upto 10 cm from surface) with the hypothesis that microbes are most pronounced at surface soil. From each plot, 5-6 plants were uprooted and soil adhering to their root were extracted and mixed properly to make composite sample before transferring to plastic bag. During the soil sampling and collection visible pieces of crop residues and gravels were removed. After collection, soil was stored in refrigerator at 4C and used for microbial as well as biochemical analysis. The following parameters were analyzed to achieve the objectives of the present study.

Counting and morphological study of spore: AMF spore counting was done by sieving and decanting method as suggested by Pacioni (1992) There are three steps under this process: (1) Preparation of soil suspension: To start the analysis 20 g of air-dried soil sample was taken in 1L of water and stirred using a magnetic or glass rod for at least 10 minutes. 4-5 minutes was given for particles to be settled at the bottom of the container. (2) Decanting of soil suspension on graded sieve and collection of debris in a petridish: Prepared soil suspension was allowed to pass by a graded sieve containing pore of size 500 µm, 250 µm, 100 µm and 45 µm and allowed to filter across it. Debris retained by the sieve resuspended in the remaining suspension, stirred and poured again on the sieve. With the aid of jet of water content of each of the sieve was transferred to a petridish containing nylon cloth and observation was taken under stereoscope. (3) Observation of collected spore under stereoscope: Peridish containing fungal spore was brought under stereoscope and observed finely to study number of spore. Morphological study of spore (under a stereomicroscope) was based on colour, shape and specific character (free or attached). Spore of diverse color (yellow, brown and hyaline), shape (round, spherical and oval) and characteristics (free and attached) were detected under different cropping system.

RESULTS AND DISCUSSION

Spore count and morphology of AMF in initial soil samples

Mean values of 109±1.25 and 122±1.00 obtained for spore count under different cropping system on two consecutive year (2018 and 2019) of experimentation (Table 1). A non-significant ($p < 0.05$) difference in spore count was observed in initial soil samples (without imposing treatments), while second year (2019) a significant difference in spore load was detected due to influence of cropping system (Verbruggen and Kiers, 2010) which was absent for initial soil sample. Comparatively higher spore count was observed under RMCp and RWGg cropping system as under this cropping systemfull principle of conservation agriculture was adopted (tillage, residue and legume incorporation) while third cropping system i.e., RCfBr followed incomplete CA adoption (Singh et al., 2021). Higher coefficient of variance (CV) 1.13% was observed with initial soil sample than 2019 (0.81 %).

Table 1: Spore count of AMF under different cropping system during 2018 and 2019

Cropping system	Spore count per 25 g of dry soil	
	2018	2019
Rice-maize-cowpea	102A	166A
Rice-wheat- green gram	113A	137B
Rice-cauliflower- bororice	111A	66C
Mean ± S.E	109± 1.25	122± 1.00
CV (%)	1.13	0.8174

Values with different cases (A–C) are significantly different for each other while means with the same letter are not significantly different.

Spore shape differed significantly across the cropping system. Compared to spherical and oval spore round spore were significantly higher and contributed 70% of the total spore count under different cropping system. Spherical and oval spore were significantly low i.e., only 16% and 12% respectively. In RMCp and RCfBr cropping system, RT showed significantly higher round shaped spore while same under RWGg cropping system were under ZT (Table 2). Spherical spore was significantly lower under RT compared to CT and RT, under all the three-cropping system. Oval shaped spore were higher under ZT in RWGg and RCfBr cropping system. Under RT yellow and brown spores were predominating. In RWGg cropping system yellow, hyaline and brown spores were significantly higher under ZT while in RCfBr yellow and brown spore were higher under ZT while hyaline spore was significantly higher under RT.

Compared to attached spore free spores were 7.5 times higher taking all the cropping system and tillage practices together. Out of the total spores 88% spores were free while only 12% spores were attached.

Table 2: Spore characteristics of AMF in initial soil samples under different cropping system and tillage practices

Cropping system	Tillage practices	Spore shape			Spore colour			Special Character		Total spore count
		Round	Spherical	Oval	Yellow	Hyaline	Brown	Attached	Free	
RMCp	CT	70b	28a	20a	34a	27b	58a	119a	33a	119
	RT	95a	11b	15c	35a	54a	20c	109b	10b	111
	ZT	39c	25a	10b	22b	29b	23b	72c	2c	74
RWGg	CT	57c	14a	13b	25c	18b	41b	84b	9c	84
	RT	85b	8b	6c	35b	44a	20c	99b	8b	99
	ZT	95a	15a	40a	53a	46a	51a	135a	15a	155
RCfBr	CT	49c	21a	9b	24c	12c	38b	77b	12b	78
	RT	100a	10b	7b	35b	57a	25c	117b	10a	118
	ZT	97b	22a	18a	49a	20b	68a	116a	21c	183
Total		687	156	120	312	307	344	122	902	**963**

(Note: CT-Conventional tillage, ZT-Zero tillage, RT-reduced tillage; RMCp: Rice-maize-cowpea, RWGg: Rice-wheat-green gram, RCfBr: Rice-cauliflower-bororice; Values with different cases (a-c)) are significantly different for each other while means with the same letter are not significantly different**)**

Influence of conservation agriculture on count and morphology of spore under rice-maize-cowpea cropping system

Under RMCp cropping systemmean value of spore count was 166. Tillage practices RT (197) and ZT (181) retained significantly higher spore load than ZT (119) (Baltruschat et al, 2019) as conservation tillage offers less soil disturbance, keep soil aggregates undisturbed and don't cause mechanical injuries to AMF hyphae what AMF suffer under conventional tillage practices (Neelam et al., 2010). Comparatively lower spore count was seen under CT in RMCp cropping system (Fig 1) as disruption of the AMF hyphal network in conventional tillage reduce spore production and roots colonization (Borie et al., 2008). Across residue and fertilizer treatment,mean values were highest under treatments with 100% residue i.e., under R2 (177) and R3 (173) while treatment than 50% (R3 andR4) or no residue (R1). This is due to supply of nutrients to AMF under residue application. Moreover, during residue decomposition release of organic acids occurs that lowers the soil pH and make soil more favourable to fungal inhabitancy (Pande et al., 2017). Among all the 15 treatments (tillage and residue interaction) spore count differed significantly (Fig1). Highest count was under RT-R1 treatment followed by RT-R1 treatments. Under ZT treatments RT-R3 and RT-R4 excelled over other treatments.retention of residues may affect AMF indirectly by altering the activity of microorganisms in the rhizosphere or changing soil chemical properties (e.g., pH and nutrient availability).

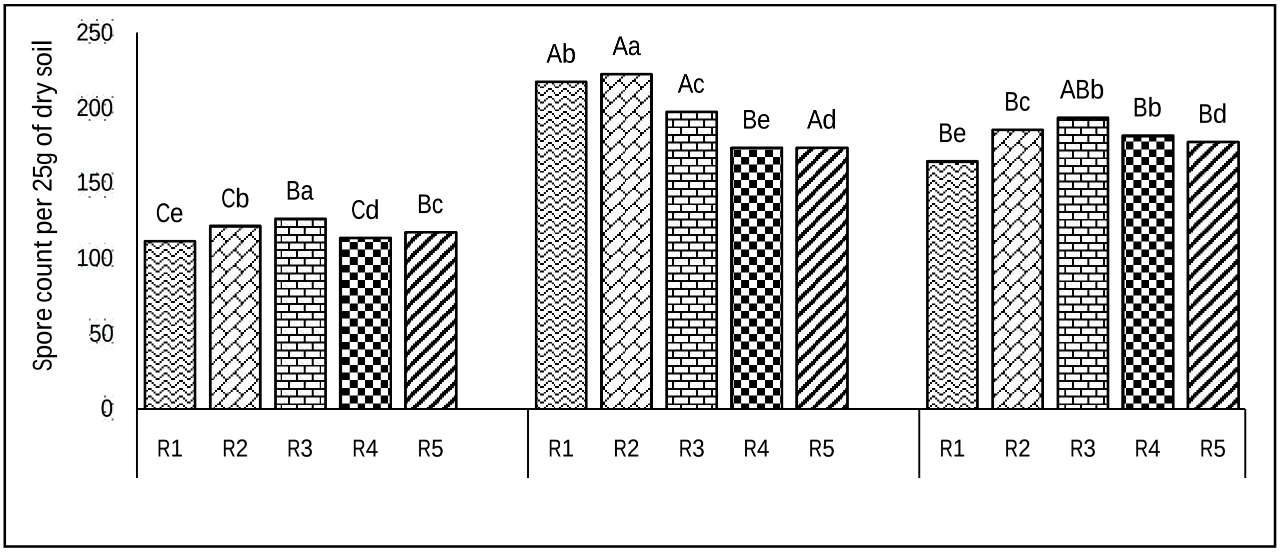

Fig1: AMF spore count under different tillage and nutrient combination in rice-maize-cowpea cropping system (Values with different lower case (a–c) superscript letters are significantly different for each residue treatment and upper case (A-C) are significantly different for each tillage treatment, at $p < 0.05$)

In RMCp cropping system, round shaped spore was predominating and varied significantly across tillage and residue interaction. Spherical and oval spore were very few and varied non significantly across tillage and residue treatments (Table 3). With respect to soil color brown spores (mean value=81) were predominant one followed by yellow (mean value= 52) and hyaline (mean value= 32) spore. Yellow and brown spores were highest in RT-R2 treatment while hyaline spores were predominant in RT-R5 and ZT-R4 treatment. Highest number of free spores were in RT-R2 treatments while attached spore in RT with R4 and R5 level of residue application. With respect to spore shape 81% spore were round, 10% spherical and 9% oval. The abundance of yellow, hyaline and brown spore was 31%, 19% and 50%, respectively.7% spores were attached while 93% of them were free.

Table 3: Spore characteristics of AMF sporesunder tillage practices and residue influence in rice-maize-cowpea cropping system and

Tillage practices	Nutrient application	Spore shape			Spore colour			Special Character	
		Round	Spherical	Oval	Yellow	Hyaline	Brown	Attached	Free
CT	R1	94Bd	13Aa	5 Aa	40Cb	18Cb	53Cd	5Cc	107Cc
	R2	94Cd	13Aa	5 Aa	40Cb	18Cb	53Cd	4Cd	118Cb
	R3	111Ca	13Aa	3 Aa	45Ca	18Bb	64Ba	4Cd	123Ba
	R4	106Cb	6 Aa	2 Aa	38Bc	17Ce	59Bc	6Cb	108Cd
	R5	103Cc	11 Aa	4 Aa	37Bd	20Ca	61Cb	8Ca	110Cc
RT	R1	144Ac	31 Aa	43 Aa	61Ac	67Aa	90Ac	15Ac	203Ab
	R2	158Ba	39 Aa	25 Aa	67Aa	40Ab	116Aa	18Ab	205Aa

	R3	148Bb	26 Aa	24 Aa	66Ab	36Ac	96Ab	18Ab	180Aa
	R4	128Bd	28 Aa	19 Aa	53Ad	34Bd	86Ad	22Aa	15Bd
	R5	128Bd	28 Aa	19 Aa	53Ad	34Aa	86Ad	22Aa	152Bd
ZT	R1	153Ae	14 Aa	3 Aa	55Bc	35Bc	78Bd	9Bc	156Bd
	R2	168Ac	12 Aa	6 Aa	56Bb	29Bd	96Ba	11Ba	175Bb
	R3	180Aa	10 Aa	4 Aa	61Ba	37Ab	96Aa	10Bb	184Aa
	R4	176Ab	10 Aa	3 Aa	50Ad	41Aa	91Ac	7Bd	175Ab
	R5	166Ad	11 Aa	2 Aa	56Ab	29Bd	92Ab	11Ba	167Ac

(Note: CT-Conventional tillage, ZT-Zero tillage, RT-reduced tillage; R1= 0%Residue +100%RDF, R2=100%Residue +50%RDF, R3= 100%Residue +75%RDF, R4= 50%Residue +100%RDF, R5=50%Residue +75%RDF; Values with different lower case (a–c) superscript letters are significantly different for each residue treatment and upper case (A-C) are significantly different for each tillage treatment, at $p < 0.05$)

Influence of conservation agriculture on count and morphology of spore rice-wheat-green gram cropping system

Tillage and residue interaction under RWGg cropping system differed significantly with mean value of 137. Significantly higher spore count noticed under RT-R2 (159), ZT-R3 (166) and ZT-R4 (159) treatments. Tillage means of AMF spore was 126, 144 and 148 for CT, RT and ZT, respectively. Treatments CT-R1, CT-R2 and CT-R3 reported lowest spore count while ZT-R5 reported lowest spore count (Fig 2). ZT systems are associated with a higher AMF spore and enhanced functioning in undisturbed soil (Borie et al., 2008). Under ploughed soil, disturbance transport external hyphae and colonized root fragments to the upper soil layer causing dilution of viable propagules for succeeding crops (Säle et al., 2015). Negative effects of CT on spore abundance and AMF community are attributed to mechanical disruption of the mycorrhizal network, dilution of AMF inoculum, changes in nutrient availability and microbial activity. Residue mean was 123, 142, 144, 150 and 128 for treatments R1 to R5 as high levels of inorganic fertilizers resulted in a shift in AMF community structure and reduced AMF diversity, thus, reduced spore production (Oehl et al., 2004). This decline is due to readily available soil P (and N).

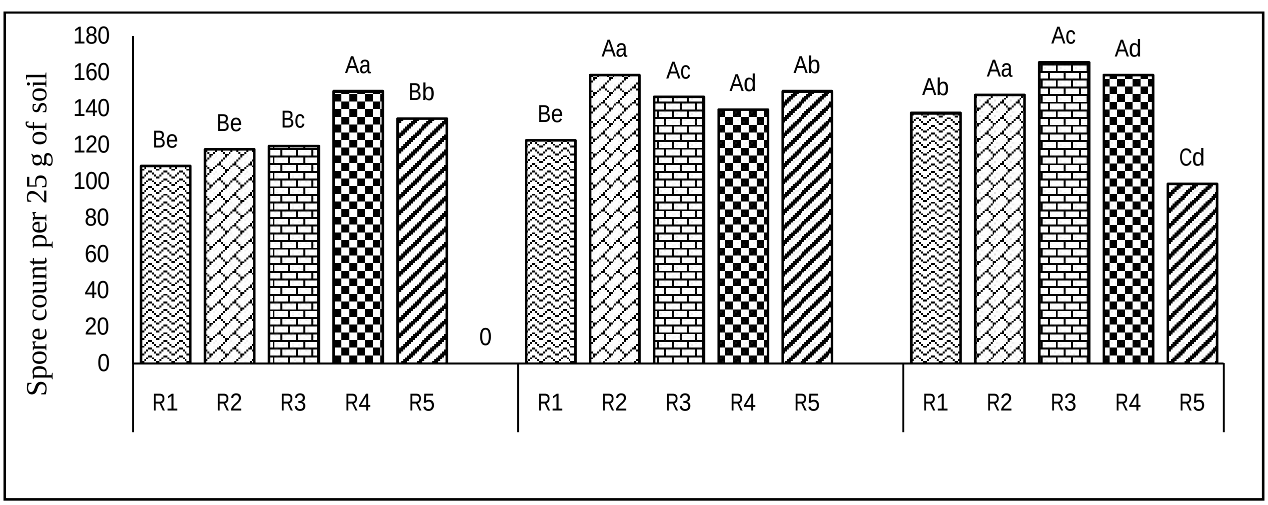

Fig 2: AMF spore count under different tillage and nutrient combination in rice-wheat-green gram cropping system (Values with different lower case (a–c) superscript letters are significantly different for each residue treatment and upper case (A-C) are significantly different for each tillage treatment, at $p < 0.05$)

Similar to RMCp cropping system in RWGg cropping system also round shaped spores were predominating than spherical and oval spores. Out of total spore count in RWGg83% spore were round shaped, 10% spherical and 7% oval. Round shaped spores were highest in RT-R5 and ZT-R3 treatment. Likewise, spherical spores were higher in ZT-R1 and oval spores were high in RT-R2 and ZT-R1 treatments. Compared to RT and CT, ZT was favored round and spherical spores.Among different colored spores, yellow spores contributed only 26%, hyaline spores 35% and 41% spores were brown. With respect to spore color all the spores were higher in count under RT. Under RWGg cropping system, in case of attached spore non-significant variation was detected across tillage and residue interaction while free spore varied significantly. 99% spores were free while free spores were <1%.

Table 3: Spore characteristics of AMF spore under tillage practices and residue influence in rice-wheat-green gram cropping system

Tillage practices	Nutrient application	Spore shape			Spore colour			Special Character	
		Round	Spherical	Oval	Yellow	Hyaline	Brown	Attached	Free
CT	R1	91Bd	13Ba	5Ce	37Ad	22Cd	50Bc	2 Aa	107Ce
	R2	100Bc	11Cb	7Bc	39Ac	19Ce	59Bb	9 Aa	10Cd
	R3	101Cc	8Bd	12Aa	26 Ce	53Ba	41Cd	9 Aa	111Cc
	R4	128Ba	10Bc	11Ab	50 Aa	37Cb	62Ba	6 Aa	144Aa
	R5	121Bb	8Cd	6Bd	49 Ab	35Cc	50Bc	6 Aa	129Bb
RT	R1	94Be	13Ba	14Bb	33Bc	31Bd	59Ac	6 Aa	117Be
	R2	122Ad	12Bb	25Aa	36 Bb	43Bb	80Aa	11 Aa	148Aa
	R3	128Bb	8Bd	11Bc	57 Aa	43Cb	47Be	11 Aa	136Bc
	R4	125Bc	7Ce	8Bd	32 Cd	42Bc	66Ab	8 Aa	132Bd
	R5	135Aa	10Bc	6Be	33Bc	62Aa	56Ad	11 Aa	139Ab
ZT	R1	107Ad	22Aa	16Aa	20 Ce	79Ab	46Cc	10 Aa	128Ad
	R2	125Ac	19Ac	6Cd	30 Cc	60Ad	58Bb	11 Aa	137Bc
	R3	145Aa	13Ae	7Cc	33 Bb	73Ac	60Aa	11 Aa	155Aa
	R4	140Ab	15Ad	5Ce	37 Ba	80Aa	43Cd	7 Aa	152Ac
	R5	65Ce	20Ab	14Ab	28Cd	45Be	25Ce	7 Aa	92Ab

(Note: CT-Conventional tillage, ZT-Zero tillage, RT-reduced tillage; R1= 0%Residue +100%RDF, R2=100%Residue +50%RDF, R3= 100%Residue +75%RDF, R4= 50%Residue +100%RDF, R5=50%Residue +75%RDF; Values with different lower case (a–c) superscript letters are significantly different for each residue treatment and upper case (A-C) are significantly different for each tillage treatment, at $p < 0.05$)

Influence of conservation agriculture on count and morphology of spore rice-cauliflower-bororice

Tillage and residue influence in RCfBr cropping system was same to those of other cropping system i.e., RMCp and RWGg, showing that intense tillage and high-input conventional farming negatively affect AMF abundance and resulting to reduction of AMF (Oehl et al., 2004). Because intensively ploughed soil hinders its ability to re-establish a functional mycorrhizal network by means of anastomosis after hyphal disruption caused by tillage. However,RCfBr cropping system,astonishinglyspore count reduced to 60 and was less than half of previous year (Table 1). The spore count under this cropping system was significantly lower compared to RMCp and RWGgcropping system. One of the causes of drastic lower spore density in RCfBr might be growing cauliflower which is non-host of AMF and having same influence on AMF as that of fallowing. AMF are obligate symbionts and cannot survive over extended periods in the absence of a host plant which provide them energy source for survival. Among different tillage and residue treatments highest spores were detected in treatments RT-R5 (89) followed by RT-R3 (79) (Fig 3). Across cropping system lowest spore count detected under CT (mean value=49), followed by RT (mean value=68) and ZT (mean value=63). Across residue application highest spore count was retained by treatments R5 (50% residue &75% RDF) and lowest by R1(0% residue & 100% RDF).

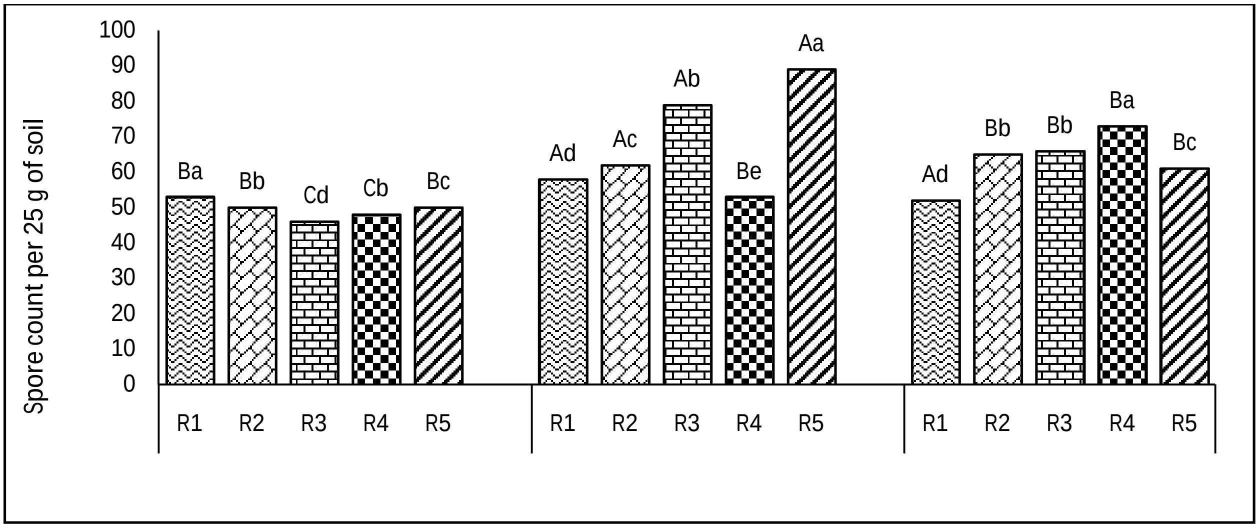

Fig 3: AMF spore count under different tillage and nutrient combination in rice-cauliflower-bororice cropping system (Values with different lower case (a–c) superscript letters are significantly different for each residue treatment and upper case (A-C) are significantly different for each tillage treatment, at $p < 0.05$)

Spore shape (round, spherical and oval) varied significantly across the tillage and residue treatment. Round shaped spores were highest in R1 R5 and R2 treatments under CT, RT and ZT, respectively. Spherical spores were higher in R1and R4 treatments under RT and ZT tillage practices, respectively while oval spores were higher in R3 and R4 treatment under RT and ZT tillage. Comparing the color of the spore, relative % of different colored spore yellow (35%)>hyaline (22%)>brown (43%). Yellow and hyaline color spore differed significantly across the tillage and residue treatment. However brown colored spores showed non-significant influence of tillage and residue (Table 4). Out of total spore 7% spores were attached and showed non-significant influence of tillage and residue combination. While majority of spores (93%) were free and differed significantly towards treatment practices. Predominance of such spore morphotypes was also reported by several workers (Ajaz et al., 2017).

Table 4: Spore characteristics of AMF under tillage practices and residue influence in rice-cauliflower-bororice cropping system

Tillage practices	Nutrient application	Spore shape			Spore colour			Special Character	
		Round	Spherical	Oval	Yellow	Hyaline	Brown	Attached	Free
CT	R1	43Aa	5Bb	5Cb	22Aba	10Ac	21Aa	2Aa	51Ce
	R2	35Bd	7Ba	7Ba	18Bc	8Cd	21Aa	8 Aa	42 Cd
	R3	32Ce	7Ba	7Ba	18Bc	3Ce	21Aa	6 Aa	40Cc
	R4	36Bc	5Bb	7Ba	15Cd	12Bb	21Aa	2 Aa	46Aa
	R5	41Cb	5Cb	4Cc	20Bb	13Ca	17Aa	3 Aa	47Bb
RT	R1	43Ad	9Aa	6Bc	23Ad	7Be	26Aa	1 Aa	57Be
	R2	48Ac	8Ab	5Cd	20Ae	11Bc	30Aa	5 Aa	57Aa
	R3	69Ab	1Ce	9Aa	34Ab	19Aa	26Aa	1 Aa	78Bc
	R4	48Ac	2Cd	3Ce	24Ac	10Cd	19Aa	0 Aa	53Bd
	R5	76Aa	6Bc	7Bb	38Aa	15Bb	36Aa	4 Aa	85Ab
ZT	R1	34Bc	9Ac	9Ab	21Ba	7Be	25Aa	5 Aa	47Ad
	R2	51Aa	6Ce	8Ac	16Bd	16Ac	33Aa	3 Aa	62Bc
	R3	50Ba	10Ab	6Cd	18Bc	11Bd	35Aa	4 Aa	62Aa
	R4	25Cd	22Aa	26Aa	20Bb	21Ab	32Aa	9 Aa	64Ab
	R5	45Bb	8Ad	9Ab	11Ce	29Aa	22Aa	5 Aa	56Cc

(Note: CT-Conventional tillage, ZT-Zero tillage, RT-reduced tillage; R1= 0%Residue +100%RDF, R2=100%Residue +50%RDF, R3= 100%Residue +75%RDF, R4= 50%Residue +100%RDF, R5=50%Residue +75%RDF; Values with different lower case (a–c) superscript letters are significantly different for each residue treatment and upper case (A-C) are significantly different for each tillage treatment, at $p < 0.05$)

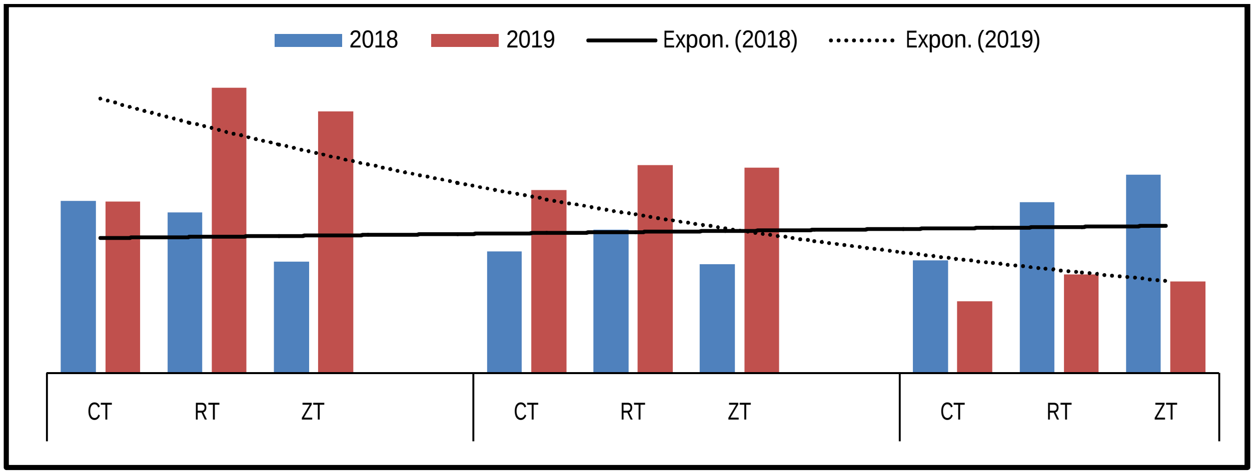

Fig 4: Trend followed by AMF spore over 2 years of experimentation

Observing total spore count under different cropping and tillage intensity over 2 years of study, showed different pattern of spore response, AMF spore count in 2018 (solid black line) were almost similar (Fig. 4). While the trend changed next year in 2019 (dotted black line). Second year of experiment under RMCp and RWGg a significant increase in AMF spore was observed due to their ability to retain huge leftover biomass in soil in case of former and ability to fix atmospheric nitrogen (Kihara et al., 2012b) particularly under conservation tillage (i.e., RT and ZT). While trend was reverse under RCfBr cropping system under all the tillage practices a significant decline in AMF spore count was there (Johnson, N.C., 2010). Crucifers crop produce a specific secondary metabolite glucosinolate (GLS: type of anion hydrophilic secondary metabolite containing nitrogen and sulfur and highly soluble in nature), relatively high in the Cruciferae, (especially Brassica). This compound act as plant defence mechanism against range of pathogens (Liu et al. 2021). Inside pathogen or parasite body GLS get hydrolyzed and hydrolytic products breakdown into glucose and unstable sugar glycoside ligands. Glycoside ligands are rearranged to form isothiocyanates, nitriles, oxazolidinethiones, thiocyanate, epithionitriles, which all exhibit a wide range of biological activity (Esteve M., 2020).

CONCLUSION

Modern agricultural practices are input intensive and highly torturous to microbial world particularly to fungi as their body is made of intensive fungal network. Approaches of conservation agriculture (CA), in this context, in terms of minimum mechanical disturbance, diversified crop rotation along with residue retention,provide a protected habitat for microbes and don't cause any injuries to fungal hyphae or mycelium network. Thus, conservation agriculture can a better plat form for restoring AMF in soil but selection of crops to increase AMF spore is a great challenge as the fungi is obligatory plant symbionts. Incorporation of cole crops or crop belonging to Cruciferae family are non-host of AMF thus having same influence on AMF as that of fallowing. Avoiding Brassicaceae or Cruciferae crop in cropping system under conservation agriculture can efficiently restore AMF in soil and benefit to arable crops. Beside this the experiment strongly support low mechanical soil disturbance and residue application (higher the better) help increasing AMF spore in soil.

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