World Applied Sciences Journal 14 (10): 1457-1468, 2011
ISSN 1818-4952

Enhanced Production of Mnp Enzyme Produced by *Pleurotus sajor-Caju* Exposed to Gamma Radiation

[1]M.A.M. Abo-State, [2]M. Othman, [2]O. Khatab and [2]E.A. Abd-ElFattah

[1]National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt
[2]Faculty of Science, Helwan University, Helwan, Egypt

Abstract: Production of MnP enzymes on different media by different white rot fungi (WRF) has been investigated. *P. ostreatus*, *P. sajor-caju*, *P. chrysosporium* 34541 and *P. chrysosporium* 24725 were used for studying their abilities to produce MnP by different submerged liquid media (I,II,III). The highest MnP (0.720 U/ml) in general have been produced by *P. sajor- caju* on medium (I) and assayed by ABTS. MnP produced and assayed by ABTS was higher than that assayed by DMP (0.720 and 0.550 U/ml) respectively. The results revealed that, MnP produced by *P. sajor-caju* on both medium (I) and medium (II) supplemented with 1000 µM $MnSO_4$ was (0.741 and 0.576 U/ml) respectively. However, MnP produced by *P. sajor-caju* on medium (I) and assayed by Mcllavine buffer (pH 5.0) proved that it was the best organic acid (0.918 U/ml) than both acetate (0.597U/ml) and citrate buffers. But MnP produced on medium (II) and assayed by different buffers proved that acetate was the best buffer (2.422 U/ml). Also, as the inoculum size increased, the MnP activities increased. So, the highest MnP (1.648 U/ml) produced by 4 discs of *P. sajor-caju* incubated at 30°C for 3 weeks incubation period. On the other hand a gradually decrease in growth of *P. sajor-caju* was observed as a result of increasing in gamma radiation doses. However, gamma irradiation by different doses enhanced MnP production on medium (II). *P. sajor-caju* exposed to 0.75 and 1.0 kGy produced MnP 4.5 times that of the parent strain.

Key words: White-rot fungi (WRF) · MnP · Media · $MnSO_4$ · Gamma radiation

INTRODUCTION

Lignin is a heterogeneous and irregular arrangement of phenyl-propanoid polymer that resists chemical or enzymatic degradation to product cellulose. Due to the lignin complexity pre-treatment is often physico-chemical but in nature to degrade lignocellulosic biologically with enzyme it include lignin- degrading peroxidases and hemicellulase [1]. Basidiomycete's species are considered to be a very interesting group of fungi given their exceptional adjustment abilities to accommodate detrimental conditions of the environment where they continue to act as natural lignocellulose destroyers and include very different ecological groups such as white rot, brown rot and leaf litter fungi [2]. The enzymes responsible for lignin degradation are mainly: lignin peroxidase (LiP), manganese peroxidase (MnP) and a copper containing phenoloxidase, known as laccase [3]. The best studied white rot fungus *Phanerochate chrysosporium* secretes two extracellular heme peroxidases which along with an H_2O_2-generating system are apparently the major components of its lignin degradation system [4, 5]. Some wood-rotting fungi having high lignolytic activities are *Pleurotus pulmonaris, Pleurotus sapidus*, *Phanerochate chrysosporium* [6)], *Phlebia radiate* [7] and *Phlebia tremellosa* [8].

Phanerochate chrysosporium is a filamentous fungus when grown in agaited liquid culture typically forms pellets [9] spore aggregation immediately before and during germination [10]. Agitation of *Phanerochate chrysosporium* cultures in the formation of mycelial pellets greatly suppresses lignin degradation and more specifically the production of LiP [11]. Fungi of the *Pleurotus* genus have an important place among the commercially employed basidiomycetes because they have gastronomic, nutritional and medicinal on a large range of substrate. The genus *Pleurotus* has also been studied by several authors with the most varied objectives including the production of liquid inoculum

Corresponding Author: M.A.M. Abo-State, Department of Microbiology,
National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt

[12], extracellular enzymes [13], flavoring agents [14], β-glucosidases [15], antimicrobials [16, 17] and vitamins [18]. A studies were carried by using the spawns *Pleurotus ostreatus* and *P. sajor-caju* where inoculated on different agricultural wastes including viticulture wastes, wheat straw, paddy straw, sesame straw, sawdust as well as the mixture of theses wastes with wheat bran The carbon and nitrogen content of substrates containing bran were the highest. *P. ostreatus* and *P. sajor-caju* grown on substrates containing wheat bran had higher biological efficiency and total yields as well as higher (CMCase) and laccase activities [19]. Manganese peroxidase (MnP) oxides Mn^{2+} to Mn^{3+}, which oxides phenolic structures to phenoxyl radicals [20]. The product Mn^{3+} is highly reactive and complex with chelating organic acid, as oxalate or malate, which is produced by the fungus [21-23]. MnP is an H_2O_2-dependent heme glycoprotein of Mwt 46.000 which like LiP exists a series of isozymes [4, 24]. Purification of Mn-peroxidase revealed the existence of two forms: MnP1 (molecular mass 43 *KDa*, *PI* 4.5) and MnP2 (42*KDa*, *PI* 3.8) [25]. MnP have been identified in the fungus *Phanerochaete chrysosporium* [26, 27], which are regulated in response to nutrient limitation, heat shock, concentration of MnP (^{22}) and hydrogen peroxidase [27-30]. The appearance of 2 manganese peroxidase (MnP) activity in nitrogen-limited cultures of *P. chrysosporium* is dependent on the presence of manganese. Cultures grown in the absence of Mn developed normally and produced normal levels of the secondary metabolite, veratryl alcohol but produced no MnP activity [31]. Thus $MnSO_4$ or $MnCl_2$ stimulated the production of Mn peroxidase and up to 24 times higher levels of the enzyme were achieved in cultures [32]. Gamma radiation causes a variety of damage to DNA in cells [33]. The vitro experiments show that high light energy transfer radiation (HLET) is more effective in inducing double strand breaks damages on the DNA than low light transfer (LLET) and higher densities of free-radicals are more effective in producing sever damage on DNA molecules [34,35]. The majority of all types of base substitutions occurred on G and C bases in other studies [36, 37].

Knowledge of microorganisms having ligninolytic activity is important to understanding of plant biomass recycling in nature, but also to the use of enzymes in biotreatment of large number of organic pollutants involving (PAHs, chlorophenols, dyes, etc.). So the increasing demand for these enzymes has intensified the search for micro- organisms having high level of enzyme activities and for improved fermentation. The aim of this study is the production of manganese peroxidase enzyme(s) in high levels to facing the increased demand on this enzyme in the field of biotechnology treatment of the environment.

MATERIALS AND METHODS

Microorganisms: Four white rot fungi (WRF) were used in this study. *Pleurotus ostreatus* CBS 411.71, *Pleurotus sajor-caju, Phanerocheate chrysosporium* BKM-F1767 (ATCC 24725) and *Phanerocheate chrysosporium* American Type Culture Collection (ATCC 34541).

Maintainance of WRF: The four WRF stains were maintained and stored on PDA media (potato dextrose agar) [38] plates and slants. The slants were kept at 4°C till need. These strains were cultivated on malt agar medium [39] and incubated at 30°C for 7 days to be used for inoculation.

Production of Mnp by Different Wrf on Different Media: Two mycelia discs (10 mm diameter) were transferred to 250 ml conical flask containing 50 ml of the production media, Medium (I) Basal medium [39], Medium (II), N-limited medium [40] and Medium (III), Defined medium [41] supplemented with 2000μM $MnSO_4$ (product of Fluka, Switzerland) and incubated at 30°C for 14 days with shaking (150rpm).Three replicates were used for each strain in each medium. At the end of incubation period, MnP activity was determined.

MnP Assay: According to Field *et al.* [42] and Garzillo *et al.* [43], MnP activity was carried out in 1 cm quartz cuvette. The reaction mixture of 1 ml contained 1 μM of 2, 6 dimethoxy phenol (DMP) (product of Merck, Germany) or 2 μM of 2, 2′-azino- bis (3-ethylbenzo-thiazoline-6-sulphonic acid (ABTS) (product of Sigma-Aldrich, USA) and 1 μM $MnSO_4$ in Mcllavine buffer (pH 5.0). To assay mixture 100μM of centrifuged (8000rpm, 15 min) extracellular fluid was added. The peroxidase activity was then initiated by the addition of H_2O_2 (product of Fluka, Switzerland) to 0.04 mM level. The enzymatic activity was estimated in IU by monitoring the absorbance changes at 469nm (DMP, î 469=27.5$mM^{-1}cm^{-1}$) or 420nm (ABTS, î 420=36$mM^{-1}cm^{-1}$) by spectrophotometer (LW-V-200-RS-UV/VIS, Germany) at 30°C.

Production of Mnp on Different Medium with Different Concentrations of Manganese Sulphate: Two discs (10 mm diameter) of WRF grown on malt agar media used

to inoculate medium (I) and medium (II) supplemented with different concentrations of $MnSO_4$ (zero, 100, 500, 1000, 2000, 3000, 4000 or 5000 μM). Three replicates were used for each concentration. At the end of incubation period (7 and 14 days), MnP activity and extracellular protein were determined.

Protein Determination: To determine the amount of soluble protein in fungal filtrate (supernatant) of any culture was determined according to Lowry *et al.* [44]. The absorbance was determined at 720 nm. To determine the concentration of protein in the samples, a standard curve of bovine serum albumin (BSA) product of Sigma, USA was measured.

Effect of Incubation Period on MnP Production: The four WRF were used to inoculate media (I and II) supplemented with appropriate concentration of $MnSO_4$ for different incubation periods (7, 14, 21, 28 days). At the end of each incubation period MnP was determined.

Effect of Different Buffers on MnP Activity: *Pleurotus sajor-caju* was used to inoculate media (I and II). The culture media was supplemented with 1000 μM $MnSO_4$. The culture was incubated for 14 days at 30°C in shaking (150 rpm) incubator. Three buffers were used for MnP assay at pH 5.0.

Optimization of MnP Production *Pleurotus sajor- caju*: was used in a number of trials to optimize MnP production. In this serial of experiments, different inoculums size of medium (I) supplemented with 1000μM $MnSO_4$ in combination with different incubation temperature for different incubation periods.

Effect of Gamma Radiation on *Pleurotus Sajor-Caju* Growth: *Pleurotus sajor - caju* grown on malt agar plates for 7 days were exposed to different doses of gamma radiation (^{60} CO, Indian chamber located at National Center for Radiation Research and Technology, Nasr city, Cairo, Egypt). The doses were (0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0 kGy). The dose rate was 1 kGy/12.5 minutes. Three plates were used for each dose. One disc (10 mm diameter) from each plate was placed on the center of malt agar plates. Three replicates were used for each dose. The plates were incubated at 30°C for 6 days. The growth diameter of each disc was recorded.

Effect of Gamma Radiation on Mnp Production by *Pleurotus Sajor-Caju:* Two discs from each plate of each dose were used to inoculate 50 ml of media (I) and (II). The medium were supplemented with 1000 μM $MnSO_4$. Three replicates were used for each dose to determine MnP activity. The flasks were incubated at 30 °C for 14 days in shaking (150 rpm) incubator.

RESULTS AND DISCUSSION

Production of MnP on Different Media: Four white rot fungi, *P. ostreatus, P. sajor-caju, P. chrysosporium* 34541 and *P. chrysosporium* 24725 were used for studying their abilities to produce MnP by different submerged liquid media (I, II, III). High production of MnP by the two *Pleurotus* species were in the following order medium (I) > medium (II) > medium (III). The highest MnP (0.374 U/ml) production was recorded on medium (II) by *P. chrysosporium* 24725 and assayed by DMP as in indicated in Table 1. Production of MnP on different media by the four different WRF and assayed by ABTS have been indicated Fig. 1. The results revealed that MnP produced on the three different media and assayed by ABTS was more than that assayed by DMP. The highest MnP (0.720 U/ml) had been recorded on medium (I) by *P. sajor-caju. P. sajor-caju* produced MnP in the following order medium I>II>III as indicated in Fig.1. Also *P. chrysosporium* 24725 produced more Mn on medium I, II and III than that produced by *P. chrysosporium* 34541.

Media composition had been played a great role in MnP production. Many studies proved that both the nature and concentration of nitrogen sources are powerful nutrition factors regulating MnP production by WRF [45-46]. In *Pleurotus* spp, the best nitrogen sources for MnP production were peptone in concentration of 0.5% and NH_4 NO_3 with nitrogen concentration of 30 mM, respectively [47]. Amino acid L-glutamate plays a great role in regulating of ligninolytic activities [48, 49]. On the other hand L-phenylalanine, L-tyrosine, L-lysine and L-leucine had a negative effect on MnP production by these amino acids [50, 51]. Carbon sources play also, another role in ligninolytic enzymes production. Previous studies of the physiology of lignin degradation in defined liquid media have shown that *P. chrysosporium* dose not degrade lignin during its primary growth phase. If growth is prevented by exhaustion of extracellular carbon, nitrogen or sulfur sources, thus fungus will degrade lignin as long as it has accessory energy source

Table 1: Production of MnP on different media by different white rot fungi (WRF) assayed by DMP.

White rot fungi	Media(III)	Media(II)	Media(I)
P. ostreatus	0.275	0.220	0.236
P. sajor-caju	0.550	0.275	0.453
P. chrysosporium	0.283	0.228	0.200
P. chrysosporium	0.330	0.264	0.374

Table 2: Effect of different concentrations of $MnSO_4$ on MnP production by *P. sajor-caju* after 1 week.

$MnSO_4$(µM)	Media (I)	Media (II)
Zero	0.036	0.018
100	0.180	0.082
150	0.324	0.082
500	0.252	0.082
1000	0.741	0.576
2000	0.504	0.216
3000	0.118	0.216

Table 3: Effect of different buffers of MnP assay produced by *P. sajor-caju.*

	Buffers		
Media	Mcllavine	Acetate	Citrate
Media (I)	0.918	0.597	0.597
Media(II)	0.360	2.422	0.309

Table 4: Effect of different doses of gamma radiation on MnP production by *P. sajor-caju.*

Doses (kGy)	Media (I)	Media (II)
Control	0.270	0.036
0.25	0.345	0.090
0.50	0.273	0.054
0.75	0.190	0.165
1.0	0.129	0.165
1.5	0.126	0.093
2.0	0.090	0.093
3.0	0.036	0.144
4.0	-	-

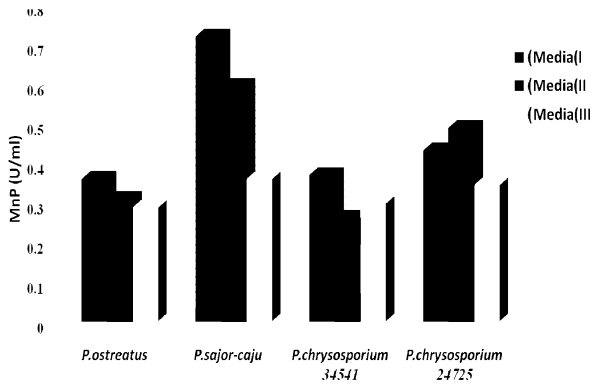

Fig. 1: Production of MnP on different media by different WRF assayed by ABTS.

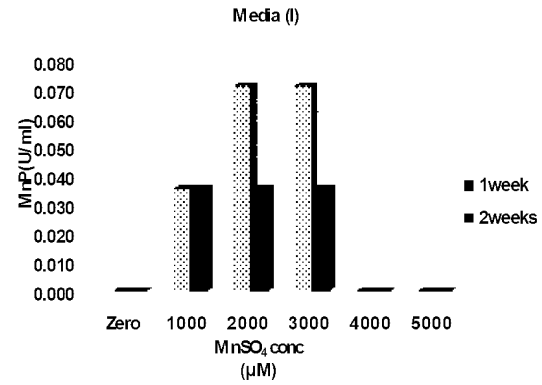

Fig. 2: Effect of different concentrations of MnSO4 on MnP production by P. ostreatus on Media (I).

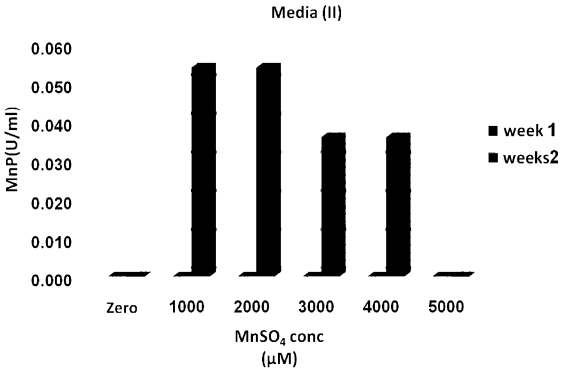

Fig. 3: Effect of different concentrations of MnSO4 on MnP production by P. ostreatus on Medium(II)

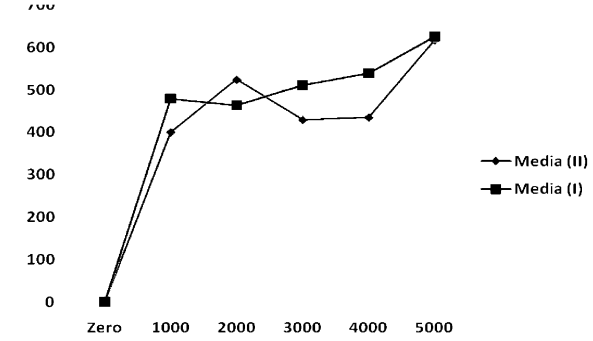

Fig. 4: Production of protein on different concentrations of MnSO4 by P. ostreatus

[11, 48, 52, 53, 54]. MnP does not oxidize veratryl alcohol, ABTS and 2, 6 DMP in the absence of Mn^{2+}. MnP is essentially strictly requiring Mn^{2+} as a substrate [54]. The MnP was able to oxidize different phenolic (DMP and Quaiacol) and non phenolic (ABTS) substrates in the presence of Mn^{2+} [56]. Oxygen tension has a stimulating effect on lignin degradation and enzymatic production by *P. chrysosporium* [11, 57, 58]. The highest MnP titers

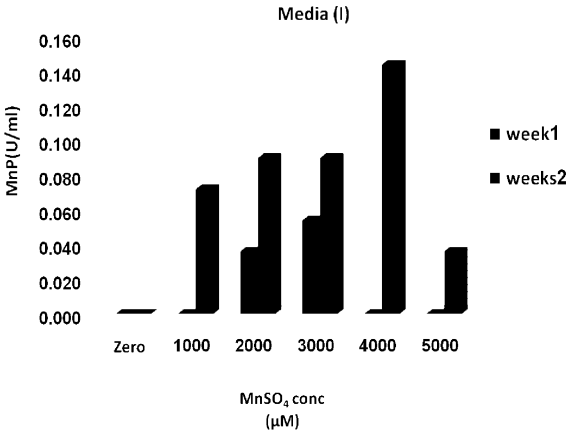

Fig 5: Effect of different concentrations of MnSO4 on MnP production by P. chrysosporium 34541 on Medium(I).

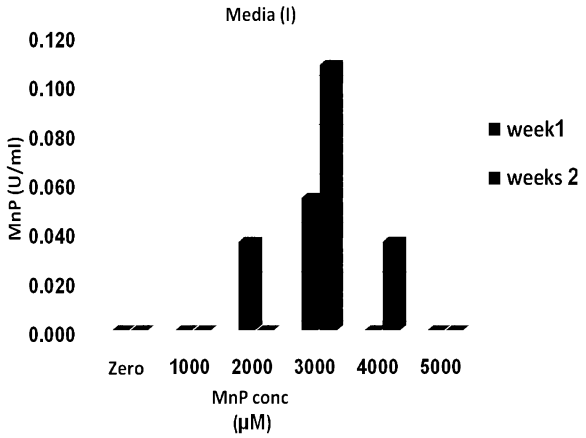

Fig 8: Effect of different concentrations of MnSO4 on MnP production by P. chrysosporium 24725 on Medium (I).

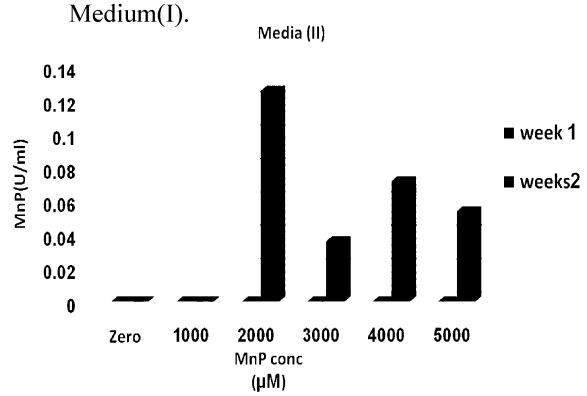

Fig 6: Effect of different concentrations of MnSO4 on MnP production by P. chrysosporium

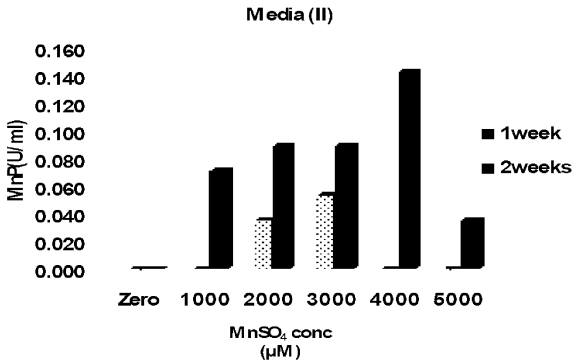

Fig 9: Effect of different concentrations of MnSO4 on MnP production by P. chrysosporium 24725 on Media (II).

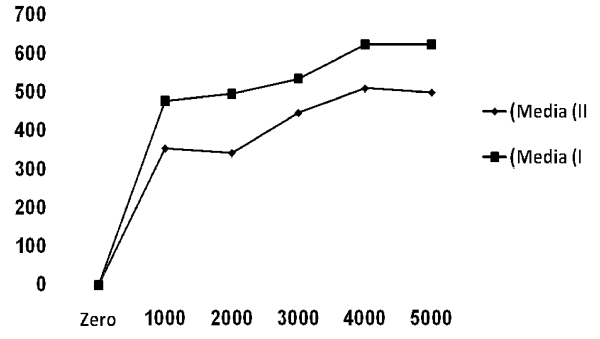

Fig. 7: Production of protein on different concentrations of MnSO4 by P. chrysosporium 34541

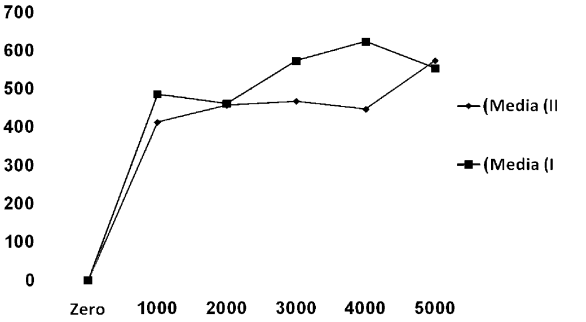

Fig. 10: Production of protein on different concentrations of MnSO4 by P. chrysosporium 24725.

obtained in an oxygen environment are not due only to the increase in MnP production by the stimulation of *mnp* gene. With increasing shaking speed the activity of the enzyme increased up to 60 rpm and then decreased. Shaking increase substrate mass transfer and oxygen transfer rates [60-62].

Effect of $MnSO_4$ Concentration on MnP Production: Effect of $MnSO_4$ concentrations on MnP production was investigated as shown in Figs 2 and 3. The results revealed that *P. ostreatus* produced the highest MnP (0.072 U/ ml) after one week at 2000 and 3000 µM $MnSO_4$ on medium (I). However, as the incubation period

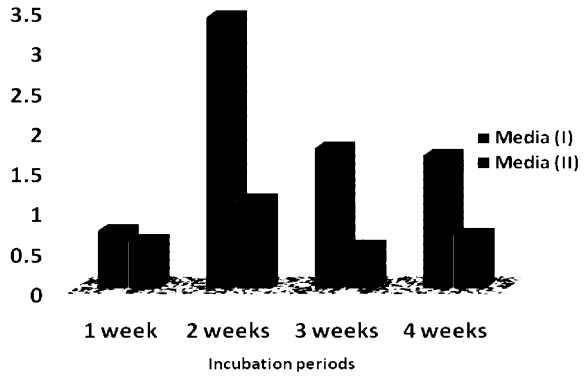

Fig 11: Effect of incubations periods on MnP production by P. sajor- caju.

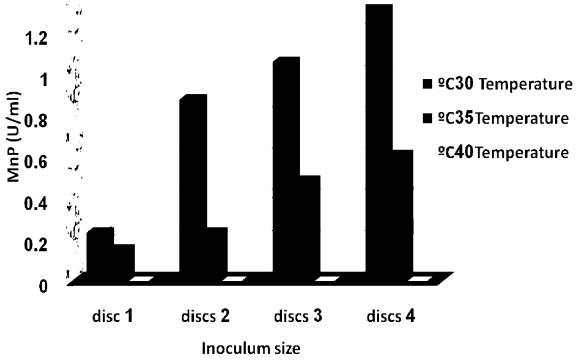

Fig 12: Effect of temperature and inoculum sizes on MnP production by P. sajor-caju after one week incubation

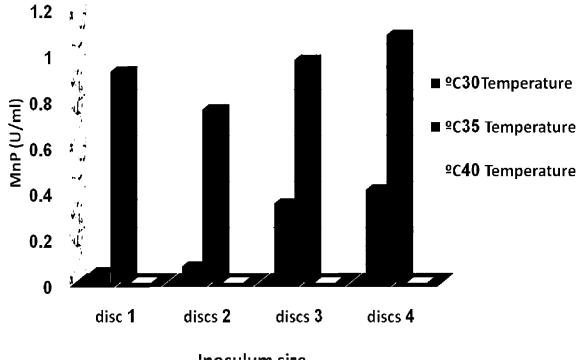

Fig. 13: Effect of temperature and inoculum sizes on MnP production by P. sajor-caju after two weeks

increased (2 weeks) MnP (0.036 U/ml) decreased. The highest concentrations could not produce MnP after 1 or 2 weeks incubation on medium (I). Without $MnSO_4$ (zero), *P. ostreatus* could not produce MnP also as indicated in Fig. 2. On the other hand,

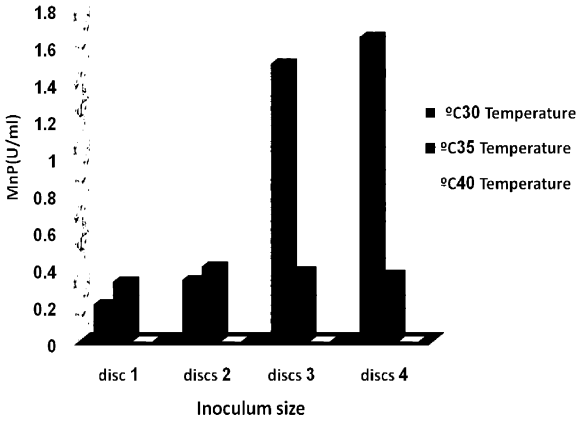

Fig. 14: Effect of temperature and inoculum sizes on MnP production by P. sajor-caju after three weeks incubation.

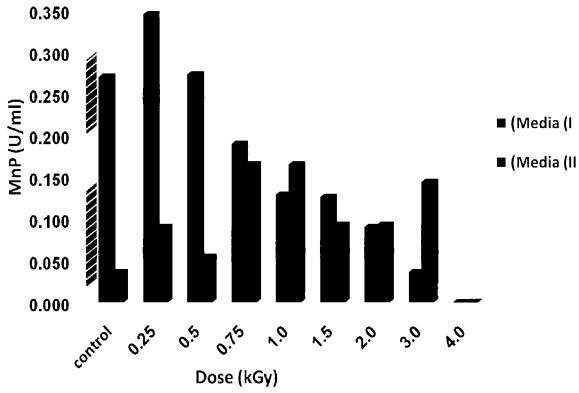

Fig. 15: Effect of different doses of gamma radiation on MnP production by P. sajor- caju

as the concentrations of $MnSO_4$ increased the protein secreted by *P. ostreatus* increased as indicated in Figure (4). This might be explained on the bases that, $MnSO_4$ induced the production of MnP and other enzymes, at the higher concentrations, the other enzymes were predominant than MnP. In case of medium (II), *P. ostreatus* produced the highest MnP (0.054 U/ml) at 1000 and 2000µM $MnSO_4$ after 2 weeks. At higher concentrations (3000 and 4000 µM) as indicated in Figure (3), *P. ostreatus* produced less MnP (0.036 U/ml). As the concentration increased more (5000 µM), no MnP had been detected. However, as the concentrations of $MnSO_4$ increased, extracellular protein increased to reach its maximum protein secretion (617 µg /ml) at 5000µM $MnSO_4$ as indicated in Fig.4.

Figures 5 and 6 indicated the effect of $MnSO_4$ concentrations on MnP produced by *P. chrysosporium* 34541. No MnP have been detected after 1 week at zero

and 1000 μM $MnSO_4$ but as the concentration increased (2000 μM), MnP was produced (0.036 U/ml), with more increase in $MnSO_4$ (3000 μM), MnP gave the highest production (0.054 U/ml). As the incubation period increased (2 weeks) and concentrations increased, MnP production increased to reach the maximum productivity (0.144 U/ml) at 4000 μM $MnSO_4$. With increasing $MnSO_4$ more (5000 μM) MnP began to decrease (0.036 U/ml). The results in Fig. 6 revealed that *P. chrysosporium* 34541 could not produce any MnP after one week at any concentration on medium (II). As the incubation period increased (2 weeks), MnP production has been detected from 2000 μM $MnSO_4$ till 5000 μM $MnSO_4$. The highest productivity of MnP was recorded at 2000 μM (0.126 U/ml). Also, as the concentration of $MnSO_4$ increased, the extracellular protein secretion increased as indicated from Fig. 7. Effect of different $MnSO_4$ concentrations on MnP production by *P. chrysosporium* 24725 on medium I and II have been indicated in Figures (8-9). The results revealed that no MnP have been detected on medium I at low $MnSO_4$ concentration (zero, 1000μM) and high concentration (4000, 5000 μM) after one week. At 2000 and 3000 μM *P. chrysosporium*24725 produced 0.036 and 0.054 U/ ml, respectively after 1 week. The highest MnP production (0.109 U/ ml) have been recorded at 3000 μM $MnSO_4$ after 2 weeks incubation. In case of medium II. *P. chrysosporium* 24725 produced the highest MnP (0.144 U/ml) after 2 weeks incubation on 4000 μM $MnSO_4$ as indicated in Fig. 9. Also, as the concentration of $MnSO_4$ increased, extracellular protein secreted increased as in Fig.10. Effect of different concentrations of $MnSO_4$ on MnP production by *P. sajor-caju* on different media has been indicated in Table 2. The results revealed that, the highest MnP produced by *P. sajor-caju* on both medium (I) and medium (II) had been recorded at concentration 1000 μM $MnSO_4$. The highest MnP was (0.741 and 0.576 U/ml), respectively. As, the concentration increased more, MnP decreased on both media. Mn (II) has been reported to be essential for the induction of MnP production in many white rot fungi [29, 32, 63].

Culture grown in the absence of Mn developed normally and produced normal levels of the secondary metabolite, veratryl alcohol but produced no MnP activity. The results indicate that Mn, the substrate of the enzyme, is involved in the transcriptional regulation of the MnP gene (31). Mn^{2+} affects *mnp*3 expression even 2 h after its addition to the cultures, suggesting a direct effect of the metal ion on expression [64]. In the absence of Mn (II) concentrations, extracellular LiP isoenzymes predominated whereas in the presence of Mn (II), MnP isoenzymes were dominant [32].

Effect of Incubation Period on MnP Induction: Figure 11 showed the highest MnP (3.384 U/ml) have been recorded at 2 weeks incubation on medium (I). Also, the highest MnP (1. 101 U/ml) on medium (II) was found at 2 weeks incubation period. As the incubation periods increased more, MnP decreased on both media. It has revealed that number of days from complete mycelial colonization to primordial formation of *Pleurotus* spp. number of days from opening the bags to first harvest, number of fruiting bodies per bag, average weight of individual fruiting bodies, total yield of fruiting bodies. The incubation time ranged from 30-41 days depending upon substrate, sawdust recorded the shortest incubation period being 29,30 and 32 days. On the other hand, sugar cane bagasse had the longest incubation period 41, 37 and 39 days which significantly differed from other substrates [65]. However it was observed that under 100% O_2 till 2h incubation in the presence of Mn resulted in a further increase in MnP activity on days 3 and 10 that resulted in substantially more *mnp* mRNA, a time when oxalate oxidation is most active and glyoxylate concentrations are highest [30].

Effect of Different Buffers on MnP Assay: Studying the effect of different buffers on MnP assay had been shown in Table (3). The results revealed that the best organic buffers used for MnP assay was Mcllavine buffer. MnP produced by *P. sajor-caju* and assayed by Mcllavine buffer (pH 5.0) was 0.918 U/ml on medium I. Medium (II) gave the highest MnP(2.422 U/ml) activity when assayed by acetate buffer, but when assayed by Mcllavine or citrate, MnP activities (0.360 and 0.309 U/ml) respectively which were less than that produced by medium (I). This indicated the important role played by the organic buffers in MnP activity determination. Oxalate serves as reducing agent of iron [66] and is capable of mediating oxidation of Mn^{2+} to Mn^{3+} via lignin peroxidase and veratryl alcohol [67]. Oxalate can support MnP catalyzed oxidations in absence of exogenous H_2O_2 and in the presence of dioxygen [68]. Lactate buffer helped to oxidize Mn^{2+} to Mn^{3+} [69, 70]. Sodium acetate stimulate large amount of MnP ($2000UL^{1-}$) when used with Mn^{2+} [71]. At pH value 4.5 was obtained as optimum for maximum ligninolytic enzymes activities [72].

Effect of Temperature, Inoculum Size and Incubation Period on MnP Production: Studying the effect of temperature, inoculum sizes and incubation periods on MnP production by *P. sajor-caju* in combination have recorded in Figs 16-19. The results revealed that, the highest MnP activities were recorded at 30°C

temperatures after one week incubation. Temperature 35 °C gave higher MnP than that recorded by 30°C, but as the temperature increased to be 40°C no MnP activities have been recorded after 2 weeks. As the incubation period increased more (3 weeks), *P. sajor-caju* produced the highest (1.648 U/ml) at temperature 30°C and 4 discs inoculum size. Also, as the inoculum size increased, the MnP activities increased in all incubation period at both 30 and 35°C. The results indicated that at low inoculum sizes (1 and 2 discs), the highest MnP productivity was found in 35°C temperature (0.324 and 0.406 U/ml), respectively after 3 weeks. As the inoculums sizes increased (3 and 4 discs), the highest MnP productivities had been recorded at 30°C (1.504 and 1.648 U/ml) respectively. This experiment indicated a clear relation between, the inoculums size and temperature of incubation. It might be explained on the bases that as the inoculum size increased, the temperature of the medium increased as a result of bioactivity reactions, so the lower temperature (30°C) might be more suitable for high MnP production. The relation between the inoculum sizes and temperature of incubation especially at long incubation period (3weeks) was complicated relationship. Temperature affects the production, activity and stability of ligninolytic enzymes in *Pleurotus ostreatus* and *Trametes veriscolor*. The fungi were able to grow and produce Laccase and MnP at 5-35°C. The highest production being reached at 25-30 °C in *P. ostreatus* and 35°C in *T. versicolor* [73, 74]. In case of the saprotrophic fungi, the failure to grow at high temperature can be partly due to excessive energy costs necessary for the replacement of heat inactivated molecules of extracellular enzymes that are indispensable for the utilization of nutrients [72].

Gamma Radiation and its Effect on Both Growth and MnP Induction: Effect of gamma radiation growth of *P. sajor-caju* had been indicated in Fig. 15 .The results showed a gradual decrease in growth diameter of *P. sajor-caju* mycelium discs as the dose of gamma radiation increased. The dose 4.0 kGy reduced *P. sajor-caju* growth completely. So, this dose of gamma radiation is considered a lethal dose. This may be explained on the bases that gamma radiation causes a variety of damage to DNA in cells [33], requiring the concerted action of number of DNA repair enzymes to restore genomic integrity [75-76]. This DNA damage caused by direct or indirect effect [77, 78]. Survival curves of *Phanerocheate chrysosporium* spores showed a rise in viability due to radiation-induced germination, before a logarithmic decline [79]. Several studies show that gamma radiation can change the genomic structure [80-83]. High light energy transfer (HLET) is more effective in inducing double strand breaks (DSBs) than low light energy transfer (LLET) [34, 35]. Aziz and Mahrous [84] reported that the dose required for complete inhibition of fungi ranged from 4.0 to 6.0 kGy. Abo-State *et al.* [85] found that gamma radiation reduced the viable count of *Aspergillus* MAM-F23 and MAM-F35 gradually. As the dose increased, the viability decreased. Doses 5.0 and 4.0 kGy reduced their viability completely (lethal dose), respectively. The results of Table 4 revealed that, the only increase in MnP (0.345 U/ml) production was recorded at dose 0.25 kGy on medium I. However, as the doses increased the MnP production decreased in case of medium (I). Surprisingly, MnP production on medium (II) at all doses were superior to the production of non irradiated (control). MnP gave the highest productivities (0.165 U/ml) when exposed to 0.75 and 1.0 kGy. This means that *P. sajor-caju* mycelial discs exposed to 0.75 or 1.0 kGy produced MnP more than the control 4.6 times. Another surprise was recorded by *P. sajor-caju* discs which exposed to 3.0 kGy gamma radiation, although their growth was reduced greatly, but it produced MnP (0.144 U/ml) more than that produced by the control by 4.0 times. So, irradiated *P. sajor-caju* produced enhanced MnP production. Gamma radiation induces not only the deficient mutants [79, 80] but also the enhanced mutants [81-86]. Lee *et al.* [86] isolated the enhanced mutants of ligninolytic ability induced by gamma ray irradiation from *P. ostreatus* Po I. The mutational spectra in the manganese (II) peroxidase gene (*mnp*) of the *Pleurotus ostreatus* mutants induced by gamma radiation (^{60}C) proved the effect of gamma on the gene [87]. El-Batal and Abo-State, [88] found enhanced productivity in CMCase, FPase, Avicelase, xylanase, pectinase by gamma irradiation at dose 1.0 kGy with increased percent 8%, 20%, 10%,4%, 31%,22% and 34% respectively as compared with unirradiated control. Also, the highest CMCase activity was recorded for *Fusarium neoceras* mutants No.1 and No.6 which exposed to 1 min UV-radiation, while the highest CMCase of *F. oxysorum* was mutant No.4 which exposed to 4 min UV-radiation [89]. Rajoka [90] reported 1.6 fold enhanced productivity of extracellular endoglucanase over the parent strain. After the optimization, the FPase in *T. reesi* MCG77 mutant was increased by 2.5 folds compared to that *T. reesi* QM9414 mutant [91]. Abo-State *et al.* [85] reported that mutant No.4 of *Aspergillus* MAM-F23 which exposed to 0.5 kGy produced higher cellulases (CMCase 372 U/ml, FPase 64 U/ml and Avicelase 39 U/ml) than parent strain (CMCase, 305 U/ml, FPase 48 U/ml and Avicelase 29 U/ml).

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