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A STUDY ON PHYSICO-CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF SUGARCANE MULCH

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ABSTRACT: The aim of this study is to investigate the physico-chemical and microbiological properties of sugarcane mulch. The results showed pH- 7.75, moisture content- 26.44%, water holding capacity- 93.4%, available phosphorus- 4.86%, organic matter- 9.54%, nitrogen- 1.54% and potassium- 0.84%. The microbial respiration was observed 0.0180 μgCg^{-1} after 35 days. 0.67 $gCm^{-2}d^{-1}$ microbial biomass carbon was found during present study. Two different media were used for the identification of mycoflora from sugarcane mulch *viz.,* Sabouraud's agar and Czapek-Dox agar media. *Aspergillus niger, Mortierella elongata* and *Mucor circinelloides* were the common species found in both the media whereas two more number of species *viz., Mortierella uniramosa* and *Mucor microsporus* were identified from Sabouraud's agar media and nine more number of species *viz., Alternaria sp., Aspergillus clavati, Aspergillus fumigatus, Aspergillus parasiticus, Helminthosporium sp., Penicillium sp., Pythium sp., Scopulariopsis sp. and Ulocladium botrytis* were observed in Czapek-Dox agar media. The analysis of the study revealed the possibility to use sugarcane mulch in agriculture purposes as an alternative to chemical fertilizer after an appropriate decomposition.

KEY WORDS: Sugarcane mulch, Microbial respiration, Microbial biomass carbon, Sabouraud's agar and Czapek-Dox agar medium.

INTRODUCTION:

India is the largest consumer and second producer of sugar in the world, with over 450 sugar factories located throughout the country. The sugar industry is amongst the largest agro-processing industries in India with average sugar production of about 176.75 lakhs tones with an annual turnover of Rs.150 billion (Kalaivanan and Hattab 2008). The sugarcane industry has several co-products of immense potential value and those include pressmud (filter cake),

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molasses, spent wash, bagasse and mulch. These co-products are produced during clarification of sugarcane juice (Bhosale, *et al.,* 2012).

Mulching is one of the management practices for increasing water use efficiency and controlling weeds in crop fields. Mulch provides a better soil environment; moderates soil temperature, increases soil porosity and water infiltration rate as well as controls runoff and erosion. The effects of organic mulches on soil properties have been studied by Herms *et al.,* (2002); Akanbi and Ojeniyi (2007). Organic mulches perform additional functions of increasing soil organic matter and CEC, enhancing biological activity, improving soil structure, increasing plant nutrients after decomposition and maintaining high crop yield, thus reducing the need for heavy application of N fertilizer (Uwah *et al.,* 2012). Sugarcane mulch is used as a fertilizer in agriculture field for enhancing the soil fertility. Various physico-chemical parameters like pH, moisture content, water holding capacity, organic matter and nitrogen have significant role in sugarcane mulch. The quality as well as quantity of organic matter in the soil has a direct correlation to growth of fungi, because most of the fungi consume the organic matter for nutrition (Bhattacharyya and Banerjee 2007). As microbes utilize high C:N carbon sources, their growth may become N-limited; conversely, decomposition of organic matter with a C:N ratio less than 30:1 may result in carbon limitation of microbial growth. When soil microbes are nitrogen limited, nutrient addition via fertilization may stimulate their growth. Therefore, soil management practices such as fertilization and application of organic mulches may have substantial effects on microbial diversity and abundance (Tiquia, *et al.,* 2002).

Soil microbiological properties such as microbial biomass carbon and soil microbial respiration may be used as early and sensitive indicators of soil quality. The amount of soil microbial respiration that occurs in an ecosystem is controlled by several factors. The temperature, moisture, nutrient content and level of oxygen in the soil can produce extremely disparate rates of respiration. The soil microbial biomass carbon is an important component of soil organic matter comprises 1-3% of total organic carbon in soil, but it has a rapid turnover rate and represents a labile reservoir of nutrients (Yadav, 2012). Nevertheless, scientists published many papers about the effect of organic mulching on soil properties and the biological parameters of mulches in many regions, but the mulch of sugarcane have rarely been studied.

The aim of the present investigation was to analyze the physico-chemical properties, identifying the myco-floral consortia of sugarcane mulch, microbial respiration and microbial biomass carbon.

MATERIALS AND METHODS:

Sugarcane mulch sample was obtained from Indian Potash Limited Sugars and Chemicals, Rohanakalan, Muzaffarnagar (U.P), India and precautions were taken so as to reduce the possibility of any error. The study was conducted during winter season for thirteen weeks *i.e.,* from January 2013 to March 2013.

Standard procedures were followed in analyzing the samples as prescribed by Jackson (1958); Trivedi and Goel (1998). Accordingly pH was measured using a digital pH meter. Fresh samples were determined after oven drying them overnight at 105°C to calculate the moisture content. Excess water was collected and measured to calculate the water holding capacity. Soil Potassium was determined using flame photometry. Organic matter was determined following the wet digestion method. Total nitrogen as nitrate and available phosphorus was analyzed spectrophotometrically as per the methods described by Anderson and Ingram (1993).

For the isolation of mycoflora from sugarcane mulch sample Sabouraud's agar and Czapek-Dox agar media were prepared. Serial dilution technique by Aneja, 2003 was adopted. 10 g of sugarcane mulch sample was weighed separately and then dissolved in 100 ml of distilled water to make a microbial suspension. Dilutions were prepared by adding 1 ml of microbial suspension into 9 ml of distilled water and were labelled as 10^{-1} to 10^{-5}. 1 ml of each dilution was dispersing throughout the petriplates by gentle rotation. Both medium were supplemented with 1% streptomycin (1 g of streptomycin was mixed in 100 ml of sterilized distilled water) was poured over the dilutions. The plates were incubated at 25°C in an inverted position for 2-7 days. After 2-7 days of incubation, the fungal colonies growing on Sabouraud's agar and Czapek-Dox agar plate with different morphology. Colonies were counted with the help of Quebec colony counter and purified on separate medium. After the isolation of mycoflora pure cultures were made. For the identification of mycoflora edge of each colony was picked up with the help of sterile needles and mounted on clean dry slide with a drop of lactophenol cotton blue stain over unteased material. The slide was gently heated over the flame so as to remove air bubbles and the excess was wiped off with the help of blotting paper and then the clean cover slip was placed over the slide and sealed with thin layer of transparent nail polish around the edge of coverslip. Then the slide was observed under microscope and the fungal colonies were identified by their shape, colour, size of their conidia and type of mycelium (Aneja, 2003; Watanabe, 2002). Microbial respiration was determined after trapping CO_2 in 0.1N NaOH solution and released CO_2 was measured titrimetrically with 0.1N HCl as per method of Parthipan and Mahadivan (1995). Results of the microbial respiration were expressed as μgCg^{-1}. Microbial Biomass Carbon (MBC) was analyzed by the chloroform fumigation method and calculated as the difference of carbon content between fumigated and non-fumigated samples (Jenkinson and Powlson 1976). Standard error was calculated as per method described by Chandel (2006).

RESULTS AND DISCUSSION:

The physico-chemical parameters of sugarcane mulch are given in Table 1. The pH indicates much about the interpretation of the mineral content and also influences the mycoflora. The value recorded for pH was 7.75±0.02 which indicates that sugarcane mulch was slightly alkaline. pH 7.58 was recorded in mulch of composted yard waste (Tiquia *et al.,* 2002). Moisture content observed was 26.44±0.33%, moisture content is one of the most important key factors which influence the microbial population in soil. It is essential for the survival and growth of the plants and other soil microorganisms (Barnett and Hunter, 1999). The availability of nutrients in soil is affected by the moisture content of the soil. Uwah, *et al.,* (2012) observed 19.01% moisture content in mulch. During the research 93.4±0.12% water holding capacity of sugarcane mulch was recorded. 65% water holding capacity in sulphinated pressmud was recorded by Tyagi, (2005). Both inorganic and organic forms of phosphorus are important sources for plant growth. The solubility of phosphorus depends on the soil pH and water content (Gupta , 2006). During present study the amount of available phosphorus in sugarcane mulch was found to be 4.86±0.15%. Tiquia, *et al.,* (2002) found 2330 mg kg^{-1} phosphorus in mulch of composted yard waste. Organic matter improves filtration rate and work as a sponge which helps in holding moisture and also improves physical properties and providing a source of energy to beneficial organisms which enhance the reservoir of the nutrients. Organic matter provides food for microorganisms and also contributes to chemical reaction in the soil (Gupta 2006). 9.54±0.01% of organic matter from mulch has been recorded during the present study. An increase in soil organic matter 25.1, 44.6 and 51.3% by addition of 2, 4 and 6 t/ha mulch was reported by Uwah, *et al.,* (2012). Ahmed, *et al.,* (2014) showed that 3.84% and 3.50% organic matter in rice husk and in ground nut shell mulch respectively. Nitrogen in the soil is present mostly in the organic form, together with small quantities of ammonium and nitrate forms. A good supply of nitrogen facilitates the growth and development of plants as well as the uptake of other essential nutrients. In nature, organic materials provide the nitrogen needed for growth however, most fungi can use sources of inorganic nitrogen as well (Barnett and Hunter, 1999). During the study 1.54±0.02% nitrogen was recorded. Tiquia, *et al.,* (2002) determined 20.30 g kg^{-1} nitrogen content in mulch of composted yard waste. Ahmed *et al.,* (2014) reported 12.44% and 16.25% nitrogen in rice husk and groundnut shell mulch respectively. Potassium occurs in nature only in ionic salts. For the function of all living cells potassium ions are necessary. Potassium ions are an essential component of plant nutrition and are found in most soil types. Potassium used as a fertilizer in agriculture and horticulture to increase yield. 0.30 mg kg^{-1} potassium content in mulch of composted yard waste was recorded during the study by Tiquia, *et al.,* (2002) and in during the analysis 0.84±0.09% potassium has been recorded. The results revealed that the sugarcane mulch is a good source of nutrients which could be added to soil to increase its fertility.

Fungal Species Composition:

Fungi play a focal role in nutrient cycling by regulating soil biological activity. However the role at which organic matter is decomposed by the microbes is interrelated to the chemical composition of the substrate as well as environmental conditions. The numbers and kinds of microorganisms present in soil depend on many environmental factors: amount and type of nutrients available, available moisture, pH and temperature (Sharma, 2010). In the present study pH (7.75±0.02) of sugarcane mulch was slightly alkaline which favours the growth of fungi. Most fungi grow optimally when the substrate is slightly acid between pH 5.0 and 6.0 however, they will generally achieve fair to good growth over a much wider range about pH 3.0 to 8.0 (Barnett and Hunter 1999). The percentage occurrences of different fungal species and total colony forming unit in different dilutions of sugarcane mulch are presented in Table 2, 3 and 4. In different dilutions of Sabouraud's agar (SDA) the colony forming unit (CFU) were found to be $3.8\times10^{-1}\pm0.82$ (CFU g^{-1}), $2.5\times10^{-2}\pm1.76$ (CFU g^{-1}), 0.8×10-3±0.35 (CFU g^{-1}), 0.6×10-4±0.49 (CFU g^{-1}) and $0.4\times10^{-5}\pm0.35$ (CFU g^{-1}). The results study revealed highest CFU value in 10^{-1} dilution when compared to the other dilutions with $3.8\times10^{-1}\pm0.82$ (CFU g^{-1}). Whereas in Czapek-Dox agar (CDA) same dilutions were used to record the CFU and it was observed that in 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} values were $4.6\times10^{-1}\pm2.82$ (CFU g^{-1}), $4.3\times10^{-2}\pm0.84$ (CFU g^{-1}), $3.7\times10^{-3}\pm1.66$ (CFU g^{-1}), $3.3\times10^{-4}\pm0.68$ (CFU g^{-1}) and $1.1\times10^{-5}\pm0.47$ (CFU g^{-1}) respectively. After comparing the two media it was found that the highest CFU were observed in 10^{-1} dilution of both the nutrient medium which showed that 10^{-1} supports the growth of the fungi.

Table 1. Values of some selected physico-chemical characteristics of sugarcane mulch (All values are mean ± S.E for 10 observations each)

S.No.	Parameters	Mean±S.E
1.	pH	7.75 ± 0.02
2.	Moisture content (%)	26.44 ± 0.33
3.	Water holding capacity (%)	93.4 ± 0.12
4.	Available phosphorus (%)	4.86 ± 0.15
5.	Organic matter (%)	9.54 ± 0.01
6.	Total Nitrogen (%)	1.54 ± 0.02
7.	Potassium (%)	0.84 ± 0.09

Different mycoflora present in both the medium were *Mucor circinelloides, Mortierella elongata, Aspergillus clavati, Aspergillus niger, Penicillium, Helminthosporium, Pythium, Alternaria, Scopulariopsis and Aspergillus fumigatus* (Figure I). In different dilutions of SDA medium five main fungal species identified were *Aspergillus niger, Mortierella elongata, Mortierella uniramosa, Mucor circinelloides and Mucor microsporus.* There is unidentified species that were observed in first three dilution only. Among all, *Mortierella elongata* was the dominant species with 23.68%, 24%, 25%, 33.33% and 75% occurrence in 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions.

Table 2. Values of colony forming unit (CFU g^{-1}) in sugarcane mulch (All values are mean ± S.E).

S.No.	SDA dilutions	CFU±S.E	CDA dilutions	CFU±S.E
1.	10^{-1}	$3.8\times10^{-1}\pm0.82$	10^{-1}	$4.6\times10^{-1}\pm2.82$
2.	10^{-2}	$2.5\times10^{-2}\pm1.76$	10^{-2}	$4.3\times10^{-2}\pm0.84$
3.	10^{-3}	$0.8\times10^{-3}\pm0.35$	10^{-3}	$3.7\times10^{-3}\pm1.66$
4.	10^{-4}	$0.6\times10^{-4}\pm0.49$	10^{-4}	$3.3\times10^{-4}\pm0.68$
5.	10^{-5}	$0.4\times10^{-5}\pm0.35$	10^{-5}	$1.1\times10^{-5}\pm0.47$

Table 3. Percentage occurrence of species in different dilutions of Sabouraud's agar medium

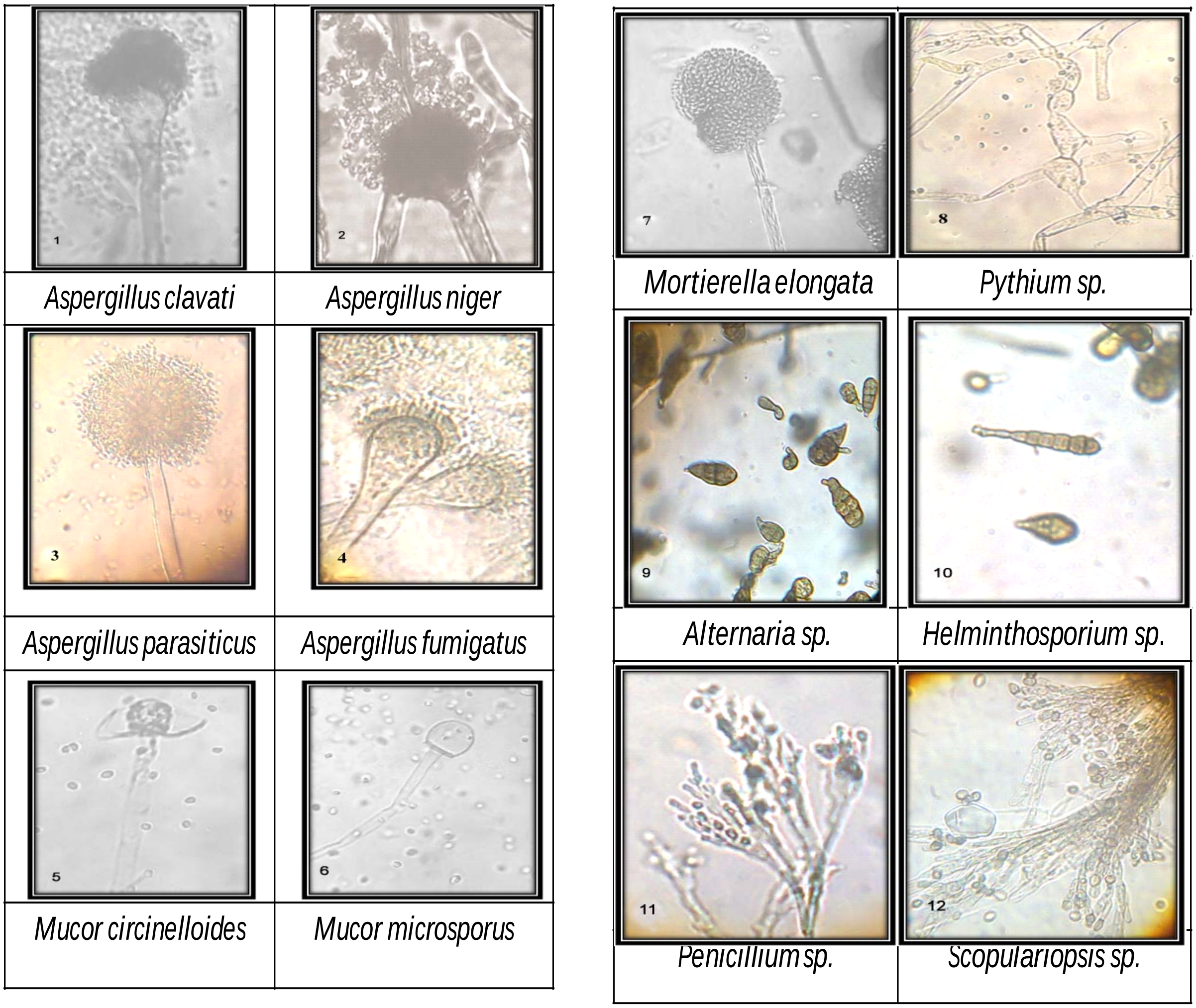
S.No.	Species	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
1.	*Aspergillus niger*	21.05	20	12.5	16.66	-
2.	*Mortierella elongata*	23.68	24	25	33.33	75
3.	*Mortierella uniramosa*	15.78	16	12.5	16.66	-
4.	*Mucor circinelloides*	13.15	12	25	-	-
5.	*Mucor microsporus*	15.78	20	12.5	33.33	25
6.	unidentified sp.	10.52	8	12.5	-	-

A total of twelve fungal species *viz., Alternaria species, Aspergillus fumigatus, Aspergillus niger, Aspergillus parasiticus, Helminthosporium species, Mortierella elongata, Mucor circinelloides, Penicillium species, Pythium species, Scopulariopsis species* and *Ulocladium botrytis* were identified in 10^{-4} of CDA media. Out of twelve fungal species except *Ulocladium botrytis* only ten species were observed in 10^{-3} and 10^{-4} dilutions. Only six species *viz., Aspergillus clavati, Aspergillus fumigatus, Aspergillus niger, Aspergillus parasiticus, Mortierella elongata* and *Mucor circinelloides* were recorded in 10^{-1} and 10^{-2}. An unidentified group of species were also observed in 10^{-1}, 10^{-2} and 10^{-5}. *Mortierella elongata* was the dominant species with 21.73% in 10^{-1} and 21.21% in 10^{-4} whereas in 10^{-2}, 10^{-3} and 10^{-5} *Mucor circinelloides* were dominant with 25.58%, 18.91% and 18.18% respectively. The results revealed that the density of fungal species is higher in CDA as compared to SDA.

Table 4. Percentage occurrence of species in different dilutions of Czapek-Dox agar medium.

S.No.	Species	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
1.	*Alternaria* sp.	-	-	-	6.06	9.09
2.	*Aspergillus clavati*	15.21	11.62	16.21	-	-
3.	*Aspergillus fumigatus*	10.86	20.93	8.10	9.09	18.18
4.	*Aspergillus niger*	17.39	16.27	10.81	12.12	9.09
5.	*Aspergillus parasiticus*	8.69	13.95	13.51	3.03	-
6.	*Helminthosporium* sp.	-	-	8.10	3.03	9.09
7.	*Mortierella elongata*	21.73	6.97	5.40	21.21	-
8.	*Mucor circinelloides*	8.69	25.58	18.91	18.18	18.18
9.	*Penicillium* sp.	-	-	5.40	6.06	9.09
10.	*Pythium* sp.	-	-	2.70	9.09	-
11.	*Scopulariopsis* sp.	-	-	8.10	9.09	18.18
12.	*Ulocladium botrytis*	-		2.70	3.03	-
13.	unidentified sp.	17.39	4.65	-	-	9.09

Figure I: Showing representative of different genus/species of sugarcane mulch fungi in Sabouraud's agar and Czapek-Dox agar media.



Microbial Respiration and Biomass Carbon: Microbial respiration is one of the earliest and most frequently used indices of microbial activity in soil. The maximum respiration rate usually proceeds, by several days to weeks. Carbon-dioxide evolution in soil is an ultimate product of degradation of organic matter (Yuste *et al.,* 2007). From Table-5 the results indicate the values in terms of CO_2 evolution in sugarcane mulch that it showed a consistent decrease in carbon-dioxide evolution up to 25 days whereas after 25 days it increased from 0.0132 μgC^{-1} to 0.0180 μgCg^{-1} on 35th day of experiment. Temperature and moisture content is the main factors affecting microbial respiration (Guntinas, *et al.,* 2013). Tiquia, *et al.,* (2002) in their study concluded that mulching with fertilized (15.3 mg CO_2 kg^{-1} per day) or non- fertilized (17.2 mg CO_2 kg^{-1} per day) composted yard waste increased microbial respiration due to degradation of organic matter. Soil microorganisms play an important role in flow of carbon and the cycling of nutrients in terrestrial ecosystems. Soil biomass, a liable fraction of organic matter, is a major sink of nutrients like N, P and S. The microbial biomass accounts for only 1-3% of soil organic carbon but it is the eye of the needle through which all organic material that enters the soil must pass. During this process these materials are converted by microorganisms in order to generate energy and to produce new cellular metabolites to support their maintenance and growth (Martens 1995). Table - 6 showed the values of microbial biomass carbon and the value was 0.67±0.08 $gCm^{-2} d^{-1}$.

Table 5. Values of Microbial respiration (μg Cg^{-1}) of sugarcane mulch.

S.No.	5th DAY	10th DAY	15th DAY	20th DAY	25th DAY	30th DAY	35th DAY
1.	0.0180 μgCg^{-1}	0.0168 μgCg^{-1}	0.0160 μgCg^{-1}	0.0156 μgCg^{-1}	0.0132 μgCg^{-1}	0.0156 μgCg^{-1}	0.0180 μgCg^{-1}

Table 6. Microbial biomass carbon ($gCm^{-2}d^{-1}$) (All values are mean ±S.E for 10 observations each)

S. No.	Parameter	Mean±S.E
1.	Microbial biomass carbon ($gCm^{-2}d^{-1}$)	0.67±0.08

CONCLUSION:

In the present study mulch sample of sugarcane industry was studied for physico-chemical and microbiological analysis. From the results it was concluded that the sugarcane mulch is a good source of nutrients which could be added in soil to increase its fertility after an appropriate decomposition and also clearly indicates that the percentage occurrence of different mycoflora were high in Czapek-Dox agar in comparative to Sabouraud's agar medium. The density of mycoflora, microbial respiration and microbial biomass carbon were found to be regulated by many factors like moisture content, time duration, organic matter, pH, and temperature.

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