CHAPTER -5
DISCUSSION

Weather condition of North Bengal regions is not suitable for cultivation of all selected edible mushrooms such as oyster, milky and button mushroom in natural condition throughout the year. In order to overcome this climatic barrier present study was focused on screening and standardization of these mushroom strains for suitable cultivation in natural condition throughout the year and to make a calendar for seasonal cultivation of these mushroom strains. Parallely the present study was also focused on the effect of different substrates on nutritional constituents of mushrooms. After the fruit body harversting, the spent substrate was also applied in different crop system to evaluate its effect on growth parameters, yield and disease suppression. Mycelial growth rate and growth pattern of the six mushrooms (oyster mushrooms, milky mushroom and button mushroom) were found to be different in three different media. The mycelial growth rate and fruit development of *C. indica* in different substrates were evaluated and it was found that minimum period for substrate colonization was recorded in paddy straw while it was maximum in bamboo leaves. Bamboo leaves contain high amounts of phenolic substances and complex lignin compounds as compared to other substrates (Sahoo *et al.*, 2009). Pani (2011) and Bokari *et al*., (2014) used different substrate and casing materials for cultivation of *C. indica* that affected spawn run and colonization period in the substrate. The casing materials and thickness play important role in fruit body development and fruiting period as recorded by Pani, (2012). Comparison of the biological efficiency of *C. indica* on different the substrates revealed that maximum biological efficiency was on paddy straw (196.1%) which was followed by paddy straw + saw dust (177.37%), paddy straw + maize 1:1 (174.33%), maize stalk (161.16%) and bamboo leaves (84.93%). This study was supported by the experiment of Amin *et al*., (2010) and Chakraborty *et al*., (2016). They also used different substrate for cultivation of *C. indica* to see the effect of substrates on yield and biological efficiency. The reason for low biological efficiency on bamboo leaves may be attributed to unavailability of necessary cellulosic compound in required amounts for fruit body formation. Paddy straw as substrate was recorded as the best suitable in response on spawn run period, pin head formation time average weight of fruit body and biological efficiency. Superiority of Paddy straw as compared to many other substrates such as sugarcane bagasse, ground nut haulms, soybean hay, black gram hay, sunflower stalk, cotton waste, sesamum stalk, coir pith and wheat straw for cultivation of *C. indica* has been reported by many workers (Chaubey *et al.*, 2010; Pani 2010; Saranya *et al*., 2011; Velusamy *et al.,* (2014a). Kalha *et al.,* (2011) and Kumar *et al*., (2012 reported that the various supplement and biofertilizer in casing layer have the effect on the yield of *C. indica.* The thickness of casing layer also affected the yield and bio-efficiency as recorded by Subramanian *et al.*, (2015).

The organic supplementation has effect on yield and nutritional parameters of *Calocybe Indica* (Sharma *et al.*, 2013). Yield performances of summer mushroom (*Calocybe indica*) on different agricultural subatrate have been carried out by Patel *et al*., (2017). The chemical constituent of the fruit body was found to be varied in different substrate. The protein content was always found to be higher in pileus than that of stipe. Velusamy *et al.,* (2014b) reported the higher protein content of pileus than that of stalk. The chemical constituent of mushroom fruit body grown in different substrate varies as reported by Shin *et al*., (2007) and Pani (2010). Pushpa *et al.,* (2010) suggested that milky mushroom is protein and fibrous rich with low fat content and may be used as protein supplementary diet. In present study the result revealed that the carbohydrate content of pileus is lesser than the stipe of the fruit body was supported by the study of Alam *et al.,* (2008). Among the substrates, the lowest total sugar content was found in stipe of fruit body grown in paddy straw and maize stalk combination as 26.30 mg/g tissue where others substrate based fruit body showed avg. total sugar content of 32.62 mg/g tissue. On the other hand in case of pileus the lowest total sugar content was found in bamboo leaves (21.67 mg/g tissue) where maximum total sugar content (24.33 mg/g tissue) was found in young coconut fiber growing fruit body followed by paddy straw (23.67mg/g tissue) paddy straw + saw dust (23.67 mg/g tissue) and maize stalk singly (23.67mg/g tissue) and paddy straw + maize stalk (22.33 mg/g tissue). Sharma *et al.,* (2013), reported that the organic supplements also effect on the non-enzymatic antioxidants and mineral expression in *C. indica.* Fruit body of *C. indica* contains lesser carbohydrate than protein in pileus as reported by Chakraborty *et al.,* (2016). In our study it has been found that the various casing materials have the effect in chemical constituent of fruit body of *C. indica*. Among the casing materials the highest protein content of the pileus of fruit body was observed as 137.34 (mg/g tissue) in spent mushroom substrate followed by young coconut fiber, soil and sand, vermicompost, hard paper, sand and saw dust, tea waste and sand, saw dust and, Tea waste. The maximum protein content of stipe of fruit body was observed in sample grown in paddy straw followed by paddy straw + maize stalk, maize stalk, young coconut fiber, paddy straw + saw dust (1:2) and the minimum protein content was found in both paddy straw + saw dust (1:2) and bamboo leaves. The maximum protein content of stipe was observed in spent mushroom compost casing material followed by Soil and sand, young coconut fiber, tea waste, Hard paper, vermicompost, tea waste, saw dust and the minimum protein content was found in sand and saw dust. Maximum protein content was observed in pileus of young fruit body compared to stipe by Velusamy *et al.,* (2014b). His study revealed that the carbohydrate content of pileus is lesser than the stipe of the fruit body. Among the casing materials, the maximum total sugar content (26.30 mg/g tissue) was found in stipe of fruit body grown in SMC followed by soil and sand (34.67), vermicompost (34.33), young coconut fiber and Tea waste (33.33 mg/g tissue), Tea waste and sand (33mg/g tissue), Sand and saw dust, hard paper (32.67mg/g tissue). On the other hand in case of pileus the lowest total sugar content was found in saw dust encased sample where maximum total sugar content was measured in spent mushroom substrate and soil and sand. Our study regarding the carbohydrate content was supported by the study of Velusamy *et al.,* (2014b) where he stated that the carbohydrate content of stipe was measured as maximum compared to pileus.

In case of button mushroom cultivation, timing of composting which make it suitable for mushroom growth is important for good result of production (Colak, 2009). Time period may be an optima activity stage of thermophilic fungi. This stage is a crucial stage for decomposition for carbohydrates necessary to produce a selective substrate environment for mushroom growing. The qualities of *Pleurotus ostreatus* such as high yielding, ability to grow on a wide variety of substrates such as coconut coir, sawdust, coconut fiber, finger millet straw, water hyacinth, rice straw, maize cobs, wheat straw and banana fiber was demonstrated by Musieba *et al*., (2012). Maio *et al*., (2003) studied the influence of substrate composition on the growth velocity of the mushroom and observed a significant correlation between substrate formulation and the nutritional composition of the mushroom. Pruned dry tea leaf based composts gave similar results compared to wheat straw based composts in terms of pin head formation time and yield. Therefore pruned dry tea leaves ca be used as for cultivation of button mushroom (Yilmaz *et al*., 2007). However, yield and nutritional values of *A. bisporus* should be investigated in more detail on cultivated waste tea leaves based composts.

The effect of casing materials on fruit body development was evaluated in *A. bisporus* cultivation and it was found that casing materials have effect on fruit body development. Different casing materials support different populations of bacteria, are related to the numbers of initials of fruiting and porosity and chemical composition also influence the primordial initiation and yield of *A. bisporus* (Taherzadeh *et al.,* 2013). High salt content, of casing layer can affect the mycelial growth, formation of primordial and mature fruit bodies (Taherzadeh *et al.,* 2013). Coirpith and vermiculture have been shown to stimulate initiation of *A. bisporus* (Fermor *et al.,* 2000, Barman *et al.,* 2016). Kalha *et al.,* (2011) and Kumar *et al*., (2012) reported that the various supplement and biofertilizer in casing layer have the effect on the yield of *C. indica.* The fresh weight of fruit body and yield was influenced by compost formulas. Similar result was recorded by Meire *et al.,* (2008) when they *cultivated A. bisporus* in two different compost formulas. Dias *et al*., (2003) analyzed the production of *P. sajor-caju* in different agricultural residues and also observed mycelium growth, production and biological efficiency differences depending on the compost used. The thickness of casing layer also affected the yield and bio-efficiency as recorded by Subramanian *et al.,* (2015). Hence it is important to create awareness among the farming community about various alternatives of paddy straw management by tapping the biodiversity and utilizes them for utilizing traditional and innovative technologies. These wastes include tea waste and paddy straw based waste may be the alternative income source for rural economy of North Bengal by using them in mushroom cultivation. One important aspect of production to help sustain the country and to create more employment is mushroom farming. Mushroom farming is a non-farm enterprise that can be integrated into small farms to increase incomes and enhance livelihoods. They can be cultivated on a part-time basis and require little maintenance.

Mushrooms are rich sources of nutraceuticals and nutritionally important mineral elements including iron, zinc, potassium, phosphorus, calcium, and sodium (Muthu *et al*., 2016; Ravikrishnan *et al.,* 2017) responsible for their pharmaceutical properties (Manzi *et al*., 2004). Edible mushrooms are considered as a valuable good quality nutritional dietary supplement for addressing certain malnutrition ailments (Salamat *et al*., 2017; Mridu *et al.,* 2017). The chemical characterization of fruit body was influenced by the composting duration of the compost in which they were cultivated (Dehariya *et al.,* 2013; Meirre *et al.,* 2014). In this study it was found that the protein content gradually increased with the age of fruiting body but there was a decrease in protein content in opened pileus form of fruit body (over mature). Protein content of *A. bisporus* mushroom has been reported to sharply decrease with the development of sporophore from young button stage to opened stages (Burton, 1988).The fruit body grown in different compost showed no significant difference in absorbance and phenolic content. Total polyphenols were reported in edible mushroom in methanolic extract by Keles *et al.,* (2011). The results of this study are supported by the study of Parihar *et al.,* (2015) in which they reported that the methanolic extract of *P. ostreatus* has high phenolic content. Four mushroom sp likely *A. bisporus*, *C. indica*, *P. sajor-caju* and *P. djamor* were subjected to GC-MS analysis. GC-MS was conducted using the data base of National Institute Standard and Technology (NIST) having more than 62,000 combinations of different compounds. The GC-MS analysis of the purified mushroom sample was reported to have compounds like alkanes and fatty acids which posses' therapeutic properties (Lakshmi *et al.*, 2011, Dhakad *et al.*, 2017). But Shao *et al.,* (2010) investigated different parts of button mushrooms at various development stages and found different fatty acid and detected non esterified ergosterol. From the GC-MS spectrum, the methanol and ethanol extracts from *C. indica* resulted in the identification of 47 major compounds. In our study, maximum unsaturated fatty acids as octadec-9-enoic acid were detected and moderate amount of steroids as ergosta was detected. The squalene a terpenoid known as super antioxidant was detected only in ethanolic extract. Phosphine which is generally responsible for aroma was detected in ethanolic extract of *C. indica*. The results were in the same line with the GC-MS study of the fruit body of *P. ostreauts* by Suseem *et al.,* (2011). The present study regarding the GC-MS analysis revealed the presence of volatile compounds which were identified as majorly as fatty acids ester and some non-polar and volatile compounds in these mushrooms species give the chemistry of the aroma. Bhupathi *et al.,* (2017) carried out the GC-MS analysis of milky mushroom in fresh and dry form and the results revealed the presence of increased levels of ergosterol indicates the availability of antioxidants and anticancer biomolecules and stated that milky mushroom contain many volatile compounds and phenolics which are potent antioxdant. Ribeiro *et al*. (2007) identified free amino acids composition and thirty fatty acids from twelve wild edible mushroom species by ion traping method in GC-MS. Mohamed *et al.,* (2014) used GC-MS analysis and reported 5 alkohol, 27 alkane, 3 amides 27 esters, 8 fatty acids, 4 terpenoid, 29 heterocyclic and 2 phenols in ethanolic extract of *Pleurotus ostreatus.* Jananie *et al*., (2012) used GC-MS analysis for detection of bioactive compounds in hydro alcoholic extract of *A. bisporus* and he recorded that 2-non-bornanone and methano-benzocyclodecene as prevailing compound. Ragasa *et al.,* (2016b) reported the presence of sterols and lipids in dichloromethane extract of *A. bisporus* detected in GCMS analysis. Some essential amino acids such as lysine, leucine, threonine and isoleusine and fatty acids identified by GC-MS analysis by Ravikrishnan *et al.,* (2017). Aromatic tumerone is a bioactive compound found in ethanolic extract of *A. bisporus* plays an important role in self-repair and recovery of brain function in neurodegenerative diseases. In adult brains of humans and mammals, the sub ventricular zone and hippocampus are the two key areas where growth of new neurons occurs when subjected to the powerful impact of Ar-tumerone (Liao *et al.,* 2013). Researchers are thinking that it may be able to get one step closer in treating neurological diseases, including Alzheimer's. This new finding is an incredible one in the scientific community. The presence of sterol and lipids was detected by GCMS in *P. florida* (Ragasa *et al*., 2015) and *P. djamor* (Ragasa *et al*., 2016a). The presence of linoleic and oleic acid derivatives as the major unsaturated fatty acids is in line with earlier report on mushrooms (Kalac, 2013). Linoleic acid is an essential omega-6 polyunsaturated fatty acid involved in the biosynthesis of arachidonic acid and prostaglandins. Oleic acid an essential omega-9 monounsaturated fatty acid is used as an emulsifying agent, reported to be hypotensive (Teres *et al.,* 2008). Research has shown ergosterol may exhibit some degree of antitumor properties (Takaku *et al.,* 2001). Ergosterols are known to act as biological precursors of vitamin D2, classified as provitamins (Rajakumar *et al.,* 2007). Stigmasterol level up to 1.54 % of the total n-hexane extract, known as the anti-stiffness factor a phytosterol, reported to have some anti-cancers, antioxidant, hypoglycemic, hypocholesterolemic and thyroid inhibiting properties was also observed (Gabay *et al.,* 2010; Panda *et al.,* 2012; Alam et al., 2011; Shao *et al*., 2015). *P. ostreatus* contain few antimicrobial bioactive compounds (Ruthes *et al*., 2016). The GC-MS analysis of the purified mushroom sample was reported to have compounds like alkanes and other fatty acids and was also reported to possess numerous therapeutic properties (Lakshmi *et al*., 2011, Chen *et al*., 2016, Zhang *et al*., 2016, Chien *et al*., 2015). Sharma *et al*., (2016) stated that *A. bisporus* has the antimicrobial, antioxidant and anticancer potential and they identified some phytochemicals from mushroom sample such as alkaloids, flavanoids, terpenoids, phenols and tannins that exhibit a wide range of medicinal properties. Mushrooms also exhibit numerous properties due to the presence of different types of polysaccharides such as lectins, prolamines, cordycepin (Sharma *et al.*, 2016). *Agaricus bisporus* and *Pleurotus spp* are also found to be a rich source of amino acids such as leucine, valine, glutamine and other essential amino acids (Sharma *et al*., 2016). The products derived Mushrooms can be used for deriving biologically active metabolites or compounds in order to design or develop therapeutically important drugs without any side effects and also helps in combating life threatening diseases (Sharma *et al.*, 2016). Atri *et al*., (2013) documented the nutritional and neutraceutical composition of five species of *Pleurotus* from Northwest India. Citronellol epoxide (R or S) is a natural acyclic monoterpenoid is good insect repellent and use in perfume industry. Pentadecanoic acid is a saturated fatty acid rare in nature, being found at low level of 1.2% in milk, has important role in human blood serum metabolism. Dianhydromannitol is a carbohydrate derivatives used as sweetener in diabetic food and as a medication; it is used to decrease high pressure in the eye (glaucoma) and to lower increased intracranial pressure (head trauma). Solanesol was detected in methanolic extract of *C. indica* have the clinical usesfor heart failure, treatment of liver injury and use in pharmaceutical industry as an intermediate for the synthesis of ubiquinone drugs. The GC-MS analysis of methanolic extract of *P. djamor* revealed that the extract contains a wide range of bioactive compounds. *Pleurotus djamor* are also found to be a rich source of amino acids such as leucine, valine, glutamine and other essential amino acids. They are also found to be a rich source of biologically active components which can be used as natural resources beneficial for human health. Chowdhury *et al*., (2015) evaluated the antimicrobial, antioxidant properties andbioactive compounds of some edible mushrooms cultivated in Bangladesh.

In the present study it was found that the methanolic, ethanolic and aqueous extracts of different edible mushrooms showed positive antioxidant activity as detected by DPPH assay. *P. djamor* showed maximum activity among the other species in respect to all the concentrations. It was found that *P. djamor* in methanolic extract showed about 84% DPPH scavenging activity at 500µg/ml concentration while *C indica* showed DPPH scavenging activity as 52% at 500µg/ml concentration. In both ethanolic and aqueous extract *P. djamor* showed highest DPPH scavenging activity then the other mushroom species while *C. indica* was recorded with lesser DPPH scavenging activity. DPPH scavenging activity was also evaluated by TLC-DOT assay where the violet colour of the DPPH scavenged by the sample extracts according to their scavenging activity to pale yellow. Given the high dietary fiber and antioxidants in button mushroom may be advantageous in lowering the dietary glycemic load (Jeong *et al.*, 2010). Jun *et al.*, (2013) evaluated the antioxidant activities of ethanolic extract of *A. bisporus* in vitro as well as in vivo. In the antioxidant assays he found that the ethanolic extract of *A. bisporus* have strong reducing power, superoxide radical, hydroxyl radical and 2,2- diphenyl-1-picrylhydrazyl radical scavenging activity and moderate hydrogen peroxide scavenging activity. This study supports my findings regarding the antioxidant activity of mushrooms. Many research work has been focused on the antioxidant activities in vitro of *A. bisporus* concerning reducing power, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and 2,2-azinobis-(-3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity (Kozarski *et al.,* 2011). Reis *et al.*, (2012) stated that the ethanolic extract of *A. bisporus* have higher reducing power capacity than methanolic extract of *A. bisporus.* Jayakumar *et al.*, (2009) evaluated the antioxidant activity andconsidered *P. djamor* as a good scavenger of superoxide radical. Chemical bioactive and antioxidant potential of twenty wild culinary mushroom species was carried out by Sharma *et al*., (2015a). In present study it was found that the scavenging activity of ethanolic and methanolic extract of mushrooms increased with increase of the concentration. My finding was supported by the study of Reis *et al*., (2012). Jun *et al*., (2013) used UHPLC-MS to determine the main phenolic compounds in ethanolic extract of *A. bisporus* and found that gallic acid, protocatechuic acid, catechin, caffeic acid, ferulic acid and myricetin were the main phenolic compounds in ethanolic extract of *A. bisporus*. Because of the nutritional composition and presence of bioactive molecules, oyster mushroom was considered to have anticancer, anti-mcrobial and antioxidant activity (Sharma *et al*., 2017).The individual phenolic profiles of *A. bisporus* was studied by Palacios *et al*., (2011); Reis *et al*., (2012); Ruthes *et al*., (2013). Palacios *et al*., (2011) investigated the main phenolic compounds in methanolic extract of A. *bisporus* were caffeic acid, catechin, chlorogenic acid, p-coumaric acid, ferulic acid, gallic acid, p-hydroxybenzoic acid, homogentisic acid, myricetin, protocatechuic acid and pyrogallol. Mattila *et al.,* (2002) suggested that button mushroom has high content of acidic polysaccharides, dietary fiber, and antioxidants including vitamins C, B12, and D, folate ergothioneine and polyphenol as well as it has potential anti-inflammatory, hyperglycemic and hypocholesterolemic effects. Boonsong *et al*., (2016) evaluated the antioxidant activity of *P. sajor-caju,* in terms of DPPH and FRAP activity and suggested that the ethanolic extract has antioxidant potential. Due to the presence of bioactive compounds including phenolic, polyketides, terpenoids and steroid some edible mushrooms was found to be medically active in therapeutic purposes (Sharma *et al.*, 2015a). Yan *et al.*, (2017) evaluated the nutritional value, chemical composition and antioxidant activity of three *Tuber* species from China. Ferric (Fe^{+3}) reducing power activity was estimated using the different concentrations of six edible mushrooms in three different solvent. All the mushroom species showed appreciable reducing power activity in different concentrations. Highest reducing power ability was observed in methanolic extract; in case of *P. djamor* at 20 µg/ml concentrations while *C. indica* showed lowest reducing power activity at 20 µg/ml concentration. The reducing power ability in case of *P. florida* is quite lower than that of the others species of *Pleurotus.* The EC_{50} value was also calculated and it was found that the *P. djamor* have the minimum EC_{50} value i.e. more effective as antioxidant among the six edible mushroom species. Alshammaa *et al.*, (2017) investigated the phytochemical constituent of *A. bisporus* and *P. ostreatus* by HPLC and GC-MS and compared the ergosterol content between the two and stated that ergosterol play important role in antioxidant activity of these two mushrooms. Sharma *et al.,* (2015b) evaluated the nutrients, bioactive compounds, and antioxidants of mycelia of five mushroom species and the results revealed that the mycelia extract have a wide range of bioactive compounds and also showed the antioxidant activity. In the present study the results regarding the antihyperglycemic activity of mushroom revealed that the water suspension of fruiting body of *C indica, A. bisporus,* and four species *of Pleurotus* have significant anti-hyperglycemic activity in diabetic male rats. The STZ induced diabetes control rats showed significant increase in blood glucose levels compared to normal control. Rat groups treated with the mushroom water suspension, showed significantly reduced glucose level at 15 days in compared to diabetic control. The metformin (Climp-M1) drug treated diabetic rats also showed significant ($p<0.01$) reduction in glucose levels compared to diabetic control. It was found that oral administration of the water suspension of mushrooms water suspension and commercial antidiabetic drug metformine reducedthe glucose level in the diabetic rats.They have the effect in lowering the serum Creatinine, serum Triglyceride (TG), total cholesterol(TC), low density lipoprotein (LDL) which is closely associated with the hyperglycemic condition. Further, it was also found that the intake of suspension resulted in controllingnormal food consumption with marked reduction in plasma glucose as well as TG and cholesterol concentration in diabetic rats.We also observed lower plasma liver enzymes alanine aminotransferase (ALT) and asperagine aminotransferase (AST) concentrations in diabetic rats fed water suspension of mushroom powder. This suggests that intake of these suspension may protect against STZ induced inflammation in the liver that was observed from earlier studies (Todesco *et al*., 2004). Lower plasma TC and TG concentration were observed in diabetic rats fed with mushroom water suspension, indicating that the consumption of these mushroom had a beneficial effect in suppressing cholesterol and TG levels.It is well documented that elevated levels of total plasma cholesterol and TG levels are associated with complication of diabetic mellitus (Feldstein *et al*., 2008; Aas *et al*., 2009). Oral administration of mushroom suspension was effective in significantly lowering low density lipoprotein (LDL) and to control HDL concentration in diabetes induced rats. A significant decrease in the mean LDL cholesterol level was observed in the test group when compared against the control in the study. The current study stated that diabetic rats had significantly increased plasma TG concentration compared with normal non diabetic rats. These elevated plasma TG concentration was decreased byoral administration of mushroom water suspension. Our findings have been supported by the study of Lai *et al*., (2001); Shanmugasundaran *et al*., (2013) where they reported that mushrooms are useful in regulating healthy cholesterol levels and improve their circulation and to help in maintaining blood sugar levels by reducing the blood glucose, elevation of plasma insulin levels and enhanced liver metabolism of glucose and increase cellular insulin sensitivity. Oxidative stress induced by hyperglycemia leads to the activation of stress sensitive signaling pathways, which worsen both insulin secretion and its action and promote the development of diabetic mellitus, (Shanmugasundaran *et al*., 2013). Taofiq *et al*., (2016) reported that the fungus *P.ostreatus and A. bisporus has* hypoglycemic effect in rats. Khan *et al*., (2011), have demonstrated that oral administration of water suspension of mushroom to rats leads to blood glucose lowering effect in diabetic conditions. Antidiabetic effect of ethanolic extract of *P. ostreatus* on alloxan induced diabetic rats was extensively studied and reported as an effective antidiabetic regimen (Johny *et al*., 2013).

The single treatment of some organic amendment and low urea treatment had the same plant health development and fruit production. SMS individually showed the maximum growth and development and fruit production in comparison to the other individual treatment. But the combination application of Vermicompost and SMC gave the highest result in plant health improvement and quality fruit production. But the excess urea treatment sowed poor development and abnormal fruit production. The pigment content was higher in the variants treated with bio-inoculants. Currently there is a gap of plant nutrients between removal of crops and supplying through chemical fertilizer. In this context, organic fertilizer and bio-inoculants would be the viable option for farmers to increase the productivity of soil. It can be concluded that the bio-inoculants can be used as alternative of inorganic fertilizer for development of sustainable agriculture. SMS amendment in soil significantly increased the bell pepper yield in addition to improving the quality. As a whole, spent mushroom substrate and its combination use helped in increasing yield by increasing the fruiting life of the plant and quality of capsicum along with decreased levels of disease incidence. Adedokun *et al.,* (2013) recorded the similar data when they apply SMS in pine apple production; the vegetative parameters are vigorously increased. This study supported by the experimental result of Tallapragada, *et al*., (2011). The nutritional content of harvested tomato was higher in SMS treated plant as similar as reported by Worthington (2001) when nutritional quality of organic versus conventional fruits, vegetables and grains were compared. The finding of this study was in the same line with the finding of Eudoxie *et al.,* (2011), who noted higher P and K concentration in spend mushroom substrate treated. They also reported that SMS incorporated medium showed greater macronutrient concentration than peat based medium. Medina *et al.,* (2012) confirmed that the SMS has been shown to increase the nutrient availability of growth medium which help to increase final yielding of crop. In the present study the results regarding the use of spent mushroom substrate (SMS) in crop system to influence the yield, biochemical constituents of crop was supported by the result of Meena *et al.,* (2014), in which the significant yield, nutrient and protein content was influenced through the application of vermi compost alone over organic manure. Application of vermicompost recorded highest yield and growth parameters as recorded by Biswas, (2014). Sarhan, *et al.,* (2011), worked on organic manures in tomato crop and reported that organic manures significantly affected growth parameters such as plant height, leaf area and fruiting time and yield. Organic manure improved the yield and components of egg-plant fruits as reported Abd *et al.,* (2001). Vermicompost have more positive effects on seed yield and oil content than cow manure as recognized by Jahan *et al.,* (2013), and Shahmohammadi *et al.,* (2014). Arancon *et al.,* (2004) have established the organic manure as an effective means of improving soil structure, enhancing soil fertility, increasing microbial diversity and populations, microbial activity, improving the moisture holding capacity of soil and increasing crop yields. Vermicompost is rich in most nutrients and consistently promote biological activity which can cause plants to germinate, flower and growth and yield better (Atiyeh *et al.*, 2000). Addition of spent substrate to agricultural or garden soil has been found to be an effective soil manure and conditioner and has been found to increase considerably the yield of some leafy vegetables crops (Kadiri *et al.*, 2010). Iwase *et al.*, (2000) observed that spent compost of *Volvariella volvacea* on addition to soil increased the yield of tomatoes 7 fold and the yields of soybean, lettuce and radish 2 fold each. Furthermore, they observed that addition of *Agaricus bisporus* spent compost to the soil produced greater yields of cabbage, cauliflower, beans and celery compared to addition of poultry manure to soil.

In the present study, regarding the application of spent mushroom substrate and spent mushroom compost in crop system it was also found that the SMS enhanced the mycorrhizal association in root system in tomato plant, capsicum and leafy vegetables such as coriander (Dhone pata), *Amaranthus* (lal shank), except spinach. The spore population was also found to be increased in SMS treated crops. These findings were on the same line of the finding of Dimitrios *et al*., (2015) in which he stated that mycorrhizal population in the rhizosphere is influenced by the organic fertilizer. Organic fertilizer influence VAM spore to germinate.The severity of root rots disease in *Citrus reticulata* caused by *F. oxysporum* was reduced by the treatment of SMS of *A. bisporus* in potted condition. Our study was supported by the result of Parada *et al*., (2012) in which they used autoclaved water extract from spent mushroom substrate and autoclaved spent mushroom substrate of the edible mushrooms *Lyophyllum decastes* and *Pleurotus eryngii* to reduce powdery mildew diseases caused by *Podosphaera xanthii* and bacteria *Pseudomonas syringae* on cucumber plants. Kwak *et al*., (2015) conducted as experiment in which they used spent substrate water extracts of edible mushrooms, *Pleurotus eryngii*, *Hericium erinaceus* and *Lentinula edodes* andpromoted growth of pepper seedling as well as mycellial growth rate of *Phythopthora capsici* and *Fusarium oxysporum* was dramatically inhibited by 100% and 70% in vitro by using SMS extract. These results have supported our study and contributed support to conclude the SMS extract have dual effects that suppress plant disease and promote plant growth. In our study the mycelial growth of *F. oxysporum* was also inhibited by the mycelial phase of six mushroom as well as the by the SMC extract.Up to 52% inhibition of mycelial growth and sporulation by the SMC water extract was recorded. Suarez *et al.*, (2012) studied *in vitro* control of *Fusarium* wilt using agro-industrial subproduct-based composts. Choi *et al.*, (2007) successfully reduced the basal stem rot of the cactus caused by *F. oxysporum* using spent mushroom compost. Ayala *et al*., (2015) and Bastida *et al.*, (2016) successfully studied comparative antibacterial activity of the spent substrate of *Pleurotus ostreatus* and *Lentinula edodes.* Antimicrobial activity of mushroom extract was also evaluated by Nehra *et al.*, (2012). Hussein *et al.*, (2016) conducted an experiment for the management of wilt disease caused by *F. oxysporum* on tomato using spent mushroom compost.

The aim of the present study was to undertake demonstration and training activities to benefit farmers, rural women and unemployed youths from seven district of North Bengal through various employment generation activities by undertaking sustainable utilization of biological resources through awareness and popularization activities. The interventions on mushroom cultivation and processing centre at village level were taken up. *Pleurotus* sp. is one of the common mushrooms cultivated throughout the North Bengal. But in absence of scientific cultivation process, its cultivation practices have been standardized by using wide range of locally available substrates which are being widely adopted by the farmers of this zone and also training on post harvest processing have also given. The achievements were possible due to contribution of six important factors namely; identification of most profitable, sustainable and location specific technologies, timely and dependable information at the easy reach of rural people, providing critical inputs free of cost at the easy access, effective functional linkage, marketing empowerment and commodity based associations. To achieve this the demonstration and training activities undertaken on mushroom cultivation has covered 300 beneficiaries with setting up of spawn production and mushroom cultivation units and training of 1213 persons of which 432 nos. are women in Coochbehar, Malda, Jalpaigiuri, North and South Dinajpur and Darjeeling districts. 149 mushroom production units have been established by the beneficiaries at Coochbehar, Jalpaiguri, Darjeeling and Malda districts and 11 spawn production units were developed at Coochbehar, Jalpaiguri, Darjeeling and Malda. The present study also encompasses the development of small entrepreneurs in the field of mushroom production, as an alternative strategy for maximizing rural revenue through agricultural waste management. The assessment and proper utilization of natural resources have benefitted the farmers with extra inputs for agriculture leading to sustainable and profitable production. Upon receiving practical experience from hands on demonstration training, they started practicing the same in their own farm lands at the same time. Constant monitoring of their performance was done to encourage them and suggestions to any problem were provided. Thus, the target population learns the technologies at first by observing the demonstrations and then by doing the same on their own.