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Research Article

MICROBIAL CHARACTERIZATION AND EVALUATION OF INDIGENOUS ORGANIC PREPARATIONS INFLUENCING GROWTH AND YIELD OF WHEAT

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Abstract- Organic farming is an emerging option to protect the soil health by replenishing the rapidly depleting organic matter under tropical soil condition to ensure production sustenance. Present study evaluates the effect of indigenous organic preparation panchagavya, cattle dung manure and biodynamic preparations on nitrate reductase activity, chlorophyll content, phyllospheric microbial count, soil microbial activity as well as growth and yield of wheat crop. Amongst, all the organic preparations *viz*. panchagavya, cattle dung manure, Biodynamic compost, BD500 and Cow Pat Pit, liquid preparation panchagavya contained low amount of plant nutrients. The highest count of bacteria (log10cfu 9.39 per ml) and aerobic nitrogen fixers (log10cfu 7.35 per ml) were found in Panchagavya. Improvement in grain yield of wheat with application of panchagavya and other organics was recorded however; compared to inorganic fertilizer treatment, reduction in grain yield to the extent of 35.42% in panchagavya 9.1% in integrated nutrient management, 12.7% in conventional organic treatment and 16.3% in biodynamic treatment indicated less nutrient supplementing potential of organics in short term as evidenced by lower leaf chlorophyll content and nitrate reductase activity. Enhancement in phyllospheric microbial population and soil dehydrogenase activity with organic application suggested favorable impact of application of organics.

Keywords- *Panchagavya, Biodynamic preparations, Nitrate reductase, Chlorophyll, Wheat*

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Introduction

Growing food demand for increasing population with limited arable land resource required use of improved crop varieties, chemical fertilizers, plant protection chemicals and good agronomic practices. Though, these high input agricultural practices increased food grain production to many times but inappropriate use of agrochemicals resulted in decline in soil quality, depletion of soil organic matter and also adversely affected the environment [1-3]. The stagnating crop yield, soil quality deterioration and fertility decline coupled with emerging micronutrient deficiency is widespread problem across the world. Continuous removal of plant nutrient and soil organic matter from soil due to intensive agriculture practices without adequate replenishment has been a major reason for loss of soil fertility [4].Organic agriculture offers a holistic production management system which ensures sustainable food production by improving soil physical and biological condition and also enhancing nutrient cycling in soil. However, with the introduction of new crop varieties and hybrids which are responsive to applied nutrient and also require high amount of plant nutrients, the supply of plant nutrient had always been a constraint in organic agriculture considering the nutrient content in organic manures or composts. Though, reductions in crop yield to the extent of 9.2% under organic nutrient management have been observed [5], the soil quality was always recorded better under organic management practice. Biodynamic agriculture is a variant of organic farming where specific fermented herbal preparations are added as compost additives and field sprays [6]. Studies have found that biodynamically farmed soils have better soil quality than conventionally farmed soils [7]. Biodynamic farms soils had higher biological and physical quality than the conventional farms, significantly greater organic matter content and microbial activity, better soil structure, lower bulk density and easier penetrability of topsoil. The biodynamic farming increasingly finds practical application, mainly as a small-scale side technique to soil organic farming to maintain crop yields and improve soil health [8]. However, translation of bio-stimulating components in biodynamics and panchagavya in terms of growth and yield could hardly be explained considering their material composition [9], sprayed and added in highly diluted concentrations on compost, plants, and soil. As the organic farming practice rely on application of decomposed organic matter, green manure and biological pest control to produce crop and maintain soil health many indigenous preparations like panchagavya and their variantswere also prepared and used by farming community, claiming to have profound effect in very small quantity. Panchagavya contains five products obtained from cow milk *viz*. cow dung, cow' urine, milk, curd and ghee along with other natural ingredients like jaggery or cane juice, coconut water, ripened banana *etc*., to enriched and make it more effective [10]. However, there has been little scientific documentation of nutrient supplementation from their use in crops. Management of crop pests and diseases in organic farming by different bio-agents are understood to some extent but nutrient supplementing preparations are not thoroughly studied. In present investigation, we examined the nutrient content and microbial compositions of different organic and biodynamic preparations. We have also evaluated the effect of application of these organic and biodynamic preparations on soil microbial activities, plant growth and yield of wheat.

Materials and methods

Panchagavya was prepared in mud pot by mixing the ingredients like cow dung, cow urine, milk, curd, ghee, cane juice, coconut water and ripened banana as described by [11]. Biodynamic compost and biodynamic preparations (BD 500, BD501 and CPP) were procured from Kurinji farm, Tamil Nadu. Microbiological enumeration from organic preparations was done on nutrient agar for bacteria, Rose Bengal agar for fungi, Kenknight and Munnaier's medium for actinomycetes, Pikovskaya agar for P solubilizers and Jensen'sagar media for nitrogen fixers by serial dilution plating technique. Soil dehydrogenase activity for pot culture soil was measured by estimating reduction of tetrazolium chloride [12]. The total NPK and micronutrient in manure and organic preparations were determined as described by Tandon [13]. Estimation of chlorophyll from wheat leaf was done by method of Hiscox and Israelstam [14] by extracting chlorophyll from freshly cut leaf in DMSO at 65°C for 4 hours followed by measuring the absorbance of extracted pigment at 663 and 645nm using spectrophotometer and expressed in terms of mg chlorophyll/g fresh weight of leaf. Total chlorophyll was calculated using equation by Arnon [15].

Nitrate reductase activity measurement in wheat leaf

Nitrate Reductase (NR) activity in flag leaf of wheat was estimated by method of Cazetta, *et al.* [16]. Briefly, 200 mgof freshly cut flag leaf was placed in a test containing 3 ml of ice cold phosphate buffer (pH 7.5), 3ml of ice cold KNO_3 solution (0.2M) and 0.2ml of n-propanol was added. The tubes were removed from ice bucket and incubatedin dark at 30°C for one hour. The reaction was terminated by placing the tubes in boiling water bath for 5 minutes. To 1 ml of aliquot 1ml suphanilamide 1% and 1ml NEDD solution (0.025%) was added and mixed well. The tubes were left for 20 minutes at room temperature. The intensity of pink colour was read at 540nm using a spectrophotometer. Calculation of nitrite (NO_2^-) formed was done by preparing standard curve with different concentration of sodium nitrite. The NR activity was expressed in terms of μmoles of NO_2^-/g fresh weight of leaf/h.

Pot culture study

Pot culture study was done in net house with plastic pot containing 12kg soil and wheat as test crop. The experimental soil was black soil of vertisols, Hypothermic family of Typic Haplusterts. Soil had pH 7.9, Available N 230kg/ha, available phosphorus (P_2O_5)-11.5 kg/ha, Potash (K_2O)-390 kg/ha and organic carbon-0.75%. The treatments consisted of T1: control (No organic manures or chemical fertilizers). T2: Recommended dose of fertilizers (80:40:40 N, P_2O_5 and K_2O). T3: Integrated nutrient management (half of the N requirement was supplemented through organic manure). T4: Total Nitrogen supplemented through organic manure. T5: Biodynamic compost @2t/acre, four spray of BD500@ 25g/13lit water/acre, four spray of BD 501 was @ 10g/13lit water/acre and Cow Pat Pit (CPP)@1kg/40 lit water/acre. T6: 5 % panchagavya spray at fortnightly interval till flowering. Three replications were maintained for each treatment. Quantity of compost and fertilizers required was calculated for 12 kg soil accommodated in the plastic pot. Biomass and yield of the crop was recorded after manual harvesting and thrashing of crop in each pot containing five plants (hills).

Statistical analysis

Statistical analyses were carried out through one-way analysis of variance (ANOVA) and the mean of treatments were compared according to Fisher's multiple comparison tests. Least significant difference (LSD) was calculated at $p<0.05$ using statistical package of SAS 9.1.

Results and discussions

Microbial load and nutrient content indifferent organic and biodynamic preparations

Nutrient content analysed in the organic and biodynamic preparation reflected relatively higher nitrogen content in biodynamic preparations (BD 500: 2.1%; Cow Pat Pit: 1.82% and Biodynamic Compost: 1.12%) compared to cattle dung manure. Panchagavya contained least nutrient among all the organics compared [Table-1]. The nutrient source used in the present study was characterized for their microbiological properties as well as nutrient content present in it. Amongst different organic sources, the highest count of bacteria and aerobic nitrogen fixers were found in Panchagavya [Table-2], the fungi were numerically dominant in BD 500 preparation whereas biodynamic compost recorded the highest count of actinobacteria. Panchagavya was totally devoid of actinobacterial population. Panchagavya characterized for its microbial and nutrient composition may vary with the earlier report since it contains heterogeneous ingredients asraw materials. The composition of microbes also varies with length of fermentation [17].

Table-1 *Chemical characterization of different organic and biodynamic preparations*

Organic preparations	pH	N	P	K	Zn	Cu	Fe	Mn
BD 500	6.89	21000	8000	800	57	16.4	823.1	134.2
Cow Pat Pit	6.55	18200	12700	2400	33.97	9.4	531.3	183.4
Cattle dung manure	7.3	8400	7300	3700	120	22.9	535.9	189
Biodynamic Compost	6.7	11200	3400	3100	50.6	20.5	600	156
Panchagavya	4.66	1700	2800	700	6.41	2.2	226.2	13.2

Nutrient content in mg/kg of preparation

Nevertheless, it contains many rich nutrient sources and microbial inoculums like milk, curd, coconut water cane juices *etc*., which constitutes a rich medium for microbial growth thus justifying the higher culturable microbial count in panchagavya. The decline in pH of panchagavya in the course of fermentation might be responsible for elimination of actinobacteria which usually prefer neutral to alkaline pH for growth, also copiotrophic group of microbe growing on rich medium might have suppressed actinobacteria due to its slow growth nature.

Table-2 *Microbiological evaluation of organic and biodynamic preparations (count in log10cfu/g of preparations)*

Organic preparations	Bacteria	Fungi	Actinomycetes	P-solubilisers	N-fixers
BD500	9.228 (0.030)	5.588 (0.078)	6.413 (0.029)	3.175 (0.017)	3.433 (0.041)
Cow Pat Pit	7.167 (0.134)	3.935 (0.032)	5.601 (0.017)	3.693 (0.051)	5.676 (0.033)
Biodynamic Compost	8 (0.054)	5.094 (0.059)	6.814 (0.022)	3.954 (0.082)	5.9 (0.045)
Cattle dung manure	8.51 (0.004)	4.78 (0.006)	4.959 (0.003)	2.04 (0.023)	5.327 (0.029)
Panchagavya*	9.397 (0.055)	4.702 (0.008)	-	3.727 (0.034)	7.351 (0.008)

**Microbial count in log10cfu/ml of preparation; Figures in parenthesis represents SEM (±) of three replications.*

Effect of organic and biodynamic preparations on growth and yield of wheat

Effect of different organic and biodynamic preparations, agronomic parameters like root length, shoot length, root dry weight, shoot dry weight at 80 days of sowing was recorded. Physiological parameters like chlorophyll content of leaf and nitrate reductase activity of leaf at various growth stages was estimated. Highest root length was observed in T3 treatment ($p\leq0.05$; 20cm) where both organic and inorganic nutrient sources were applied which was significantly higher than control ($p\leq0.05$; 14.4cm) and biodynamic a ($p\leq0.05$; 16.0cm). Shoot length was recorded highest in Panchagavya treatment ($p\leq0.05$; 40.3cm) which was statistically at par with control, inorganic and organic treatment, however, visually the plant appeared slender in case of control and panchagavya. The root and shoot dry weight per pot was recorded the lowest in control which was at par with panchagavya treatment. No significant difference in root and shoot dry weight was observed in conventional organic and biodynamic nutrient management. Grain yield was noticed highest in inorganic nutrient treatment ($p\leq0.05$; 13.75g/pot) followed by integrated nutrient management ($p\leq0.05$; 12.50g/pot) and conventional organic nutrient management ($p\leq0.05$;12.00g/pot).

Table-3 *Agronomic parameters of wheat influenced by different nutrient sources*

Treatments	Root length (cm)	Shoot length (cm)	Root dry weight/ pot(g)	Shoot dry weight/ pot(g)	Straw weight(g) / Pot	Grain weight(g) / Pot
T1	14.4c	38.4abc	1.63d	3.57d	13.19d	6.52e
T2	17.6abc	38.4abc	4.66a	7.22a	21.22b	13.75a
T3	20.0a	32.8c	3.33b	6.31b	24.76a	12.50b
T4	19.2ab	39.3ab	2.77c	6.60ab	21.85b	12.00bc
T5	16.0bc	33.2bc	2.55c	4.78c	17.00c	11.50c
T6	19.8a	40.3a	1.91d	4.05d	12.67d	8.88d

Column bearing same alphabet are statistically non-significant at $p\leq0.05$

Conventional organic and integrated nutrient management performed at par to each other and no significant difference was observed between organic and biodynamic treatment in terms of grain yield [Table-3]. Compare to unfertilized control, there was improvement in yield with application of panchagavya and organics however, compare to inorganic fertilizer, reduction of grain yield to the extent of 35.42% in panchagavya 9.1% in INM, 12.7% in conventional organic treatment and 16.3% in Biodynamic treatment was recorded. Reduction ingrain yield under organic nutrient management has been well documented [5, 18-19]. Based on global meta-analysis of yields data, 15% reduced yield than conventional management was reported by Seufert, *et al.,* [20] under optimal organic management. Shah, *et al.,* [21] reported 21-34 % average yield gap between conventional and organic systems. Lower content of major plant nutrient in organics and slow release during peak crop growth period might be responsible for reduction in crop yield under organic system. Nitrate reductase activity in flag leaf was found to be the highest in inorganically fertilized treatment (T2) followed by integrated nutrient management system (T3) and the lowest in unfertilized control (T1), remaining all the treatments were at par [Fig-1]. Similarly, total chlorophyll content at 80 days after sowing was found lower in organic treatment compare to inorganic fertilizer [Fig-2]. Chlorophyll being major photosynthetic pigment determines primary production and leaf nitrogen is incorporated in chlorophyll, quantification of chlorophyll content gives an indirect measure of nutrient status [22-24]. Similarly, nitrate reductase (NR) and glutamine synthetase (GS) are the key enzymes of N metabolism and are also involved in carbohydrate metabolism [25]. The nitrate reductase activity is positively correlated with available nitrogen in soil and influences grain yield and protein content of grain. In this present study, the maximum nitrate reductase (NR) activity was observed in treatment receiving recommended dose of inorganic fertilizers nutrients.

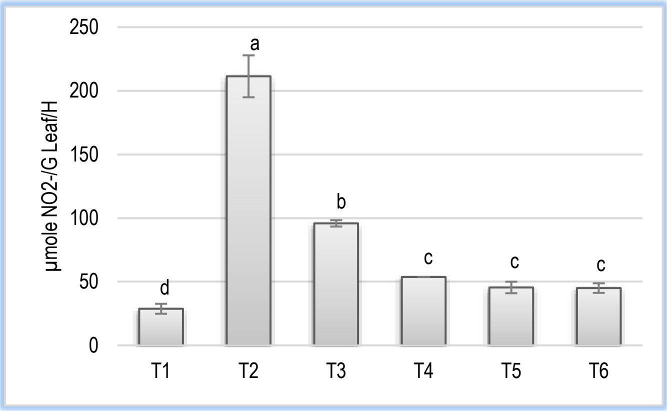

Fig-1 Nitrate reductase activity of flag leaf of wheat

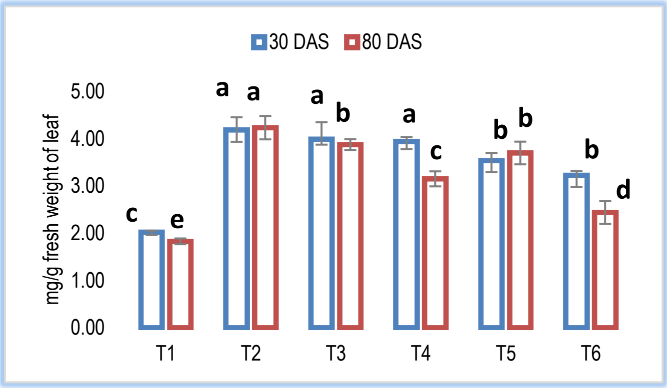

Fig-2 Chlorophyll content in wheat leaf at different growth stage(Error bar represents ± Standard deviation of three replications; similar column bearing different alphabet are statistically significant at p≤0.05)

Reduction in NR activity in control and organics indicated lower availability of N from soil which was in turn manifested in terms of reduction in yield. Lower NR activity and total chlorophyll content during later stage of crop growth in organic, biodynamic and panchagavya treatment is suggestive of lower nutrient supplying capacity of organics and inability of any organic preparation alone in meeting the crop demand for nutrients compared to inorganic fertilizers in short term.

Effect of application of organic preparation on phyllospheric microbial count and soil enzymes

Total count of phyllospheric microbes including fungi and nitrogen fixers were initially highest in panchagavya, biodynamic and organic treatment which decrease with time [Fig-3a,b,c]. Despite regular application of these organic preparations and higher count of phyllospheric microbes, very limited effect in terms of yield and nutrient supplementation was recorded as evidences by lower chlorophyll content and NR activity in organic treatments. Microbial analysis of the organic preparation used in diluted form for foliar spray found to contain abundant number of different groups of microbes which was reflected in the initial hours of phyllospheric microbial count however, the decline in the count after 15 days of application is due to their inability to survive and establish on leaf surface. The leaf epiphyte could not offer the benefit to the harboring host plant under the extreme habitat as phyllosphere represents hostile environment for bacterial colonists due to direct exposure to solar radiation, rapid fluctuation in temperature, humidity, moisture and nutrient resources available to bacterial colonists [26] thus limiting their efficiency.

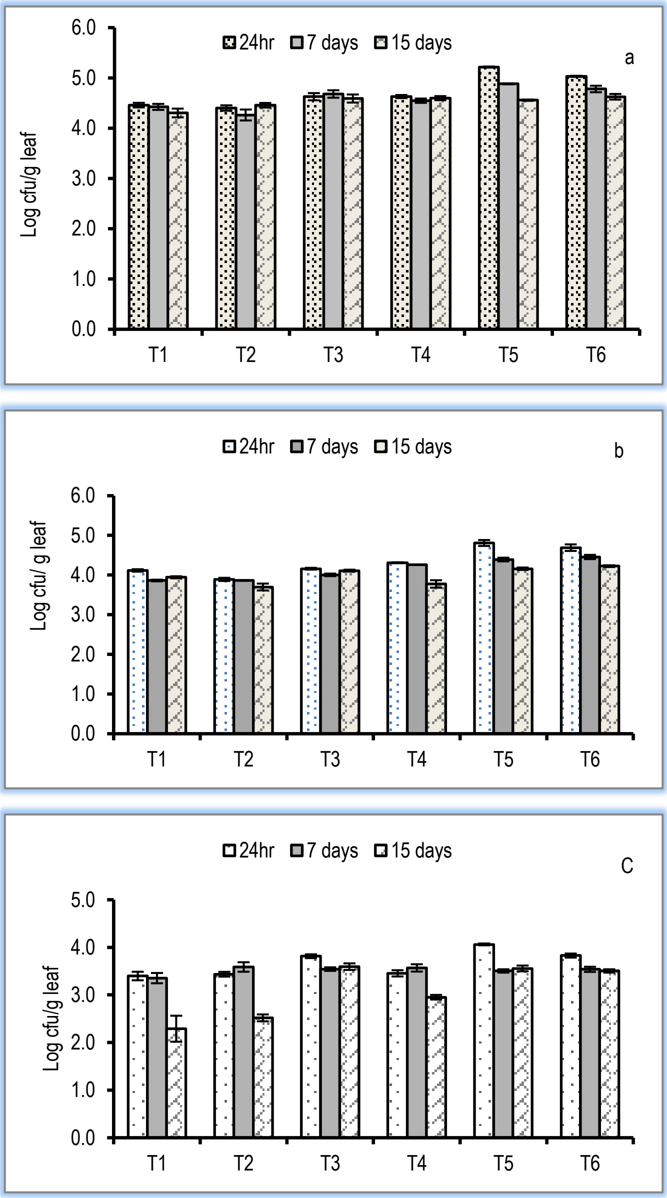

Fig-3 Phyllospheric Microbial count at different time of application of organic preparations

a: Bacteria; b: Fungi; c: Aerobic N_2^- Fixers

The soil microbial activity measured in terms of dehydrogenase enzyme was observed highest in the conventional organic treatment (T4) receiving organic manure as nutrient source followed by biodynamic treatment (T5) and integrated nutrient management (T3). No difference was observed in panchagavya (T6) and integrated nutrient source and the lowest dehydrogenase activity was recorded with control followed by chemical fertilized treatment. Overall, there was improvement in soil dehydrogenase activity compared to control in all the treatments (Fig. 4). The highest dehydrogenase activity ($p \leq 0.5$; 95.2μg TPF/g soil/day) was recorded in soil receiving farmyard manure followed by biodynamic treatment ($p \leq 0.5$; 82.5μg TPF/g soil/day) which was statistically at par with integrated nutrient management ($p \leq 0.5$; 75.0μg TPF/g soil/day). The result indicated the favourable impact of addition of organic material to the soil. Addition of organic matter to the soilserves as source of carbon and energy for soil microbes actively participating in nutrient transformation processes. In soil more than 90% of the energy flow passes from the decomposition of organic matter by heterotrophic microbes [27]. Improvement in soil microbial activity with application of organic manure is well established [19,28-29].Improvement in soil microbial activity with application of biodynamic preparation is well reported by authors [6-7].Though, the dehydrogenase activity of biodynamic soil was higher than control and inorganic fertilizer treatment but statistically equal to INM possibly due to less quantity of biodynamic preparation per unit soil mass required to be applied in finely-diluted form like homoeopathic medicine [30] to activate the soil processes. Faust, *et al.,* [31] did not find additional benefit of application of biodynamic preparations over the composted farmyard manure, where the contribution of bacteria to MBC and microbially-derived SOC was increased with farmyard manure supports our finding.

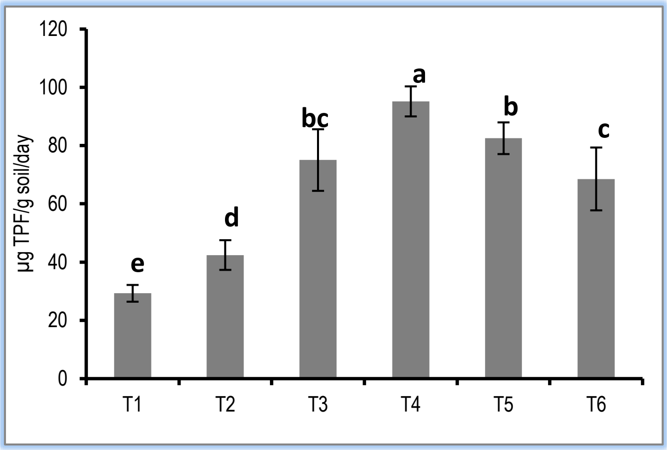

Fig-4 Soil Dehydrogenase activity influenced by different nutrient sources

Conclusion

Microbial and chemical evaluation of organic and biodynamic preparations indicated abundance of culturable microbes in panchagavya, cattle dung manure, Biodynamic compost, BD500 or CPP. Chemical analysis of various organic nutrient supplementing preparations revealed the low NPK and micronutrient content in liquid organic preparations panchagavya compared to cattle dung manure, Biodynamic compost, BD500 or CPP. Pot culture experiment set up with wheat as test crop to evaluate the effect of these organic preparations showed the increase in soil microbial activity in the organic and Biodynamic treatment compared to control and chemical treatment. No difference was observed between conventional organic treatment and Biodynamic system of organic farming. Total count of phyllospheric microbes was highest in panchagavya, biodynamic and organic treatment however, there was little effect of these microbes since lower chlorophyll content and Nitrate reductase activity was seen in the panchagavya, organic and biodynamic treatment despite regular application of preparations. The results indicated that these preparations can be used as complementary component in organic farming as none of the preparation could meet the crop demand alone in one season. There was always improvement in the biological activity of soil upon application of organic preparations or biodynamic preparations.

Application of research: The finding describes the worth of different preparation to rationalize the application of organics from available choices for economic and ecofriendly organic production.

Research Category: Organic farming, Soil Microbiology

Abbreviations: BD: Biodynamic; CPP- Cow Pat Pit; NR- Nitrate reductase

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References

[1] Prashar P., Shah S. (2016) *Sustainable Agriculture Reviews*, vol 19. Springer, Cham, 331-361.
[2] Singh B., Ryan J. (2015) *Managing Fertilizers to Enhance Soil Health. IFA, Paris, France*
[3] Bhandari G. (2014) *Applied Ecology and Environmental Science*, 2(2), 66-73.
[4] Moharana P.C., Sharma B.M., Biswas D.R., Dwivedi B.S., Singh R.V.(2012) *Field Crops Research*, 136, 32-41.
[5] Ramesh P., Panwar N.R., Singh A.B., Ramana S., Yadav S.K., Shrivastava R., Rao A.S.(2010) *Current Science*, 98(9),1190-1194.
[6] Carpenter-Boggs L., Kennedy A.C., Reganold J.P. (2000) *Soil Science Society of American Journal*, 64(5), 1651-1659.
[7] Reganold J.P. (1995) *American Journal of Alternative Agriculture*, 10, 36-45
[8] Spaccini R., Mazzei P., Squartini A., Giannattasio M., Piccolo A. (2012) *Environmental Science and Pollution Ressearch*, DOI10.1007/s11356-012-1022-x
[9] Zaller J.G. and Köpke U. (2004) *Biology and Fertility of Soils*, 40(4), 222-229.
[10] Vijayakumari B., Yadav R.H., Gowri P., Kandari L.S. (2012) *Asian Journal of Plant Science*, 11(2), 83-86
[11] Natrajan K. (2008) *Book on Panchagavya: Panchagavya manual*.
[12] Casida L.E.(1977) *Applied and Environ Microbiology*,34, 630-636.
[13] Tandon H.L.S. (2009) *Methods of Analysis of Soils, Plants, Waters, Fertilisers and Organic Manures. FertiliserDevelopment and Consultation Organisation, New Delhi*
[14] Hiscox J.D., Israelstam G.F. (1979) *Canadian Journal of Botany*, 57(12), 1332-1334.
[15] [15] Arnon D.I. (1949) *Plant Physiology*,24, 1-15
[16] Cazetta J.O., Villela L.C.V. (2004) *Scientia Agricola*, 61(6), 640-648.
[17] Mathivanan R., Edwin S.C., Viswanathan K., Chandrasekaran D.(2006) *International Journal Cow Science*, 2(2), 23-26.
[18] Benaragama D., Shirtliffe S.J., Gossen B.D., Brandt S.A., Lemke R., Johnson E.N., Zentner R.P., Olfert O., Leeson J.,Moulin A., Stevenson

C.(2016) *Field Crops Research*, 196, 357-367.
[19] Cesarano G., De Filippis F., La Storia A., Scala F., Bonanomi G. (2017) *Applied Soil Ecology*, 120, 254-264
[20] Seufert V., Ramankutty N., Foley J.A. (2012) *Nature*,485(7397), 229-232.
[21] Shah A., Askegaard M., Rasmussen I.A., Jimenez E.M.C., Olesen J.E. (2017) *European Journal of Agronomy*, 90, 12-22.
[22] Richardson A.D., Duigan S.P., Berlyn G.P. (2002) *New phytologist*, 153(1), 185-194.
[23] Ibrahim M.H., Jaafar H.Z.E. (2013) *Journal of Plant Nutrition*, 36,1366-1372.
[24] Santosh Kumari (2011) *Journal of Agricultural Science*,3(1),170-182
[25] de Oliveira Ferreira E.V., Ferreira Novais R., Aparecida dos Santos F., Ribeiro C., Barros N.F. (2015) *Australian Journal of Crop Science* 9(6), 561.
[26] Lindow S.E., Brandl M.T. (2003) *Applied and Environmental Microbiology*, 69(4), 1875-1883.
[27] Schnürer J., Rosswall T. (1982) *Applied and Environmental Microbiology*. 43(6),1256-1261.
[28] Ramesh P., Panwar N.R., Singh A.B., Ramana S., Rao A.S. (2009) *Journal of Plant Nutrition and Soil Science*, 172,577-585.
[29] Aher S.B., Lakaria B.L., Swami K., Singh A.B., Ramana S., Ramesh K., Thakur J.K. (2015) *Journal of Applied and Natural Science*,7(1), 67-71.
[30] Heimler D., Isolani L., Vignolini P., Romani A. (2009) *Food Chemistry*, 114,765-770.
[31] Faust S., Heinze S., Ngosong C., Sradnick A., Oltmanns M., Raupp J., Geisseler D., Joergensen R.G.(2017) *Applied Soil Ecology*,114, 82-89.