ABSTRACT

Calocybe indica a new introduction in mushroom world but not yet popularized like other mushrooms such as *Pleurotus sp* have been successfully cultivated in North Bengal and standardized the technique for better production. Cultivation techniques using simple composting method as well as suitable casing materials for *Agaricus bisporus* in foot hills of Darjeeling district have also been successfully standardized for the first time. Easy, low cost and less time consuming composting processes which were standardized and these techniques were provided to the rural mushroom growers in selected farms. Four species of another most popularly grown edible oyster mushroom such as *Pleurotus ostreatus, P. sajor-caju, P. djamor* and *P. florida* were selected for cultivation in this environmental condition throughout the year. Several lignocellulosic wastes and casing materials were evaluated for their suitability for cultivation of milky mushroom (*Calocybe indica*). Paddy straw, wheat straw, sugarcane bagasse, young coconut fiber, tea leaf, maize stalk as growing medium and vermicompost, coconut fiber, spent mushroom substrate (SMS), sand soil were used as casing materials to evaluate their effect on yield. Different compost formulations as well as composting duration were further evaluated for fruit body production of *A. bisporus* and for improvement of their quality. Morphological and histological characteristics were studied with special reference to their fruiting body structure, gill and spore characters. Scanning electronic microscopy (SEM) of basidiospores of oyster and button mushrooms were studied. Mycelial growth pattern as well as growth rate of these mushrooms were studied on three different media. Molecular characterization (18S rDNA sequencing) of four species of *Pleurotus*, *Agaricus bisporus*, and *Calocybe indica* were carried out using primer pairs - ITS 1 and ITS 4 (for oyster and milky mushroom) ITS1and ITS 6 (for button mushroom) . BLAST query of 18S rDNA sequences of these mushrooms were analyzed and identified as *Pleurotus ostreatus*, *P. sajor-caju, P. djamor* and *P florida, Agaricus bisporus and Calocybe indica*. These sequences of the identified mushroom species were submitted in NCBI Gen-Bank under the accession number KT768095, KT818506, KT 768094 and KT826605 for *P. ostreatus, P sajor-caju, P. djamor* and *P. florida* ; KT818508 for *A. bisporus* and MF804327 for *C. indica* respectively. In North Bengal, *P. ostreatus*, *P sajor-caju* and *P. florida* are commonly being cultivated by the farmers. However, a new species of *Pleurotus* namely *P*

djamor, the pink oyster mushroom have been introduced in this area and being successfully cultivated in this area during winter season. The seasonal productivity of all these mushrooms was evaluated. The results clearly indicate the variable seasonal productivity throughout the year. Depending upon the temperature, relative humidity and rainfall, the productivity was found to be different. In case of *P. ostreatus* and *P. sajor-caju*, it was found that throughout the year it can be cultivated with good productivity rate. But the production rate was less during June-September due to high humidity. In summer time the *C. indica* can be cultivated as it requires high temperature and humidity. It was found that winter season is suitable for cultivation of *A. bisporus* in foot hill region of Darjeeling district but throughout the year in hills regions. Seasonal productivity of *P. florida* and *P. djamor* was found to be quite similar. It was observed that the production rate decreased during May to September, while production rate increased during winter season in North Bengal. Economy of North Bengal is based on tea productivity and leaf pruning is one of the important practices of tea plantation. Here the pruned tea leaves basically used as fuel by the tea workers. In the present investigation pruned tea leaves were dried and utilized for mushroom cultivation. Besides, tea leaf based compost was further used for cultivation of *A. bisporus* successfully. Tea waste was also used as casing of materials and it was found that it gave good result for mushroom cultivation especially for *A. bisporus* and *C. indica*. Biochemical constituents of the fruit body of mushroom were influenced by the substrate on which it grows. It was found that the casing materials have the effect on biochemical constituents of fruit body of milky and button mushrooms.

During the cultivation of *Pleurotus* sp. several fungal species like *Coprinus, Fusarium* as well as *Trichoderma*; bacterial species like *Pseudomonas* and pastes like Sciarid, phorid and Beetle fly drastically affected the growth and yield of *Pleurotu*s. Management strategies were developed using Bavistin, phenyl, carbendazim and formaldehyde which reduces contamination effect. Racks in production unit were covered with nylon nets which helped in reducing the attack of flies on the substrates.

Antioxidant activity of selected four species of *Pleurotus, A. bisporus and C. indica* were also estimated and it was found that all four species showed very high amount of antioxidant activity such as DPPH scavenging activity, ferric reducing power activity. Among the cultivated *Pleurotus* species, *P. djamor* and *P. ostreatus* showed comparatively higher antioxidant activity. The oral administration of mushroom

suspension helps in regaining the body weight as well lowering the plasma glucose level. Blood urea, creatinine, triglyceride, cholesterol as well as liver enzymes like serum glutamic pyruvate transaminase (SGPT) and serum glutamic oxaloacetic transaminase were also estimated and the oral administration of *P. djamor* and *P. ostreatus* found to be very effective in recovering the other blood parameters.

Chemical constituents of three edible mushrooms, *Agaricus bisporus*, *Calocybe indica* and four species of *Pleurotus* were evaluated by GC-MS analysis. The common compounds such as phenolic, flavonoid and total free amino acid content responsible for antioxidative activity were determined in methanolic extract of these mushrooms. The GC-MS analysis revealed that the presence of phenolic derivatives, organic acids and essential fatty acids in these three mushrooms which are associated with some beneficial health activities like antioxidant. Fumaric acid, acetic acid, malic acid were detected as organic acid in the mushroom samples. Pyrazin, a phenolic derivative responsible for antidiabetic activity was detected in the mushroom samples.

Spent mushroom substrate is an important byproduct of mushroom cultivation and this spent mushroom substrate were applied as fertilizer for crop improvement. *Capsicum chinense, C. annuum, Solanum lycopersicum* and some leafy vegetables *Coriandrum sativum*, *Amaranthus* sp. and *Spinacia oleracea* were tested using the spent mushroom substrates (SMS) which was found to be very effective as a soil conditioner and also helped in increasing growth and yield of crop plants in compare to untreated plants. Spent mushroom compost (SMC) amendment in soil significantly increased the bell pepper yield in addition to improving the quality. Incidence of soil borne fungal diseases decreased to a considerable level due to the incorporation of SMS in soil. As a whole, SMC and its combination helped in increasing yield by increasing the fruiting life of the plant and quality of *Capsicum* along with decreased levels of disease incidence. SMC of button mushroom was further used in mandarin plants (*Citrus reticulata*) as soil amendment against root rot pathogen (*Fusarium oxysporum*). Disease severity was reduced by application of SMC which was evident with increased activity of defense enzymes (PAL, POX, and β-1,3-glucanase and chitinase) and cellular localization of β-1,3-glucanase and chitinase were confirmed by indirect immunofluorescence using PAbs of chitinase and glucanase and FITC conjugates.

Large number rural educated unemployed youth as well as women self-help groups of North Bengal were facilitated with proper training about mushrooms cultivation and spawn preparation. Publicity and awareness camp about mushrooms and its health benefits were also displayed at the Krishi Mela organised by the Department of Agriculture, Govt. of West Bengal.