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RESEARCH ARTICLE

INCORPORATION OF PALM OIL FOR THE ENHANCEMENT OF BIOMASS AND POLYSACCHARIDES PRODUCTION THROUGH SUBMERGED FERMENTATION FROM LOCALLY ISOLATED *PLEUROTUS SP.* MYCELIUM.

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Abstract

Therapeutic properties of *Pleurotus* sp. mushroom are associated with the polysaccharides present in the mycelium cell wall and culture broth of submerged culture. Submerged fermentation of *Pleurotus* sp. mushroom mycelium was carried out through one-factor-at-a-time method for mycelial growth, extracellular (EPS) and intracellular (IPS) polysaccharide production. The mycelial growth, EPS and IPS concentration was high when sucrose was used as carbon source which produced 4.66 g/L of mycelium dry weight, 7.16 and 4.76 mg/ml of exopolysaccharide (EPS) and endopolysaccharide (IPS) concentration respectively. Manipulation of sucrose concentration up to 100 g/L resulted in an increasing of mycelium dry weight with 6.3 g/L and 7.9 mg/mL of EPS concentration. The effect of carbon/ nitrogen source ratio in the liquid medium to the biomass and polysaccharides production was also investigated. C/N ratio of 80:1 resulted in high IPS concentration (11.89 g/L). Crude palm oil (CPO) and palm kernel oil (PKO) were incorporated with sucrose to serve as carbon source in the medium at different ratio. Both CPO and PKO have enhanced the yield of biomass and polysaccharides. The highest biomass obtained was 47.9 g/L when the PKO:Sucrose ratio at 100:0. At PKO:Sucrose ratio 25:75, highest EPS concentration (5.39 g/L) was obtained whereas the highest IPS concentration (34.02 g/L) was obtained at PKO:Sucrose ratio at 30:70. Instead of enhancing the growth of *Pleurotus* sp. mycelium in liquid culture, the used of palm oil mill effluent (POME) residues as carbon source had reduced the biomass and polysaccharides production.

Introduction:-

Mushroom is known to be a fungal growth which is in the form of filaments called as hyphae that can only be seen through microscope; with time, these hyphae grow into long network that are known as mycelium [1,2]. Mushrooms

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produce spores that are spread by the fruiting body; this late may only survive for few days before it get rotten but the mycelium that produced it can survive for years, in fact hundreds of years. Mushroom cell walls are mostly made of chitin which is a form of carbohydrate that plays a crucial role in providing a structural support to plants and it is the same material that is found in the exoskeleton of insects [3]. Mushrooms have over 2000 species that are available and can be found in nature, the production of mushrooms has effectively increased compared to the last several decades and this is simply due to their applications which with the combination of modern technology results into an incredibly positive impact in so many aspects including long term food nutrition, health care, regeneration, environmental conservation, social, and economic changes worldwide [4,5,6].

Nowadays, mushrooms are being used for their unique flavor and nutritious importance but most importantly they have a global impact in medical, nutritional, and pharmaceutical purposes. It was found that the majority of the used polysaccharides are frequently originated from fungi especially mushrooms because they are known for their property of containing various biochemical and physiological activities such as immune-stimulating activities, antitumor activities, hypoglycemic activities and even as a tonic to promote longevity by improving the quality of life [7].

The production of mushrooms can be done in two different ways: traditional technique or modern techniques. It is necessary to keep in mind that the traditional technique is associated with a number of disadvantages including a relatively long period of cultivation and a variation in the product quality caused by the variation in growing conditions which cannot be avoided. However, the modern technique whereby mushrooms and their derivatives are produced by submerged fermentation under controlled conditions has clearly proved itself to be advantageous not only by giving a consistent product composition but also is much more faster [8,9,10].

Exopolysaccharides can be obtained from mushrooms as the secondary metabolites whereby their function, structure, as well as their physical properties are dependent on the components of the fermentation medium for growth. Thus, the fermentation medium conditions are a crucial factor that highly affects the amount of exopolysaccharide produced [5]. This research is consequently focused on the optimization of the submerged fermentation medium conditions by the use of one-factor-at-a-time method which involves changing one independent variable (such as nutrients, temperature, pH, etc.) while fixing others at a certain level, in order to produce simultaneously a higher mycelium biomass and polysaccharides.

Materials and methods:-

Microorganisms:-

Mushroom mycelium of *Pleurotus* sp. were maintained on potato dextrose agar (PDA), 30 g/L added with 2% yeast extract, stored at 4°C for a long term use. The mycelium from stock culture was placed in the center of PDA plate and incubated for 7 days prior to submerged liquid fermentation.

Submerged fermentation:-

The fermentation medium used in this experiment was Glucose Yeast Extract (GYE) medium composed of 30 g glucose, 3 g yeast extract, 0.5 g $Mg.SO_4.7H_2O$, 1 g KH_2PO_4 with an initial pH of 5.5 [11]. 10 plugs of mycelium were inoculated into each flask containing 50 mL of liquid medium (GYE), incubated at 28°C, 150 rpm for 7 days. Several parameters were investigated included the effect of carbon sources, nitrogen sources, manipulation of carbon source concentration, different initial pH medium, different C/N ratio, and incorporation of CPO, PKO and POME residue.

Effect of carbon sources:-

Various carbon sources such as glucose, lactose, sucrose, and fructose were used. Initially all carbon sources were screened at 30 g/L, after that carbon source concentration were varied from 20 to 100 g/L.

Effect of nitrogen sources:-

The effect of nitrogen sources was carried out by replacing yeast extract with other organic and inorganic nitrogen sources namely peptone, meat extract, urea, ammonium chloride, ammonium sulfate, and ammonium nitrate at 3 g/L.

Effect of initial pH medium:-
The initial pH medium were varied from 4 to 8.

Effect of carbon to nitrogen ratio (C/N ratio):-
The C/N ratio was manipulated at the ratio of 0, 2, 5, 10, 15, 20, 40, 60, 80 and 93 (control) in order to increase the biomass as well as the polysaccharide production.

Effect of crude palm oil (CPO):-
The composition of carbon sources were manipulated by combining sucrose with CPO while maintained the C/N ratio at 80:1

Effect of palm kernel oil (PKO):-
The composition of carbon sources were manipulated by combining sucrose with PKO while maintained the C/N ratio at 80:1.

Effect of palm oil mill effluent (POME) residues:-
The C/N ratio of sucrose and meat extract was maintained at 80:1 with the addition of POME residues at different concentration from 3 to 21 g/L.

Determination of biomass, EPS and IPS concentration
After 7 days of cultivation, cultured broth was filtered through filter paper (Whatman No.1) and wash with distilled water twice. Culture filtrate from fermentation broth was mixed with absolute ethanol 4:1 (v/v), stirred vigorously and kept overnight at 4°C [12]. Ethanol was removed using centrifugation (10 000 rpm, 10 min) and the precipitation of polysaccharide was suspended in distilled water (1 mL) and sample solution was subjected to Phenol sulfuric acid assay [13]. In this assay, the sample were slowly mixed with 2.5 mL of H_2SO_4 (96-97%) and 0.5 mL phenol reagent (4%). The mixtures were then left at room temperature for 5-10 min before read the absorbance at 490 nm [13]. D-glucose was used as a standard. Cultured mycelia (after filtration) were dried using oven at 60°C until constant weight for biomass. Dried mycelia were then used for IPS extraction using hot water extraction method. Briefly, 40 g of dried mycelia powder was extracted twice with distilled water (600 mL) at 100°C for 3 h in water bath. The culture filtrate were then subjected to phenol sulfuric acid assay as described above [12,13] for IPS concentration.

Results and Discussion:-

Effect of carbon sources:-
The use of different compounds in a medium such as different carbon sources, different nitrogen sources and different pH medium could create different culture conditions and thus resulting into different polysaccharides production of the growth mycelium. In order to examine the suitable carbon source that is required for a maximum production of mycelium dry weight and polysaccharides (EPS and IPS), a number of various carbon sources including glucose, sucrose, lactose and fructose were tested. The obtained result in Figure 1.0 shows that amongst four carbon sources tested, the mycelium dry weight was high in a medium containing glucose as carbon source with 7.0 g/L followed by lactose (6.3 g/L) and sucrose with 4.5 g/L. Fructose gave the lowest mycelium dry weight with only 2.53 g/L.

The effect of glucose and lactose on the growth of mycelium and EPS was not significantly different. However, the effect of sucrose and fructose on both mycelium dry weight and polysaccharides (EPS and IPS) were both significantly different at $p<0.05$ from each other and also different from the effect given by glucose and lactose. In this study, sucrose was found to be the suitable carbon source for high production of EPS and IPS with 7.2 and 4.8 mg/mL respectively. The obtained results was supported by previous research which also proved that sucrose is an efficient carbon source for the growth of mycelium and polysaccharides production [14,15]. It was also reported that sucrose, glucose and fructose to be the best three carbon sources for mycelium dry weight and polysaccharides production in submerged fermentation of Tuber sinense in shake flask cultivation [16]. Previous research by [17] reported that out of ten different carbon sources tested sucrose was suitable for high polysaccharide while lactose the most un-favorable carbon source for submerged fermentation of *Lentinus squarrosulus*.

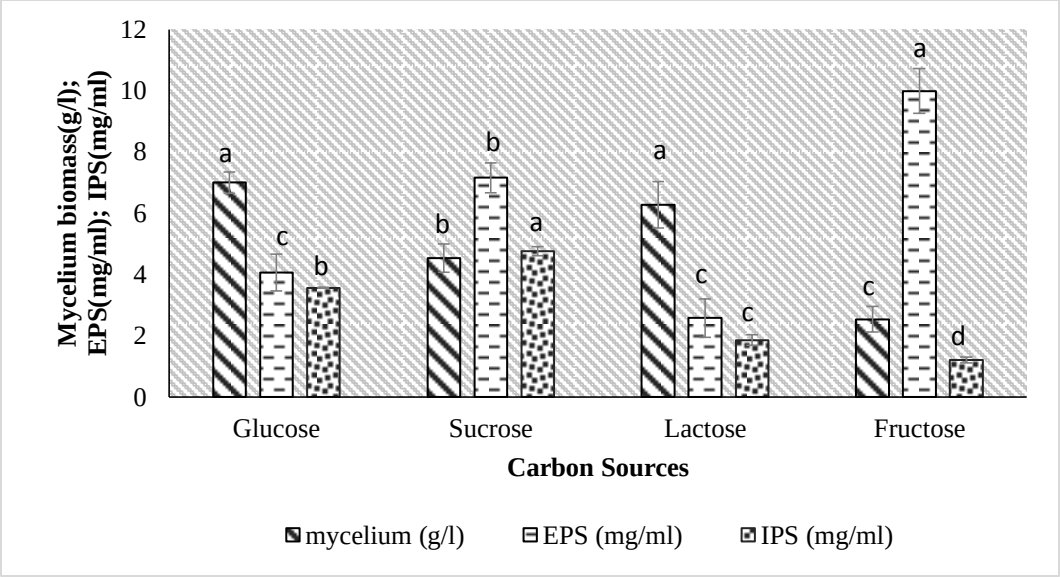

Figure 1.0:- Effect of carbon sources on mycelium dry weight, EPS and IPS production.

Fermentation was carried out for 7 days, at 28°C, carbon source concentration of 30 g/L, yeast extract as nitrogen source (3g/L) with initial pH of 5.5. Values are mean of ± standard deviation for three replicates. [abcd]Mean value with different superscript shows significance different at $p<0.05$.

Effect of sucrose concentration:-

The effect of sucrose concentration for mycelium dry weight and polysaccharide production were tested in different sucrose concentrations range from 20 to 100 g/L. As shown in Table 1.0, the mycelium dry weight increased as the concentration of sucrose increased. However, the production of EPS and IPS were fluctuated probably due to the different carbon concentration supplied in the medium. The highest mycelium dry weight (6.3 g/L) obtained when sucrose concentration reached 100 g/L. Sucrose concentration of 80 and 100 g/L exhibited significant results on the EPS concentration with 5.39 and 7.89 mg/L respectively. However, the effect given by the concentration of 50 and 60 g/L sucrose on the growth of mycelium and production of EPS were not significantly different. Previous research conducted by [15] also demonstrated that the increased of carbon source concentration greatly affect the increase of mycelium biomass as well as the production of polysaccharides.

Table 1.0:- Effect of sucrose concentration on mycelium dry weight, EPS and IPS production through submerged fermentation

Sucrose concentration (g/L)	Mycelium dry weight (g/L)	EPS concentration (mg/mL)	IPS concentration (mg/ml)
20	1.00 ± 0.70 d	2.68 ± 0.41 c	0.61 ± 0.11 b
40	3.33 ± 0.64 b	5.45 ± 0.25 b	1.69 ± 0.09 a
60	3.86 ± 0.50 b	4.65 ± 1.12 b	0.54 ± 0.1 b
80	6.08 ± 0.32 a	5.39 ± 0.14 b	1.04 ± 0.3 b
100	6.37 ± 0.59 a	7.89 ± 0.38 a	0.81 ± 0.05 b

Fermentation was carried out for 7 days, at 28°C, sucrose as carbon source, yeast extract as nitrogen source (3 g/L) with initial pH 5.5. Values are mean of ± standard deviation for three replicates. [abcd]Mean value with different superscript shows significance different at $p<0.05$.

Effect of nitrogen source:-

The effect of various organic and inorganic nitrogen sources also were investigated in this study. Table 2.0 shows that mycelium dry weight and polysaccharides production were remarkably higher when organic nitrogen sources were used as compared to inorganic nitrogen sources. In particular, the highest polysaccharides production was obtained from the used of meat extract as nitrogen source with 24.2 mg/mL concentration of EPS and 5.24 mg/mL

of IPS concentration. Although yeast extract exhibited the highest mycelium dry weight with 27.3 g/L, but the EPS (16.2 mg/mL) and IPS (3.76 mg/mL) concentration were relatively low as compared to meat extract.

It was proved here that the utilization of organic nitrogen source such as meat extract, yeast extract and peptone could enhance the growth of *Pleurotus* sp. mycelium and the production of EPS. This result was similar to previous study which stated that organic nitrogen sources were better than inorganic nitrogen sources for *Agaricus blazei* Murrill M21 in submerged fermentation [18]. Another previous research also has reported that the highest mycelial growth of *Antrodia cinnamomea* were obtained when organic nitrogen sources such as yeast extract and polypeptone were supplied in the medium [19].

For inorganic nitrogen sources, the highest mycelium dry weight obtained when ammonium sulphate (NH_4SO_4) was used as nitrogen source which gave 16.9 g/L of mycelium biomass. This is because the NH_4^+ ion played a central role in nitrogen metabolism as the form in which nitrogen incorporated into organic cell components or biomass [20]. However, both the EPS and IPS concentration were high in medium supplemented with $NaNO_3$ with 19.1 and 3.75 mg/mL concentration respectively. The obtained results suggested that yeast extract and NH_4SO_4 might contain the components necessary for mycelial growth whereby meat extract and $NaNO_3$ are more suitable for enhancing the production of polysaccharides. The high mycelium dry weight and polysaccharides production from inorganic nitrogen sources obtained from this study were contrast with most of previous study which reported that medium supplied with solely inorganic nitrogen sources enhanced poor growth of mycelial [21]. This result clearly showed the advantages of the isolated mycelium strain of *Pleurotus* sp. which could tolerate and utilized both organic and inorganic nitrogen sources thus resulting in high growth of mycelium and polysaccharides production.

Table 2.0:- Effect of various nitrogen sources on mycelium dry weight, EPS and IPS production

Nitrogen sources	Mycelium dry weight (g/L)	EPS concentration (mg/mL)	IPS concentration (mg/mL)
Meat extract	25.5 ± 1.1 a	24.1 ± 0.8 a	5.23 ± 0.07 a
Yeast extract	27.3 ± 2.1 a	16.2 ± 2.9 b	3.76 ± 1.19 b
Peptone	23.3 ± 2.3 b	22.9 ± 1.2 a	3.39 ± 0.59 b
NH_4NO_3	15.6 ± 2.8 c	13.4 ± 6.0 c	2.72 ± 0.71 c
NH_4SO_4	16.9 ± 1.7 b	14.5 ± 1.6 b	2.00 ± 1.18 c
NH_4Cl	15.2 ± 0.7 c	6.9 ± 2.2 d	0.77 ± 0.19 d
$NaNO_3$	12.5 ± 1.4 d	19.1 ± 3.6 c	3.75 ± 0.69 b
Urea	8.13 ± 1.3d	22.6 ± 2.3 a	1.55 ± 0.69 d

Fermentation was carried out for 7 days, at 28°C, sucrose as carbon source (100 g/L) with initial pH 5.5. Values are mean of ± standard deviation for three replicates. ^{abcd}Mean value with different superscript shows significance different at $p<0.05$.

Effect of initial pH medium:-

The initial pH value of medium also plays an important role in determining the biosynthetic potential of a production method as it may affect cell membrane function, uptake of various nutrients, cell morphology and structure, solubility of salt, ionic state of substrates, enzyme activity and product biosynthesis [22]. The pH value of this study only recorded at initial part of the fermentation process, thus it is only possible to examine the influence of initial pH on mycelial growth and metabolite production. By using results obtained in previous parameter where sucrose was used as carbon source with the concentration of 100 g/L, meat extract as nitrogen source (3 g/L), experiment was further carried out for different initial pH medium.

According to Table 3.0, results clearly showed that the highest mycelium dry weight obtained when initial pH of 5 was applied in the medium with 27.7 g/L followed by pH 5.5 with 23 g/L. Although the mycelium dry weight was high in pH 5, but the EPS and IPS were found to be in high concentration at pH 5.5 with 3.4 and 9 mg/mL respectively. The further increased of initial pH medium resulted in decreasing of mycelium dry weight, EPS as well as IPS concentration. Similar pH profiles were obtained with other previous study which cultivated *Ganoderma lucidum* in synthetic medium where highest yields were obtained with an initial pH of 5.5-6.5 for the production of ganoderic acid, pH 6.5 for biomass and pH 5.5-7.0 for IPS production [23]. Results obtained from this study can be supported by previous research carried out by [24] which reported that the optimum pH range for the growth of mushroom mycelium was 5.0 to 5.8.

Table 3.0:- Effect of initial pH values on mycelium growth and polysaccharides production

Initial pH value	Mycelium dry weight (g/L)	EPS concentration (mg/mL)	IPS concentration (mg/mL)
4	18.7 ± 1.2 c	1.98 ± 2.4 e	2.3 ± 0.5 e
5	27.7 ± 6.3 a	2.80 ± 2.4 b	5.0 ± 1.3 c
5.5	23.0 ± 4.0 b	3.40 ± 3.9 a	9.0 ± 0.7 a
6	19.5 ± 3.5 c	2.56 ± 2.4 c	6.8 ± 0.7 b
7	12.0 ± 1.4 d	2.32 ± 3.1 d	5.9 ± 0.19 c
8	11.2 ± 0.8 d	2.02 ± 1.7 d	4.87± 0.43 d

Fermentation was carried out for 7 days, at 28°C, sucrose as carbon source (100 g/L), meat extract as nitrogen source (3g/L). Values are mean of ± standard deviation for three replicates. [abcd]Mean value with different superscript shows significance different at $p<0.05$.

Effect of carbon to nitrogen ratio (C/N):-

Carbon and nitrogen source are important in all microorganisms for their growth. Concentration of both carbon and nitrogen sources and their balance in medium are very important for metabolites optimal production [25]. Based on the previous experiment of submerged fermentation of *Pleurotus* spp. mycelium, 100 g/L of carbon source (sucrose) and 3g/L of nitrogen source (meat extract) which is equivalent to C/N ratio of 93:1 provide the highest biomass and EPS production. As the C/N ratio is crucial for the growth of microorganism in submerged fermentation, the combined effect of carbon source (sucrose) and nitrogen source (meat extract) were investigated. The C/N ratio was manipulated at ratio of 0, 2, 5, 10, 15, 20, 40, 60, 80, and 93 (control) in order to increase the biomass as well as the polysaccharide production. The results of biomass production, extracellular polysaccharide (EPS) and intracellular polysaccharide (IPS) production of *Pleurotus* sp. at different C/N ratio are showed in Table 4.0.

Table 4.0:- Effect of different C/N ratio on the mycelium dry weight, EPS and IPS production from *Pleurotus* sp. mycelium.

C/N ratio	Mycelium dry weight (g/L)	EPS (mg/mL)	IPS (mg/mL)
0	1.80 ± 0.40^{f}	0.11 ± 0.13^{c}	0.87 ± 0.90^{c}
2	4.33 ± 2.00^{e}	0.15 ± 0.11^{c}	1.01 ± 0.08^{c}
5	4.47 ± 0.42^{e}	0.21 ± 0.13^{c}	1.16 ± 0.93^{c}
10	6.07 ± 1.94^{e}	0.37 ± 0.09^{c}	1.23 ± 0.13^{c}
15	5.67 ± 0.76^{e}	0.42 ± 0.19^{c}	2.02 ± 1.46^{c}
20	6.67 ± 0.64^{e}	0.91 ± 0.46^{c}	5.37 ± 0.81^{b}
40	9.60 ± 0.60^{d}	1.14 ± 0.98^{b}	8.68 ± 1.00^{a}
60	14.0 ± 0.80^{c}	2.71 ± 0.60^{a}	10.29 ± 0.81^{a}
80	20.07 ± 1.10^{b}	3.38 ± 1.21^{a}	11.89 ± 1.15^{a}
93	22.60 ± 1.20^{a}	3.03 ± 0.46^{a}	9.81 ± 0.38^{a}

Fermentation was carried out for 7 days, at 28°C, sucrose as carbon sources with respective ratio, meat extract as nitrogen source. Values are mean of ± standard deviation for three replicates. [abcd]Mean value with different superscript shows significance different at $p<0.05$.

It was clearly shown that as the C/N ratio increased, the mycelium dry weight also increased. Among the 10 different C/N ratios tested, the C/N ratio of 80:1 yielded the highest IPS and EPS production and the highest biomass production was achieved at C/N ratio of 93:1 (control) which is 22.6 g/L for 7 days of cultivation. However, the result is not significantly different from C/N ratio at 80:1 which also gave high biomass production at 20.1 g/L. C/N ratio at 80:1 yielded the highest amount of both EPS and IPS production at 3.38 and 11.9 g/L respectively which were higher than the EPS and IPS produced from *Pleurotus* sp. in medium of 93:1 C/N ratio (control). This result is comparable to research conducted by [26], which 30 g/L of biomass was obtained at day 14 of submerged cultivation of *Pleurotus ostreatus* with raffinose as carbon source and the production of EPS obtained were 3 mg/mL. Comparatively to this study, 3.38 mg/mL of EPS was successfully obtained from mycelium culture only after 7 days of cultivation which shows higher EPS production in shorter period of time.

Incorporation of CPO, PKO and POME residues as additional carbon sources:-

By maintaining the C/N ratio of 80:1 as obtained in the previous experiment (Table 5.0), the composition of carbon source was manipulated by combining sucrose with CPO, PKO and POME residues. Previous studies have reported on the utilization of several plant oils for the enhancement of mycelial growth and polysaccharides production [27,28,29,30]. To date, this is the first results reported on the stimulatory effect of palm oil and palm oil mill effluent (POME) residues for the enhancement of the mycelium biomass and the polysaccharide production by *Pleurotus* sp. mycelium. Palm oil is one of the commodity in Malaysia, thus it was selected because of the easy accessible for large scale production and also cost effective.

Based on the results obtained in Table 5.0, it was clearly shown that the incorporation of CPO had a remarkable effect on the mycelium dry weight as well as the polysaccharide production. At the ratio of CPO:Sucrose (30:70), the mycelium dry weight managed to increase from 19.6 g/L (ratio 80:1) to 25.1 g/L. Further increased of CPO:Sucrose ratio of 100:0 resulted in maximum mycelium dry weight with 30.8 g/L but the IPS was lowest with only 6.54 mg/mL as compared to the others ratio. Incorporation of CPO with sucrose at the ratio of 30:70 also resulted in highest IPS concentration with 28.7 g/L and this value was two times higher as compared with the ratio of 80:1 which was without additional of carbon source. The high EPS was also been produced at the ratio of 20:80 (CPO:Sucrose) with 3.45 mg/L which was also higher than C/N 80 which only gave 3.1 mg/mL of EPS. Overall, it was suggested that submerged fermentation of *Pleurotus* sp. mycelium incorporated with CPO was favorable in production of IPS compared to EPS as shown in Table 5.0.

Table 5.0:- Mycelium dry weight and polysaccharides production with the incorporation of CPO as additional carbon source.

CPO:Sucrose	Mycelium dry weight (g/L)	EPS (mg/mL)	IPS (mg/mL)
100:0	30.80 ± 2.1^{a}	1.05 ± 0.1^{d}	6.54 ± 1.40^{g}
75:25	19.07 ± 1.2^{c}	1.23 ± 0.1^{d}	7.74 ± 2.84^{g}
50:50	17.73 ± 0.6^{d}	1.53 ± 0.12^{c}	22.53 ± 3.10^{b}
30:70	25.07 ± 2.2^{b}	1.71 ± 0.24^{c}	28.67 ± 3.74^{a}
25:75	19.20 ± 1.0^{c}	2.87 ± 0.30^{b}	20.50 ± 3.02^{c}
20:80	16.13 ± 0.7^{d}	3.45 ± 0.29^{a}	16.72 ± 2.94^{d}
10:90	15.07 ± 2.5^{d}	3.41 ± 0.10^{a}	14.82 ± 1.20^{e}
0:100 (Control)	19.60 ± 1.6^{c}	3.10 ± 0.10^{a}	11.26 ± 2.27^{f}

Fermentation was carried out for 7 days, at 28°C, sucrose and CPO as carbon sources with respective ratio, meat extract as nitrogen source. Values are mean of ± standard deviation for three replicates. ^{abcd}Mean value with different superscript shows significance different at $p<0.05$.

The stimulatory effect of PKO as additional carbon source seems to give much higher mycelium dry weight as well as polysaccharides. According to Table 6.0, it was clearly showed that the mycelium dry weight increased with the gradually increased of PKO:Sucrose ratio. At the ratio of PKO:Sucrose (100:0), highest mycelium dry weight of 47.6 g/L was achieved, compared to PKO:Sucrose (0:100) as a control where only 22.93 g/L mycelium dry weight was obtained. The incorporation of PKO with sucrose at the ratio of 30:70 resulted in the increased of IPS concentration from 12.8 mg/mL (control) to 34.02 mg/mL. Highest EPS concentration was produced at the ratio of PKO:Sucrose (25:75) which was as high as 5.39 mg/mL as compared with control (3.1 mg/mL).

Table 6.0:- Mycelium dry weight and polysaccharides production with the incorporation of PKO as additional carbon source.

PKO / Sucrose	Mycelium dry weight (g/L)	EPS (mg/mL)	IPS (mg/mL)
100:0	47.60 ± 2.42^{a}	1.052 ± 0.16^{g}	13.67 ± 0.88^{e}
75:25	45.33 ± 1.60^{a}	2.097 ± 0.29^{f}	24.54 ± 0.63^{c}
50:50	42.33 ± 1.22^{b}	3.476 ± 0.66^{d}	28.78 ± 1.45^{b}
30:70	41.47 ± 1.86^{b}	4.989 ± 0.13^{b}	34.02 ± 1.38^{a}
25:75	40.13 ± 0.83^{b}	5.390 ± 0.48^{a}	24.55 ± 1.72^{c}
20:80	36.13 ± 1.72^{c}	4.413 ± 0.37^{c}	21.55 ± 1.98^{d}

10:90	30.93 ± 2.14^{d}	3.509 ± 0.25^{d}	20.96 ± 1.26^{d}
0:100 (Control)	22.93 ± 1.86^{e}	3.090 ± 0.39^{e}	12.81 ± 1.67^{e}

Fermentation was carried out for 7 days, at 28°C, sucrose and CPO as carbon sources with respective ratio, meat extract as nitrogen source. Values are mean of ± standard deviation for three replicates. [abcd]Mean value with different superscript shows significance different at $p<0.05$.

The increased of cell growth by PKO in this study might be caused by the partial incorporation of lipids in the fungal cell membrane, which facilitated the uptake of nutrient from the medium [31]. The pattern of this result was similar to the previous experiment (Table 5.0) with CPO, where the mycelium dry weight increased proportionally with the ratio of oil to sucrose, and the best polysaccharide production were obtained when the ratio of oil to sucrose were in the range of 30:70 to 20:80. This result is comparable to the previous research conducted by [28] who investigated the effect of oils on the production of EPS and biomass in submerged culture of *Schizophyllum commune*. Based on the result obtained, additional of castor and olive oils have stimulated the cell growth of *Schizophyllum commune* and the concentration of 0.5% produced high EPS [28]. Previous research also reported that various different media supplemented with coconut, cotton, groundnut, butterfat, palm kernel and palm oil had significantly affect the mycelial growth of *Psathyrella atroumbonata* and *Lentinus squarrosulus* [27]. It is also stated that oil which has the function of an antifoam agent in fermentation has been favourable to mycelial growth in several medicinal mushrooms and to increase the production of bioactive metabolites [31,32,33]. The stimulation of mycelial growth by oil might be caused by the partial incorporation of lipids in the cell membrane, which facilitated the uptake of nutrients from the medium [31].

Experiment was further carried out by testing different concentration of POME residues (Table 7.0). POME residues were obtained after separation of POME water using specific formularized chemicals (unpublished data). In this study, the C/N ratio of sucrose and meat extract were maintained at 80:1 with the addition of POME residues at different concentration range from 3 to 21 g/L. Control was prepared according to previous study, C/N ratio at 80, without any additional carbon source. As compare with control (19. 87 g/L), the mycelium dry weight obtained were lower at all concentrations of POME. This indicates that although POME was added in the medium as additional carbon source, instead of enhancing the mycelial growth, it reduced the growth of *Pleurotus* spp. in submerged fermentation. This is probably due to the toxicity and acidic properties of POME. According to [33], POME has a pH value of approximately 4.84 in general and the acidity of POME is due to the presence of volatile fatty acids such as acetic acid, propionic acid. Besides, POME has a general characteristic which will harm the living organisms [34]. The addition of POME had also decreased the production of EPS and IPS in the submerged fermentation. The highest EPS concentration (2.52 mg/mL) obtained was at POME concentration of 15 g/L, however it was still lower than control (2.84 mg/mL). For IPS production, the highest concentration was achieved at 18 g/L of POME concentration, which was 14.57 mg/mL of IPS. This result was slightly higher than the control (14.53 mg/mL) even though 18 g/L of POME has been added into the medium as additional carbon source. Hence, it can be concluded that addition of POME do not cause significant effect on the mycelium growth of *Pleurotus* spp. as well as the polysaccharides production in submerged fermentation.

Table 7.0:- Mycelium dry weight and polysaccharides production with the incorporation of POME as additional carbon source.

POME (g/L)	Mycelium dry weight (g/L)	EPS (mg/ml)	IPS (mg/ml)
Control	19.87 ± 0.90^{a}	2.84 ± 0.66^{a}	14.53 ± 2.31^{a}
3	13.60 ± 0.71^{c}	2.29 ± 0.26^{a}	8.10 ± 0.88^{c}
6	13.40 ± 0.92^{c}	2.44 ± 0.05^{b}	7.14 ± 0.12^{c}
9	14.20 ± 0.72^{c}	1.57 ± 0.41^{c}	7.99 ± 0.92^{c}
12	15.73 ± 0.31^{b}	1.94 ± 0.10^{d}	10.25 ± 0.64^{b}
15	15.33 ± 0.95^{b}	2.52 ± 0.37^{a}	13.28 ± 2.15^{a}
18	15.1 ± 1.45^{b}	1.74 ± 0.30^{e}	14.57 ± 2.38^{a}
21	13.27 ± 1.51^{b}	1.49 ± 0.43^{e}	13.78 ± 1.30^{a}

Fermentation was carried out for 7 days, at 28°C, sucrose and POME as carbon sources with respective concentration, meat extract as nitrogen source. Values are mean of ± standard deviation for three replicates. [abcd]Mean value with different superscript shows significance different at $p<0.05$.

Conclusion:-

In conclusion, the production of biomass, EPS and IPS of *Pleurotus* sp. mycelium in submerged fermentation was successfully enhanced by incorporation of CPO and PKO as alternative carbon source. C/N ratio of 80:1 resulted in the highest EPS and IPS production (3.4 g/L and 11.9 g/L respectively). Incorporation of CPO has successfully increased the biomass, IPS and EPS production. PKO has further increased the growth and polysaccharides production of *Pleurotus* sp. mycelium in submerged fermentation. Instead of enhancing the growth, addition of POME has decreased the mycelium growth and its polysaccharides production**.**

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