4- RESULTS

4.1. Isolation of red pigment producer fungi:

Six rhizosphere soil samples from different cultivated areas and one sample each from barley, wheat and maize, as well as, one silage sample were used for the isolation of red pigment producer fungi on specific red pigment production solid medium (MPI).

The results obtained in Table (1) indicate that there were twenty-two fungal isolates belonging to 4 genera namely: *Aspergillus* (11 isolates), *Penicillium* (7 isolates), *Fusarium* (3 isolates) and *Monascus* (one isolate) were isolated from different sources on MPI medium. The *Monascus* strain was isolated from the silage sample. From the results of Table (1), it could be observed that, *Aspergillus* contributed the greatest number of isolates.

4.2.Screening of isolated fungi for red pigment production

The aim of the present experiment is to demonstrate the ability of the isolated fungi to grow in specific red pigment solid and liquid media (MPI) as pure culture. The experiments were designed to select the most active fungi for red pigment production.

Mycelial disc of each fungal isolate (8 mm diameter) of 7 days cultures grown on PDA medium, were transferred to the surface of MPI solid medium, and the colony growth was observed daily at 30°C. The results obtained in Table (1) revealed that, out of 22 fungal isolates tested 8 only showed diffusible red pigment on the

solid medium. The other isolates can grow on the surface of the solid medium without formation of any pigment color (Table 1).

Also, each of the isolated fungi (1 ml spore suspension, 2 x 10^7 CFU/ml) was grown in Erlenmeyer flasks containing 50 ml MPI medium (pH 5.5). The inoculated flasks were incubated at 30°C for a period of 4 days on a shaker incubator (150 rpm). Biomass and concentration of red pigment production were determined and the results were shown in Table (1).

The results obtained in Table (1) showed that all the fungal isolates (except the first isolate of *Aspergillus* sp.), which have the ability to produce red pigment on the solid medium, can be grow in the broth medium with production of red pigment. The concentration of extra-red pigment produced by these 7 isolates were in the range of 0.03-0.31 g/l. The highest extra-red pigment concentration was produced by *Monascus* sp. (0.31 g/l), while the sixth and third isolates of *Penicillium* and *Fusarium* spp., respectively, showed moderate concentration of extra-red pigment production (Table 1). The other 4 isolates showed lower secretion of extra-red pigment. On the other hand, two isolates only from the above 7 isolates showed positive production of very little amount of intra-red pigment (Table-1).

Table (1): Screening of different fungal strains isolated from different sources for their potentiality of red pigment production.

Strain No.	Fungal sp.	Source of isolation	Red pigment			Biomass
			On plates	In Broth g/l*		
				Extra	Intra	
	Aspergillus					
1		Soil	+	ND**	ND	6.25
2		Soil	ND	ND	ND	7.71
3		Soil	ND	ND	ND	6.42
4		Barley	+	0.03 ±0.006	ND	5.93
5		Barley	ND	ND	ND	7.52
6		Silage	+	0.08 ±0.003	ND	8.20
7		Soil	ND	ND	ND	6.91
8		Soil	ND	ND	ND	7.24
9		Wheat	ND	ND	ND	5.53
10		Silage	ND	ND	ND	7.80
11		Corn	ND	ND	ND	7.12
	Penicillium					
1		Soil	+	0.06± 0.003	ND	6.50
2		Soil	ND	ND	ND	6.04
3		Soil	ND	ND	ND	6.60
4		Soil	ND	ND	ND	7.81
5		Soil	ND	ND	ND	6.32
6		Corn	+	0.16 ±0.01	0.01	7.70
7		Corn	ND	ND	ND	8.34
	Fusarium					
1		Soil	+	0.05 ±0.002	ND	5.43
2		Soil	ND	-	ND	6.25
3		Barley	+	0.16 ±0.005	ND	6.14
	Monascus					
1		Silage	+	***0.31 ±0.006	0.03	7.20

* Mean ± SE

** ND = Not Detected

*** Significant from all values ($P < 0.05$)

4.3. Isolation and simple identification of red pigment produced by isolated *Monascus* sp.:

The pigment produced by the local isolate of *Monascus* sp. was extracted by pure ethanol and scan spectrophotometery was carried out for the precipitated pigment. The pigment isolated in this work showed a unique light absorption characteristic at 498 nm, indicating red pigment (Fig.4). The maximum absorbance of culture filtrate (0.6) was recorded at 498 nm which corresponded to 0.31 g/l from the extracted red pigment (Table 1).

4.4. Identification of the *Monascus* isolate:

The morphological and cultural characteristics of the highly red pigment producer fungus isolate (*Monascus* sp.) were recorded in Table (2) and plate (1). The morphological diameter of *Monascus* colonies on NA, PDA, malt extract agar, sabouraud dextrose agar, czapek's dox agar, and muzutani media after 7 days, were 22, 38, 66, 32, 54 and 48 mm, respectively, plane eventually with small aerial development, sparse and have flocculent superficial texture. Mycelium was initially white (1-2 days), turning to orange and then to red (5-7 days). The red soluble pigments that diffuse through the agar were observed after 36h of incubation. Colonies diameter on NA medium were 11-22 mm in diameter after 7 days of growth, white, flat with short white-red aerial mycelium. For the above characteristics and according to the identification procedure of, **Hawksworth and Pitt (1983) and Carvalho*, et al*. (2003)**, we can suggest that this red pigment producer strain belongs to the *Monascus* genera and coded as *Monascus purpureus*.

Fig. (4): Absorption spectra of pigments produced by the local strain of *Monascus* sp. isolated from silage. Table (2): Morphological and cultural characterization of the isolated *M. purpureus*.

Cultural and morphological characters	Culture media, 7d of cultivation at 28 °C					
	NA	**PDA**	**Czapek's**	**Sabourad**	**Muzutani agar**	**Malt extract**
Conidia						
Shape	Globose	Globose	Globose	Globose	Globose	Globose
Type of conidia chain	Straight	St	St	St	St	St
Number of conidia	1	1-2	1-3	1-2	1-3	1-3
Size μm	4-6	6-7	9-10	6-7	11-12	10-11
Ability to form conidia	±	+++	++++	+++	++++	++++
Ascospore						
Shape	Not observed	Oval	Oval	Oval	Oval	Oval
Formation capability	Not observed	++	+++	++	+++	++++
Diameter μm	Not observed	4-5	5-6	4-5	5-6	5-6
Colony						
Diameter mm	22	38	54	32	48	66
Color	White, orange	Yellow, orange	Red	Orange, red	Red	Red
Shape	Flat	Flat sparse	Lava	Flat sparse	Raised	Lava
Aerial mycelium	Short	Abundant	Long	Abundant	Long	Long
Exudates	Colorless	Orange	Red	Yellow	Red	Dark red

++++ = Heavy
+++ = High
++ = Moderate
± = Rare

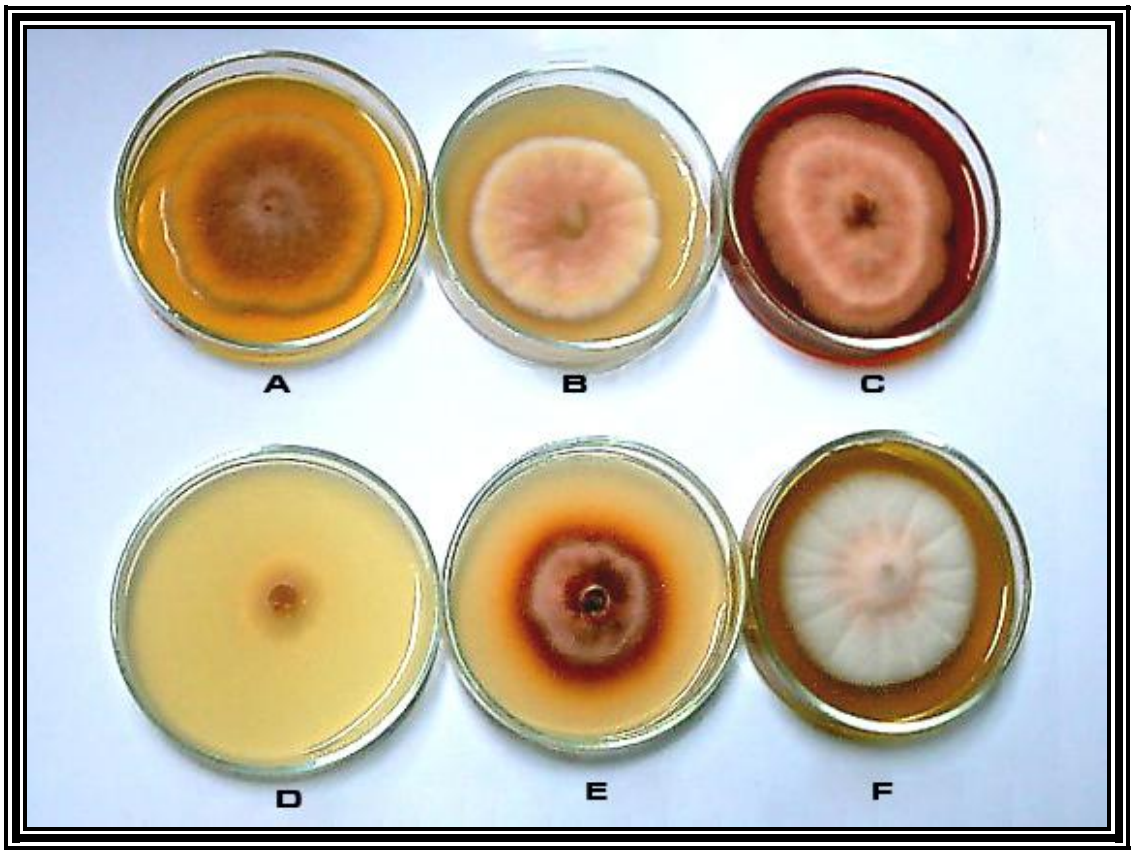


Plate (1) Morphological characterization of isolated red pigment producer *M. purpureus* strain on different solid culture media. A= Sabouraud dextrose agar, B= PDA, C= Czapek's dox agar, D= Nutrient agar, E= Mizutani medium, F= Malt extract agar

4.5. Factors affecting red pigment production by *M. purpureus:*

4.5.1. Influence of specific media:

The fungus was cultured on different specific red pigment production media (MPI, MPII and MPIII), in 250 ml Erlenmeyer flasks, each containing 50 ml medium. The flasks were inoculated with 1 ml spore suspension (2 x 10^7 CFU/ml) and incubated at 30°C under shaking (150 rpm) for 4 days.

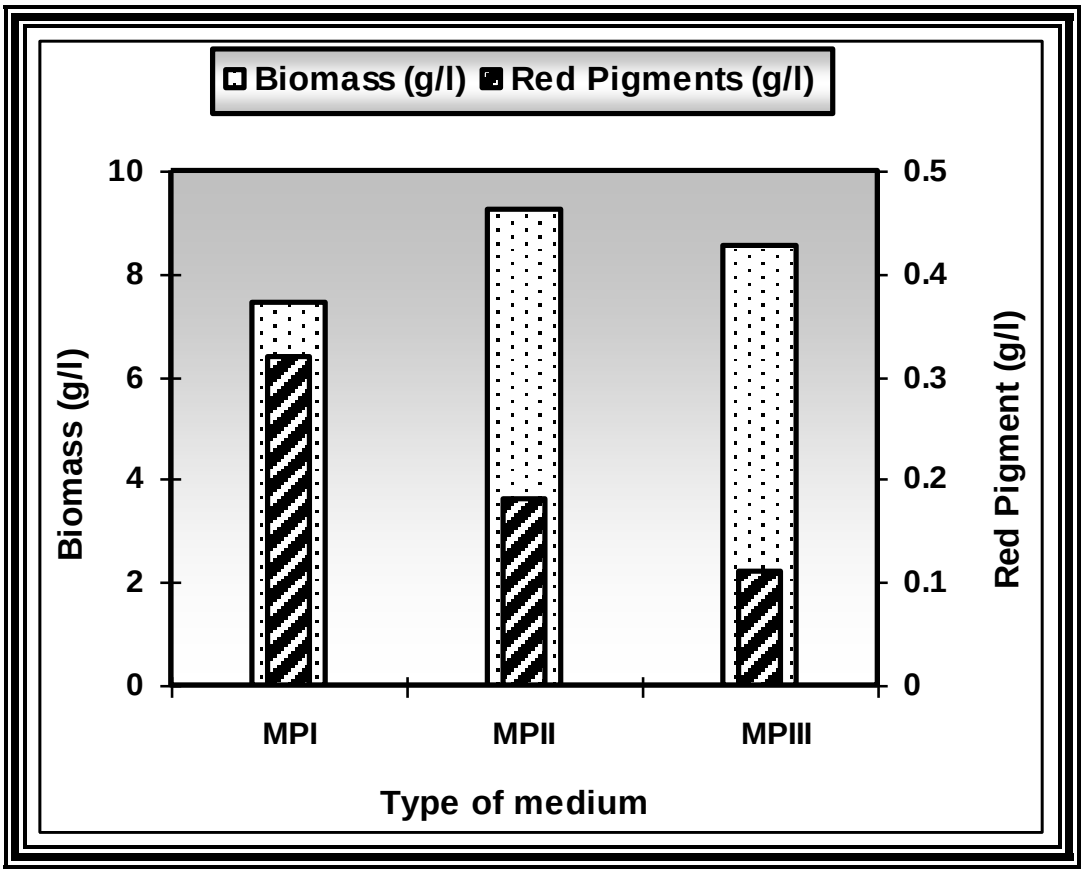
The results in table (3) and figure (5) showed clear variation in both growth and red pigment production among the tested media. The highest red pigment concentration was obtained by MPI medium which recorded 0.32 g/l. While, the lowest red pigment concentration 0.11g/l was obtained when grown on MPIII medium. On the other hand, MPII and MPIII media showed highest fungus biomass 9.25 and 8.53 g/l, respectively, compared with the biomass formed by MPI medium (7.44 g/l).This results indicated that the MPI medium was the best medium for red pigment production by this *M. purpureus* strain.

During this experiment very slight amount of intra-red pigment was recorded by this fungus (Table 3), so we were only concerned by extra-red pigment production in the following experiments.

Table (3): Red pigment production by *M. purpureus* (local isolate) grown on various media.

Type of medium	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Extra-Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)	Intra-Red Pigment (g/l)
MPI	15.52	7.44	**0.32 ± 0.006	47.93	2.06	3.33	0.03
MPII	16.63	9.25	0.18 ±0.002	55.62	1.08	1.87	0.01
MPIII	14.80	8.53	0.11 ±0.003	57.47	0.74	1.14	0.01

* Mean ± SE
** Significant from all values ($P < 0.05$)



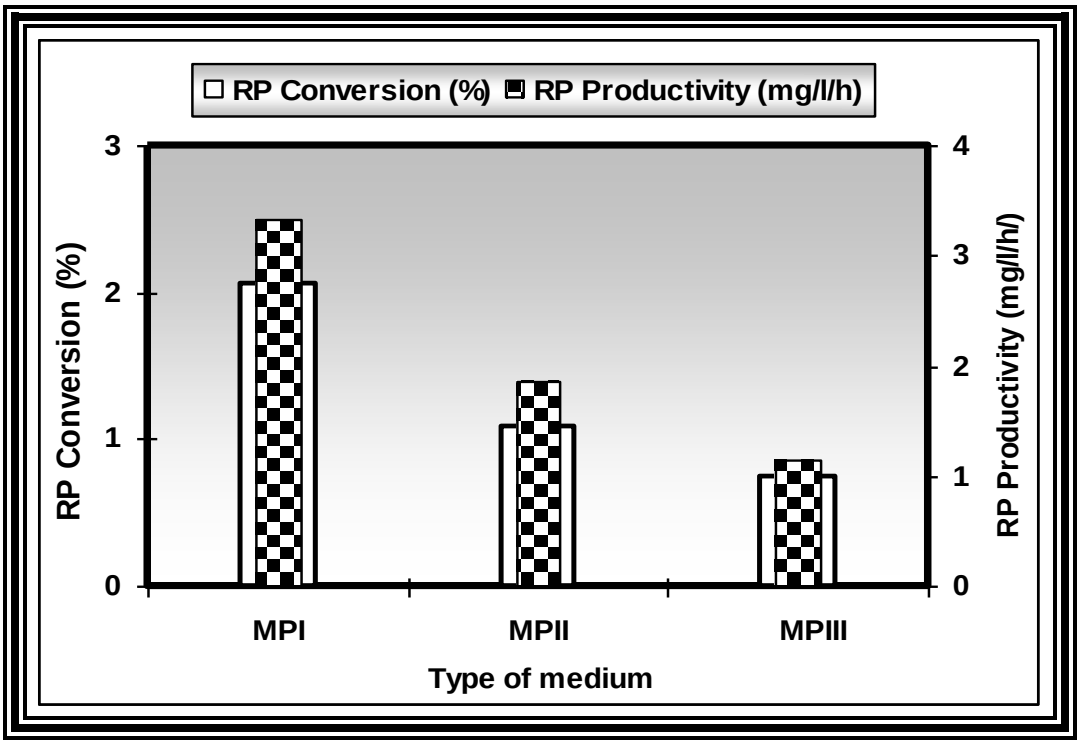


Fig. (5): Extra red pigment production by *M. purpureus* (local isolate) grown on various media.

4.5.2. Influence of incubation period:

This experiment was carried out to determine the rate of growth and red pigment production on MPI medium by *M. purpureus* at different time intervals in order to obtain the maximum red pigment production at a proper time.

As shown in table (4) and figure (6) the biomasses were increased as the incubation periods increased, they were ranged from 2.5 to 8.3 g/l. The maximum biomass content (8.3 g/l) and conversion value (48.14%) were obtained after 5d, followed by a relatively constant value and then decline slowly. Red pigment production started from the beginning of growth (24 h) and increased gradually reaching its maximum value (0.35 g/l) after 4d

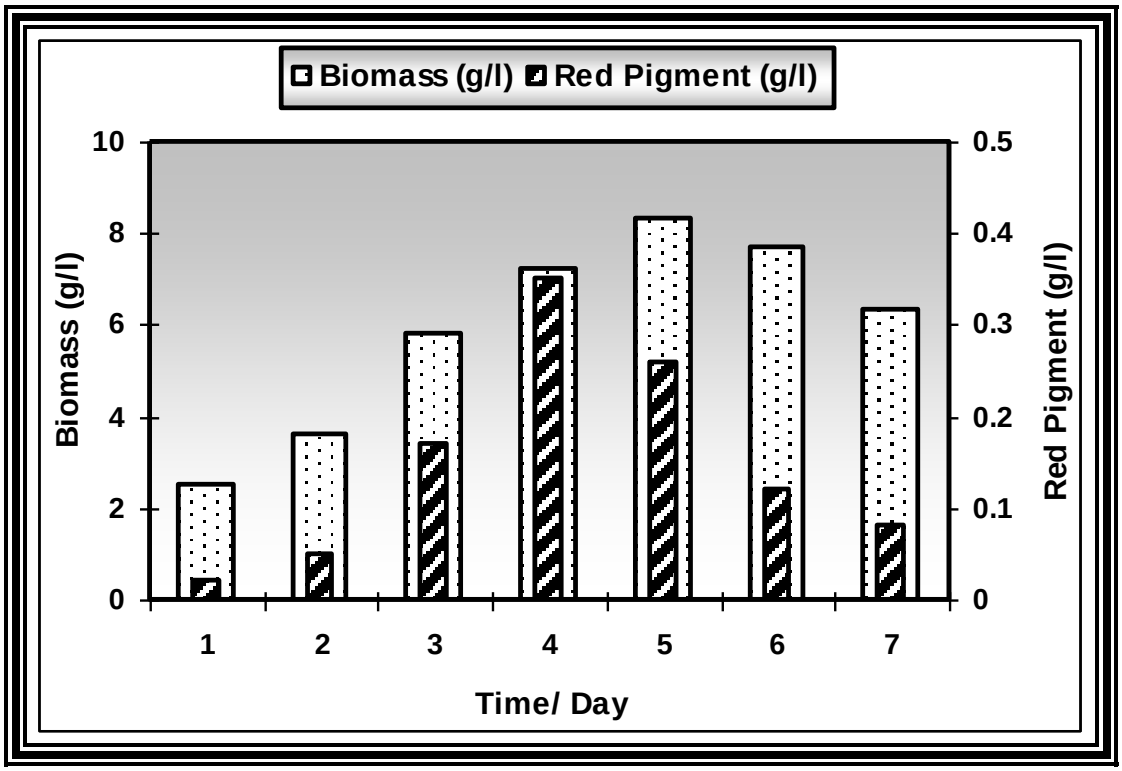
with conversion content (2.21%) and productivity rate (3.64 mg/l/h), then decreased thereafter.

Table (4): Time course of red pigment production by *M. purpureus* grown on MPI medium.

Time/day	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
1	3.41	2.50	0.02	73.31	0.58	0.83
2	7.52	3.62	0.05 ±0.003	48.13	0.66	1.04
3	12.50	5.81	0.17 ±0.001	46.48	1.36	2.36
4	15.82	7.22	**0.35 ± 0.002	45.63	2.21	3.64
5	17.24	8.30	0.26 ±0.003	48.14	1.27	1.83
6	18.30	7.71	0.12 ±0.002	42.13	0.65	0.83
7	18.45	6.32	0.08 ±0.002	34.25	0.43	0.47

* Mean ± SE

** Significant from all values ($P < 0.05$)



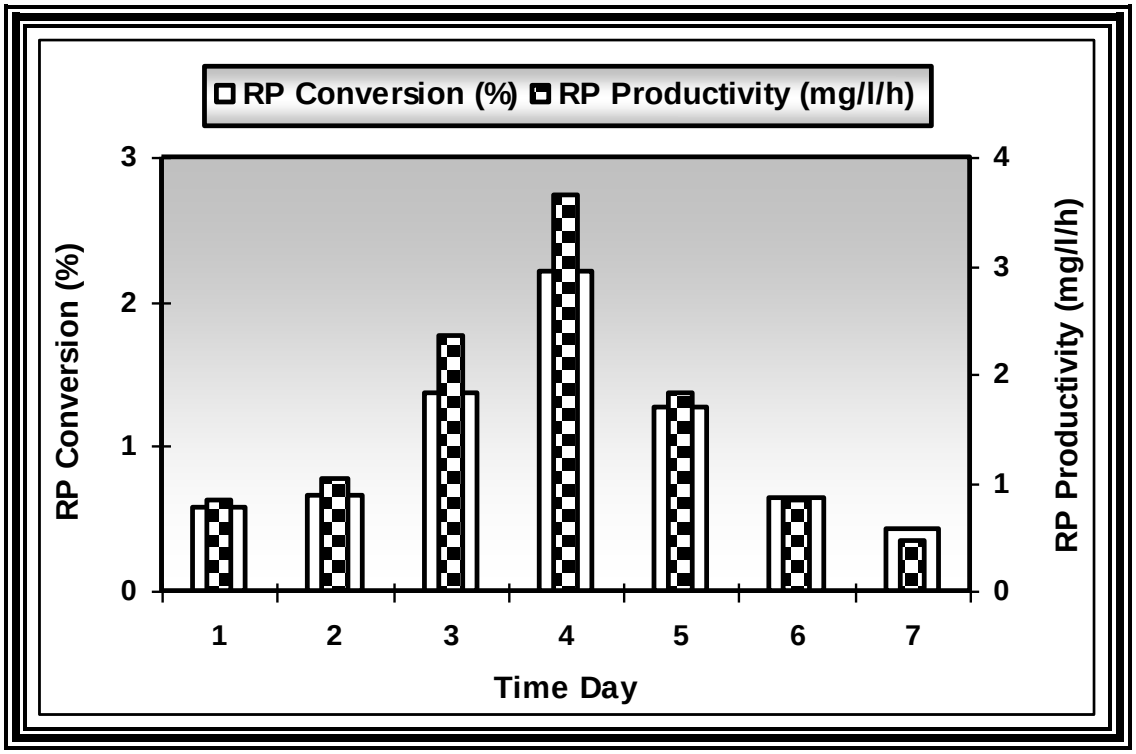


Fig. (6): Time course of red pigment production by *M. purpureus* grown on MPI medium.

4.5.3. Effect of incubation temperature:

To elucidate the effect of incubation temperature on mycelial growth and pigment production, *M. purpureus* was cultivated under various temperature degrees ranging from 20 to 40 °C at pH value 5.5 for 4d under shaking condition (150 rpm).

The results in table (5) and figure (7) showed that the optimum incubation temperature for fungus growth was observed at 25 °C (7.52 g/l) with conversion value (52.58%). While, gradual increase of the incubation temperature from 20 to 30 °C enhanced red pigment production by tested fungus. The maximum pigment concentration produced (0.32 g/l) was obtained at incubation temperature of 30°C with conversion content (2.06%) and productivity rate (3.33 mg/l/h). In addition, pigment concentration highly decreased (0.03 g/l) at incubation temperature of 40 °C.

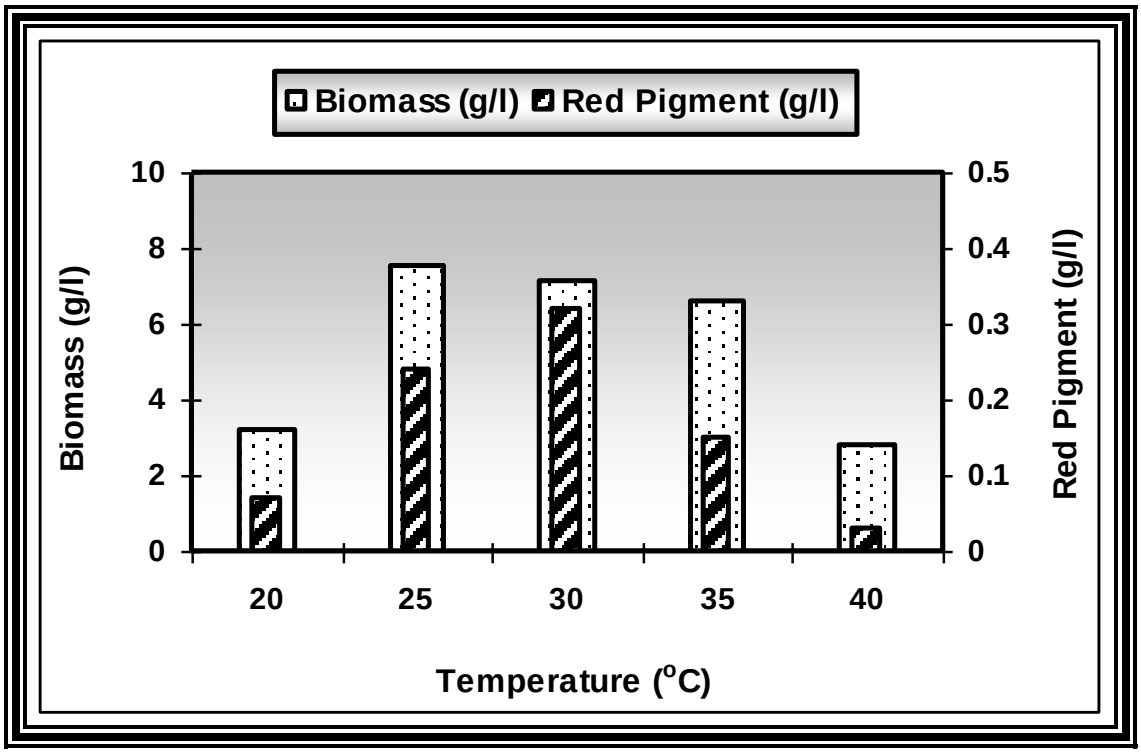
Table (5): Effect of different incubation temperature (20-40 °C) on red pigment production by *M. purpureus* grown on MPI medium for 4 days.

Temp. (°C)	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
20	7.25	3.22	0.07 ±0.002	44.41	0.96	0.72
25	14.30	7.52	0.24 ±0.002	52.58	1.67	2.50
30	15.52	7.13	**0.32	45.94	2.06	3.33

			±0.006			
35	13.24	6.62	0.15 ± 0.004	50.00	1.13	1.56
40	6.61	2.80	0.03 ±0.001	42.36	0.45	0.31

* Mean ± SE

** Significant from all values (P < 0.05)



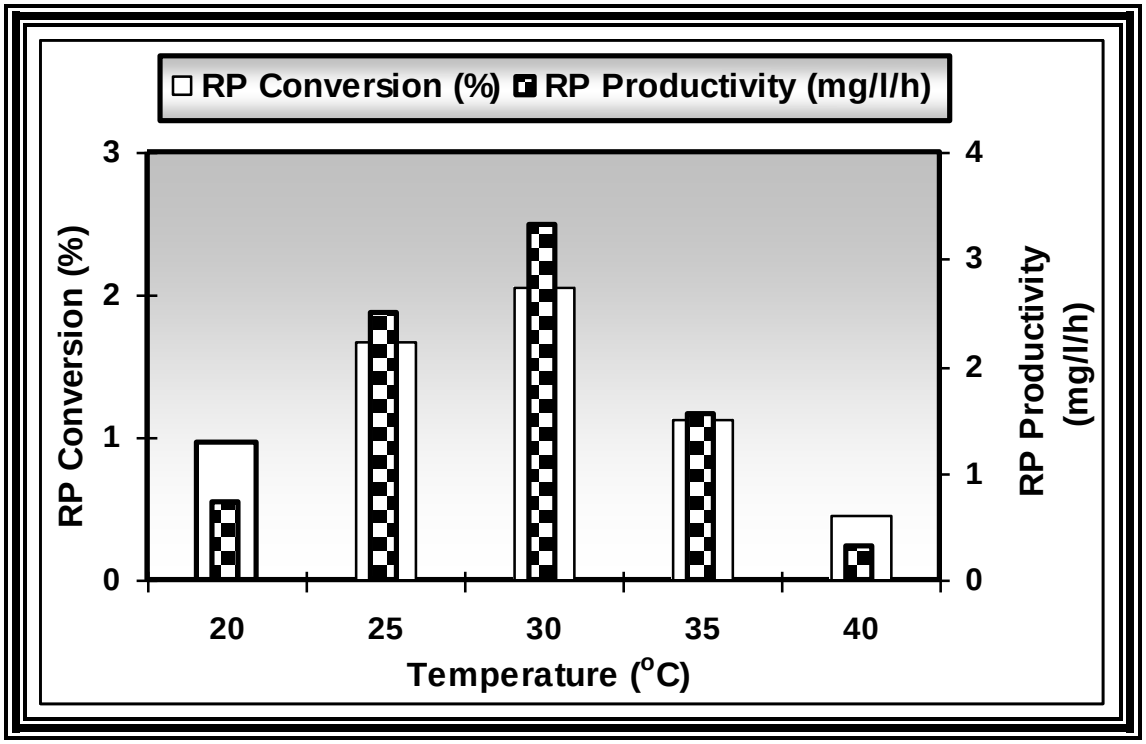


Fig. (7): Effect of different incubation temperature (20-40 °C) on red pigment production by *M. purpureus* grown on MPI medium for 4 days.

4.5.4. Effect of initial pH:

The initial pH of the growth medium plays an important role in pigment production, therefore, the effect of different pH values on growth and red pigment production by *M. purpureus* was studied over range 3.0-7.0.

Clearly, as shown in table (6) and figure (8), *M. purpureus* was able to grow with different degrees in all pH values. The maximum growth was obtained with an initial pH value of 5.0 which recorded 7.82 g/l. Meanwhile, the pH 3.0 and 7.0 resulted in lower growth (3.20 and 2.72 g/l, respectively). On the other hand, the results indicated that the maximum red pigment production was achieved at

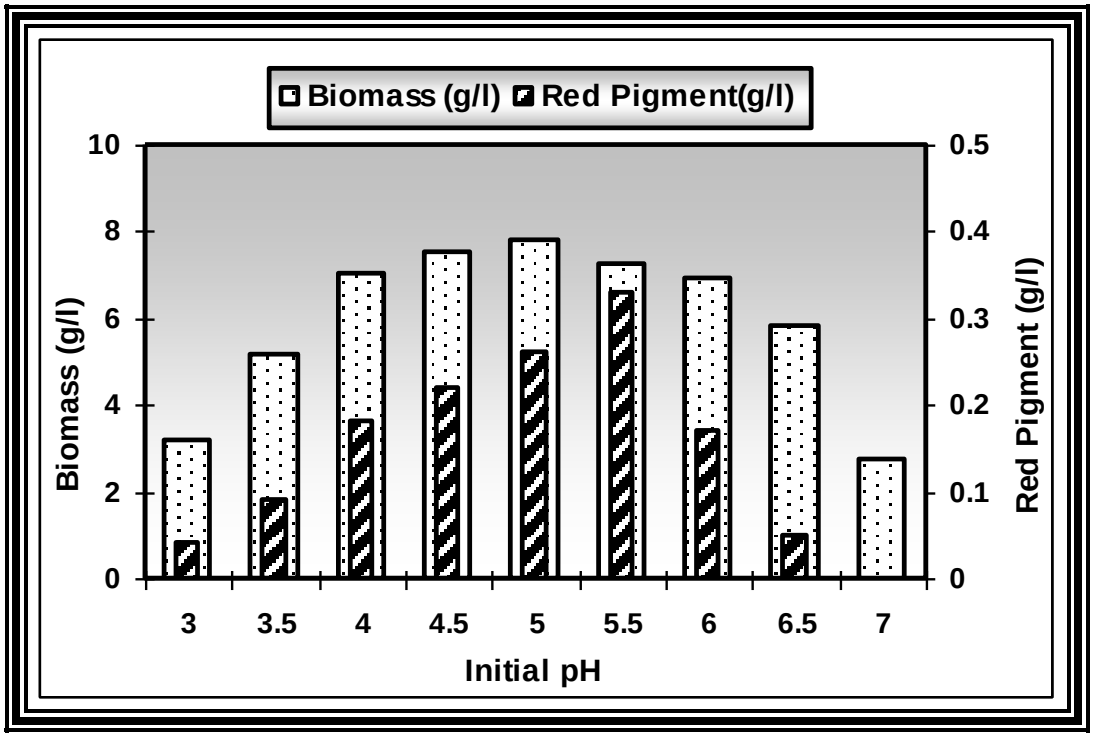
pH 5.5 reaching to 0.33 g/l with conversion content (2.16%) and productivity rate (3.43 mg/l/h). Whereas, lower concentrations of pigment production (0.04 and 0.05 g/l) were recorded at pH 3.0 and 6.5, respectively. Moreover, complete inhibition of red pigment synthesis was observed at pH 7.0.

Table (6): Effect of different pH values (3-7) on red pigment production by *M. purpureus* grown on MPI medium for 4 days at 30 °C.

Initial pH	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
3	3.41	3.20	0.04 ±0.002	93.84	1.17	0.41
3.5	8.60	5.14	0.09 ±0.001	59.76	1.04	0.93
4	10.51	7.03	0.18 ±0.001	66.88	1.71	1.87
4.5	11.73	7.51	0.22 ± 0.003	64.02	1.87	2.29
5	13.62	7.82	0.26 ±0.001	57.41	2.05	2.91
5.5	15.21	7.24	** 0.33 ±0.002	47.60	2.16	3.43
6	14.60	6.90	0.17 ± 0.008	47.26	1.16	1.77
6.5	10.34	5.81	0.05 ±0.003	56.18	0.48	0.52
7	3.46	2.72	-	78.61	-	-

* Mean ± SE

** Significant from all values ($P < 0.05$)


Biomass (g/l)
Red Pigment(g/l)
Biomass (g/l)
Red Pigment (g/l)
10
8
6
4
2
0
0.5
0.4
0.3
0.2
0.1
0
3
3.5
4
4.5
5
5.5
6
6.5
7
Initial pH

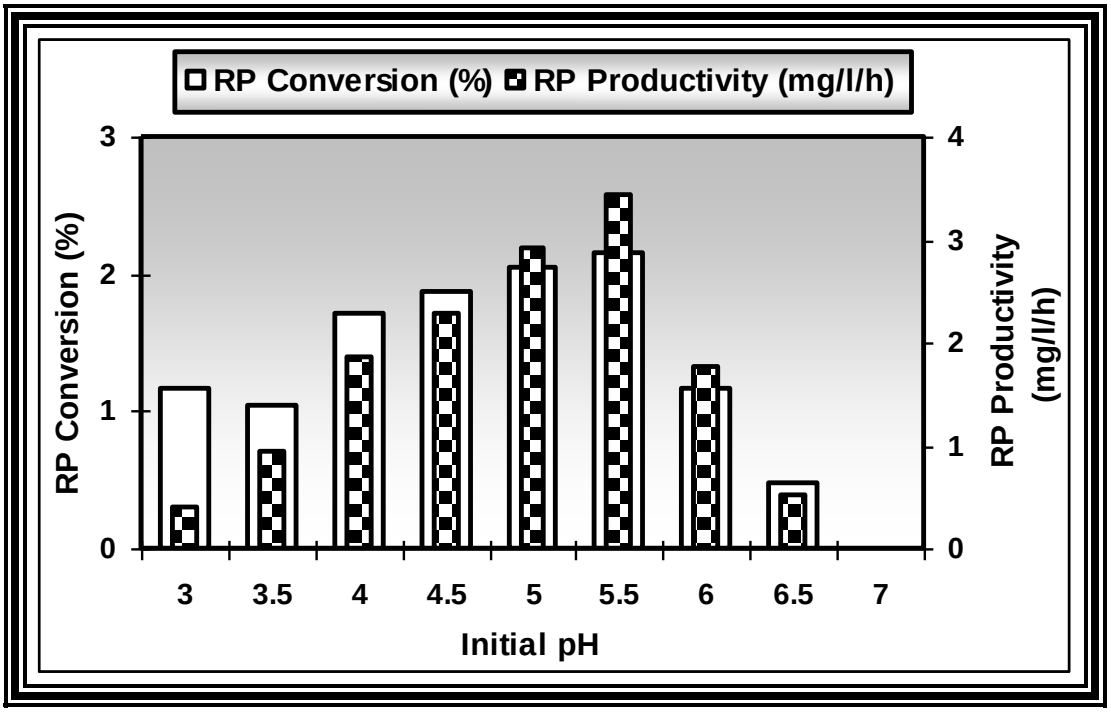

RP Conversion (%)
RP Productivity (mg/l/h)
RP Conversion (%)
RP Productivity (mg/l/h)
3
2
1
0
4
3
2
1
0
3
3.5
4
4.5
5
5.5
6
6.5
7
Initial pH

Fig. (8): Effect of different pH values (3-7) on red pigment production by *M. purpureus* grown on MPI medium at 30 °C for 4 days.

4.5.5. Effect of agitation speed:

The effect of agitation on growth and red pigment production was studied by incubating the inoculated flasks (pH 5.5) at different agitation speeds (0 to 300 rpm) on rotary shaker at 30 °C for 4d.

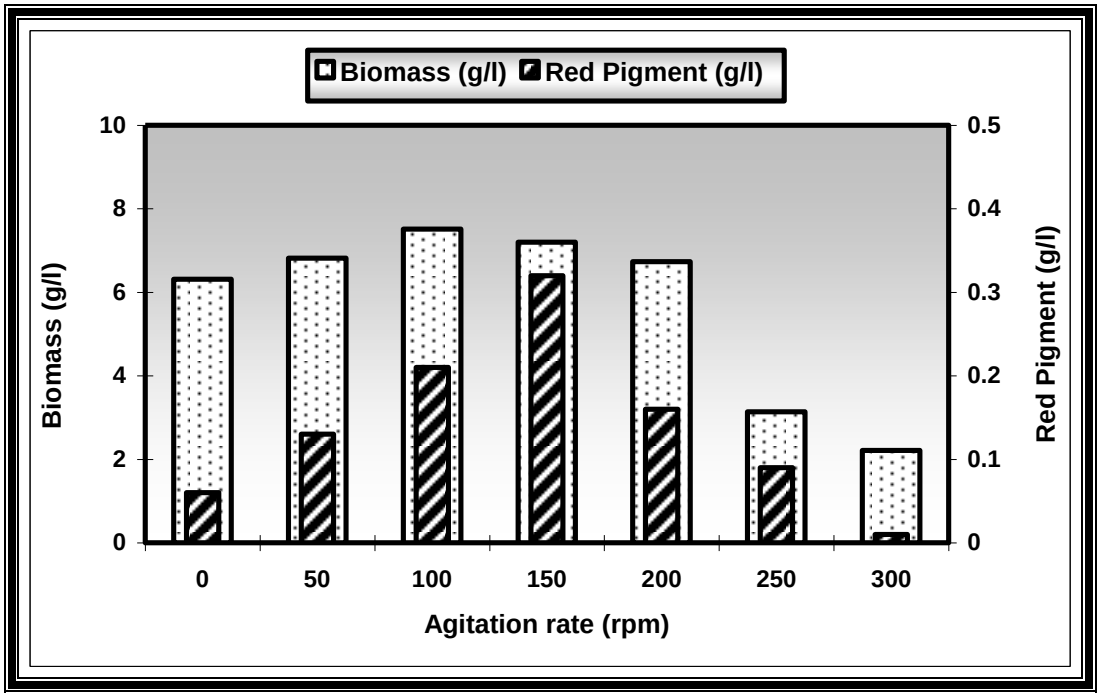
From the data represented in table (7) and figure (9), it was noticed that the fungus isolate under investigation could grow and synthesis red pigment under static and shaking conditions. The cell growth increased by increasing the agitation speed up to 100 rpm reaching the maximum dry weight (7.51 g/l) with biomass conversion value (48.70%). Similarly, red pigment production increased by increasing agitation speed but reached its maximum at 150 rpm (0.32 g/l) with conversion content (2.04 %) and productivity rate (3.33 mg/l/h). While, cells dry weight and pigment production decreased with further increase in agitation speed. On the other hand, lower concentration of red pigment production (0.06 g/l) was observed under static condition.

Table (7): Red pigment production by *M. purpureus* grown on MPI medium at different aeration regimes (growth conditions: temp.30°C, pH 5.5 and incubation time, 4days).

Agitation rate (rpm)	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
0 (static)	12.80	6.31	0.06 ±0.002	49.29	0.46	0.62
50	13.32	6.82	0.13 ±0.003	51.20	0.97	1.35
100	15.42	7.51	0.21 ±0.001	48.70	1.36	2.18
150	15.61	7.20	**0.32 ±0.001	46.12	2.04	3.33
200	14.23	6.73	0.16 ±0.002	47.29	1.26	1.87
250	11.51	3.14	0.09 ±0.001	27.28	0.78	0.93
300	6.42	2.21	0.01 ±0.002	34.42	0.15	0.10

* Mean ± SE

** Significant from all values ($P < 0.05$)



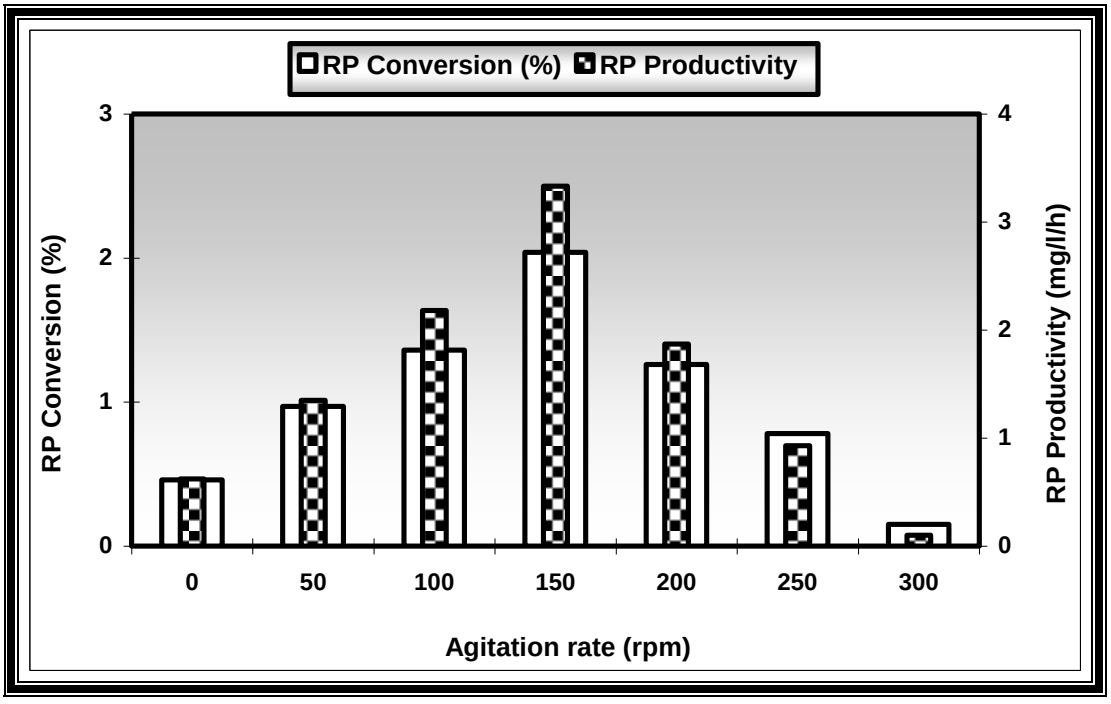


Fig. (9): Red pigment production by *M. purpureus* grown on MPI medium at different aeration regimes (growth conditions: temp. 30°C, pH 5.5 and incubation time, 4 days).

4.5.6. Effect of carbon sources:

To investigate the effect of carbon sources on red pigment production by *M. purpureus*, the fungus was cultured on MPI medium (pH 5.5) containing different carbon sources (2% w/v) at 30 °C for 4 days under shaking (150 rpm).

The data presented in table (8) and figure (10) showed that the maximum cell growth obtained using starch and glucose were 7.63 and 7.13 g/l, respectively, followed by fructose (6.9 g/l). Consequently, all the carbon sources used were suitable for growth except manitol which gave the lowest biomass concentration (3.6 g/l).

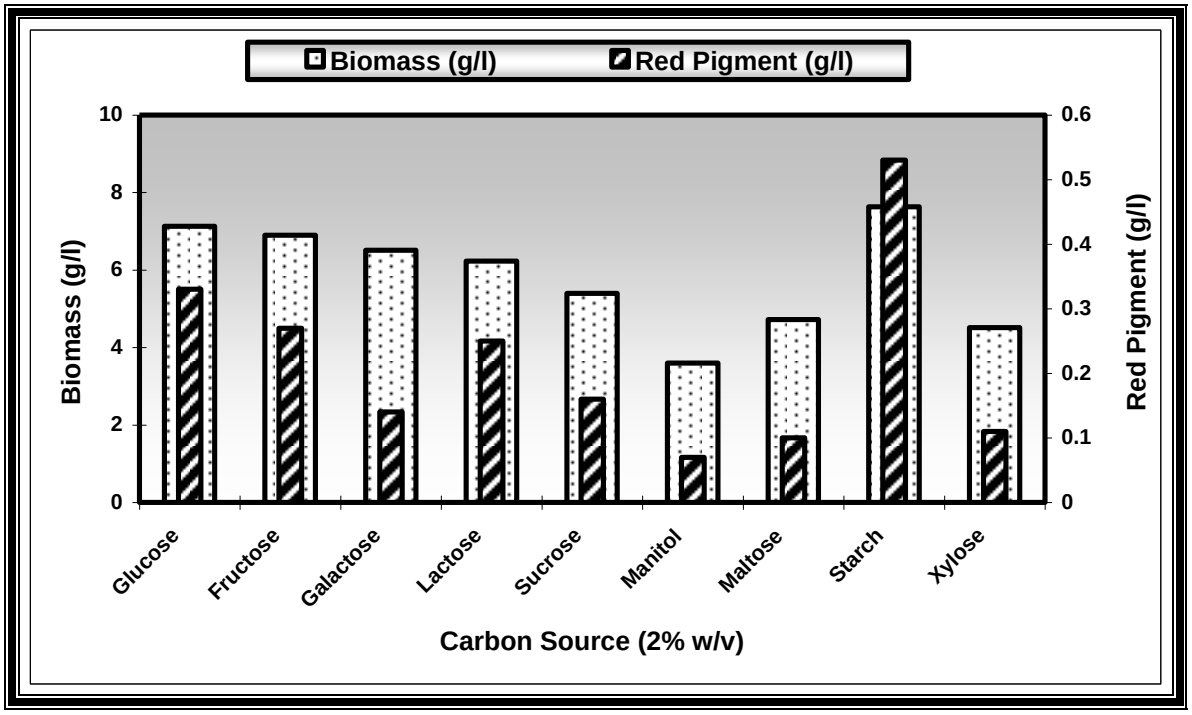
Also, the results revealed that the best carbon sources for red pigment production were starch and glucose that recorded 0.53 and 0.33 g/l, respectively. Whereas, manitol and maltose represented the poorest carbon sources for pigment production (0.07 and 0.1 g/l, respectively). In addition, the highest values of biomass and red pigment conversion (49.16% and 3.41%, respectively) as well as, pigment productivity (5.52 mg/l/h) were recorded when starch was used as the sole carbon source.

Table (8): Effect of different carbon sources on red pigment production by *M. purpureus* grown on MPI medium (growth conditions as in table,7 under agitation rate,150 rpm).

Carbon sources (2%w/v)	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
Glucose "control"	15.32	7.13	0.33 ±0.002	46.54	2.15	3.43
Fructose	15.61	6.90	0.27 ±0.002	44.20	1.72	2.81
Galactose	13.70	6.51	0.14 ±0.002	47.51	1.02	1.45
Lactose	13.14	6.23	0.25 ±0.001	47.41	1.67	2.29
Sucrose	12.40	5.40	0.16 ±0.002	43.54	1.29	1.66
Manitol	10.51	3.60	0.07 ±0.002	34.25	0.66	0.72
Maltose	11.30	4.72	0.10 ±0.003	41.76	0.88	1.04
Starch	15.52	7.63	**0.53 ±0.012	49.16	3.41	5.52
Xylose	11.23	4.51	0.11 ±0.002	40.08	0.97	1.14

* Mean ± SE

** Significant from all values ($P < 0.05$)



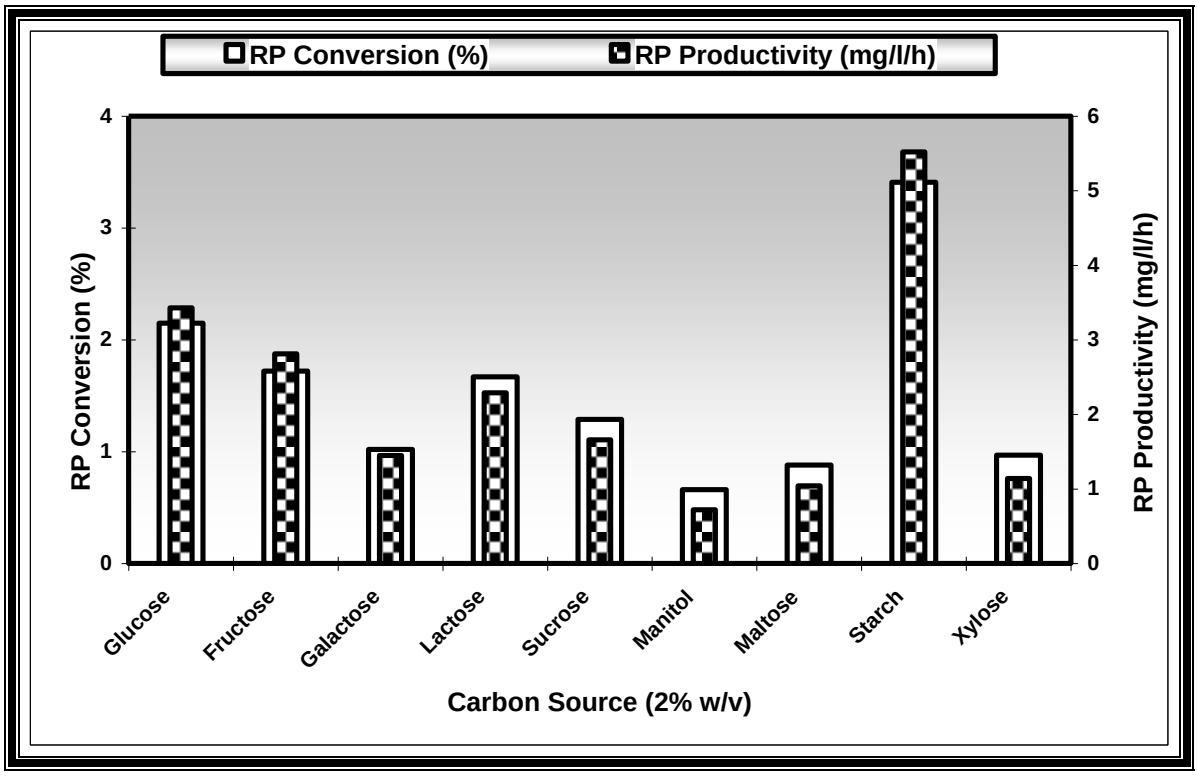


Fig. (10): Effect of different carbon sources (2% w/v) on red pigment production by *M. purpureus* grown on MPI medium (growth conditions as in table,7 under agitation rate,150 rpm).

4.5.7. Effect of starch concentration:

Since starch was the best carbon source for red pigment production by the microorganism under investigation, an experiment was performed at various starch concentrations ranged from 0.5-3.0% (w/v) to determine the optimum starch concentration for both growth and pigment production.

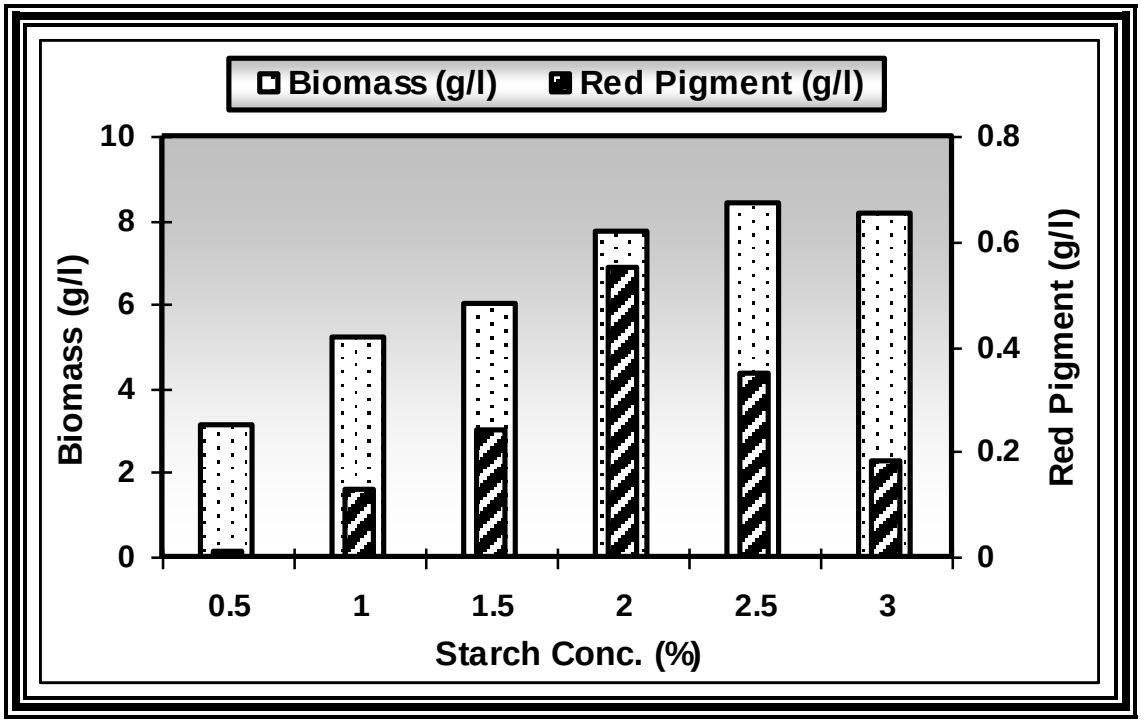
As illustrated in table (9) and figure (11) the biomass content was increased by increasing the starch concentration, and the maximum mycelial growth (8.4 g/l) was recorded at 2.5 % starch concentration. Meanwhile, the highest value of biomass conversion (64.86%) was recorded at 0.5% starch concentration. On the other hand, 2.0 % starch concentration gave the best production of red pigment (0.55 g/l) with conversion and productivity rate (3.52% and 5.72 mg/l/h, respectively), and increasing the starch above this limit did not stimulate the pigment production.

Table (9): Effect of different starch concentrations (w/v) on red pigment production by *M. purpureus* grown on MPI medium (growth conditions as in table 8).

Starch conc.(% w/v)	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
0.5	4.81	3.12	0.01 ±0.002	64.86	0.20	0.10
1.0	9.23	5.21	0.13 ±0.002	56.44	1.40	1.35
1.5	13.40	6.04	0.24 ±0.001	45.07	1.79	2.50
2.0	15.62	7.72	**0.55 ±0.003	49.42	3.52	5.72
2.5	18.31	8.40	0.35 ±0.001	45.87	1.69	3.22
3.0	21.50	8.13	0.18 ±0.003	37.81	0.83	1.87

* Mean ± SE

** Significant from all values ($P < 0.05$)



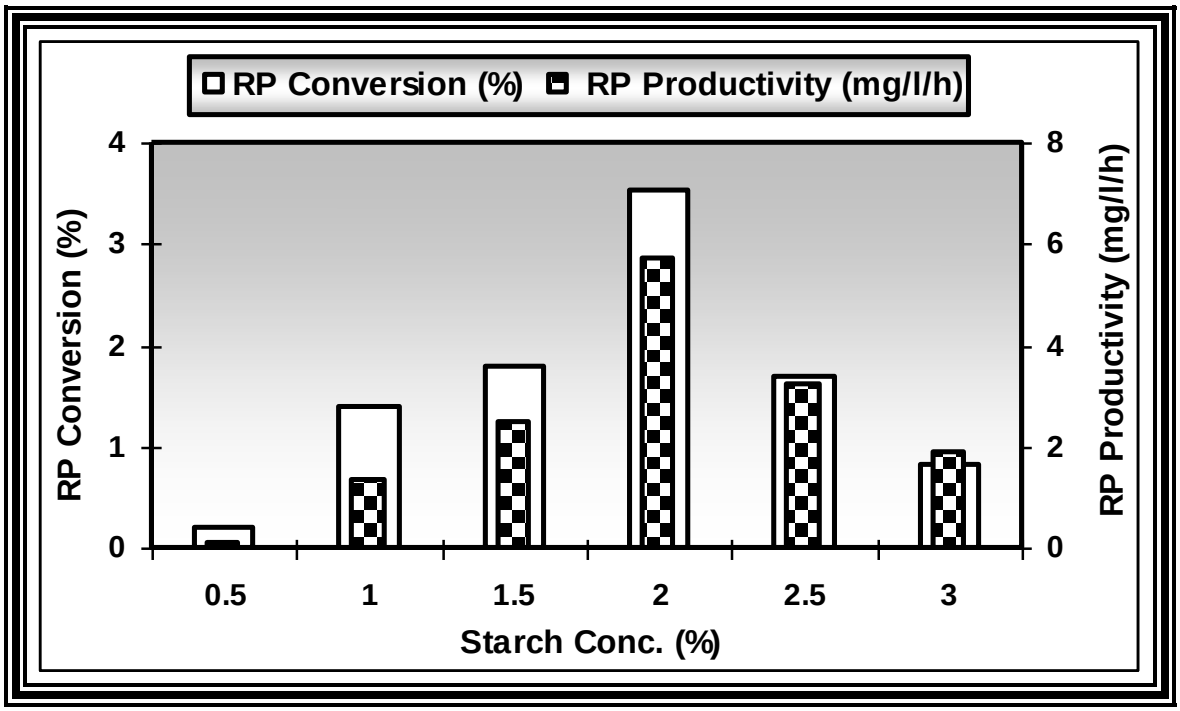


Fig. (11): Effect of different starch concentrations (w/v) on red pigment production by *M. purpureus* grown on MPI medium. (Growth conditions as in table 8).

4.5.8. Effect of nitrogen sources:

In order to investigate the effect of nitrogen sources on the growth and red pigment production by *M. purpureus* several types of both organic and inorganic nitrogen sources (at concentration 0.5 g/l, as N_2) were used.

The data represented in table (10) and figure (12) showed that the biomass concentrations ranged from 4.8 to 8.41 g/l and from 2.91 to 7.72 g/l when organic and inorganic nitrogen sources were used, respectively. It is worthily to mention that yeast extract was the best nitrogen source for growth of *M. purpureus* (8.41 g/l).

The present results also indicated that the maximum red pigment production (0.65 g/l) was obtained when ammonium sulfate used as sole nitrogen source, while tryptone showed lower pigment production (0.09g/l). Also, the highest values of red pigment conversion (4.27%) and productivity (6.77 mg/l/h) were recorded with ammonium sulfate. On the other hand, bacteriological peptone, soy peptone and potassium nitrate inhibited pigment production by *M. purpureus*.

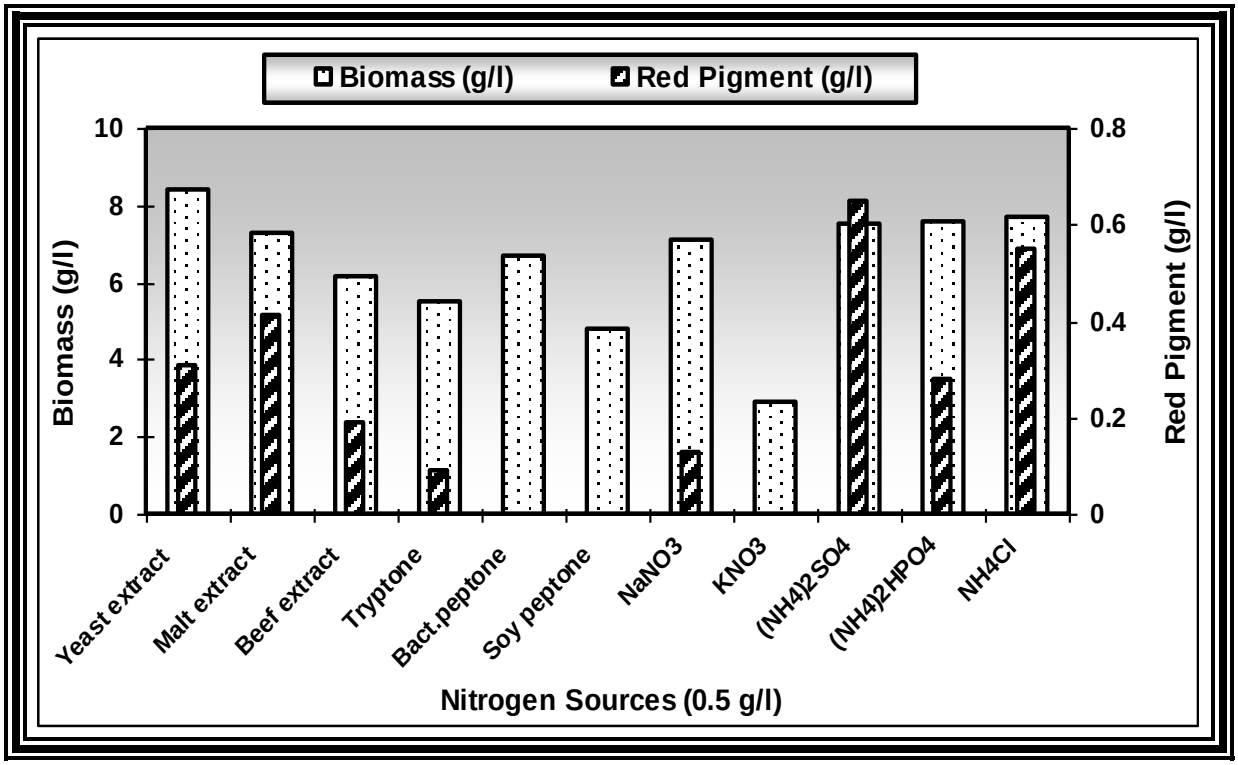
Table (10): Effect of different nitrogen sources on red pigment production by *M. purpureus* grown on MPI medium (growth conditions as in table 8 at 2% w/v starch conc.).

Nitrogen sources (0.5g/l, as N_2)	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
Yeast extract	17.22	8.41	0.31 ±0.002	48.83	1.80	3.22
Malt extract	15.61	7.30	0.41 ±0.001	46.76	2.62	4.27
Beef extract	11.43	6.14	0.19 ±0.002	53.71	1.66	1.97
Tryptone	10.50	5.52	0.09 ±0.001	52.57	0.85	0.93
Bact.peptone	11.62	6.71	ND**	57.74	-	-
Soy peptone	8.81	4.80	ND	54.48	-	-
$NaNO_3$	14.52	7.13	0.13 ±0.002	49.10	0.89	1.35
KNO_3	6.30	2.91	ND	46.19	-	-
$(NH_4)_2SO_4$	15.22	7.50	***0.65 ±0.003	49.27	4.27	6.77
$(NH_4)_2HPO_4$	15.43	7.60	0.28 ±0.008	49.25	1.81	2.91
NH_4Cl control	16.12	7.72	0.55 ±0.002	47.89	3.41	5.72

* Mean ± SE

** ND = Not Detected

*** Significant from all values ($P < 0.05$)



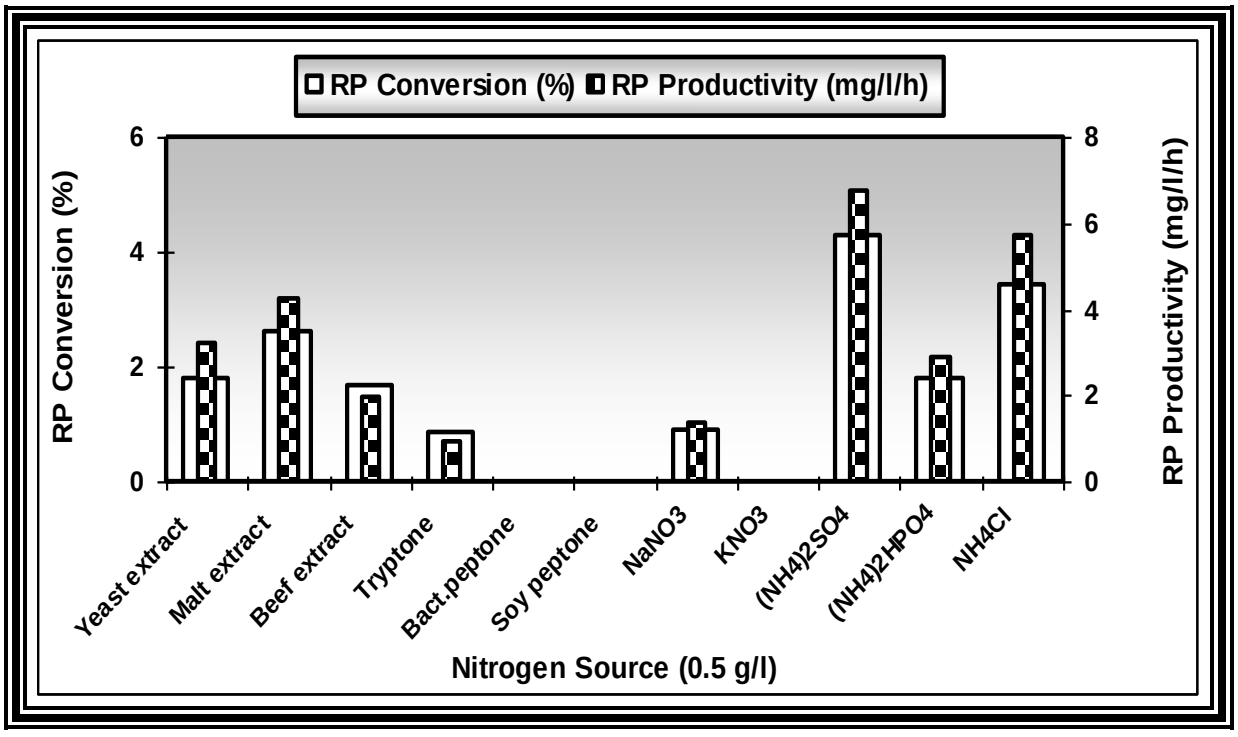


Fig. (12): Effect of different nitrogen sources on red pigment production by *M. purpureus* grown on MPI medium (Growth conditions as in table 8 at 2% w/v starch conc.).

4.5.9. Effect of ammonium sulfate concentrations:

Studying the effect of different nitrogen sources revealed that ammonium sulfate was the best nitrogen source for pigment production, therefore an experiment was performed with various concentrations of ammonium sulfate ranged from 0.2 to 1.0 g/l, as N_2, to determine the optimum concentration for pigment production.

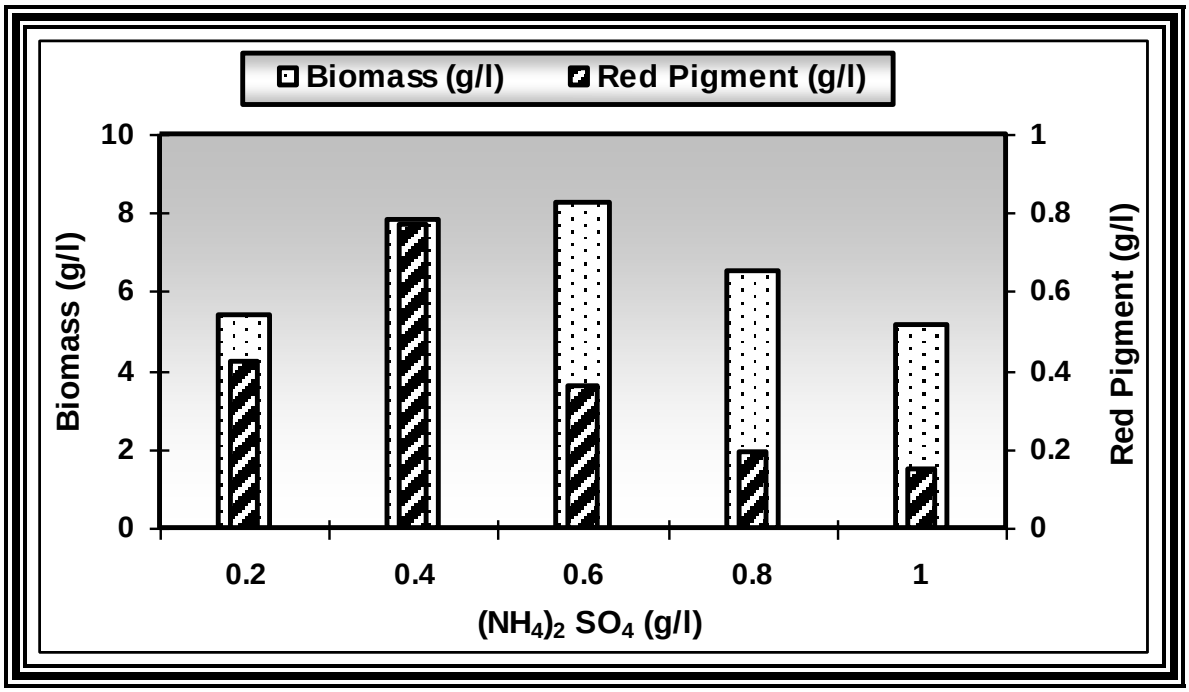
Table (11) and figure (13) illustrated that the biomass increased gradually by increasing the amount of ammonium sulfate reaching its maximum (8.23 g/l) at 0.6 g/l ammonium sulfate concentration. While, the maximum red pigment production (0.77 g/l) was obtained at 0.4 g/l ammonium sulfate concentration. In addition, the best concentration of ammonium sulfate for biomass and red pigment conversion (51.21% and 5.06%, respectively) as well as red pigment productivity (8.02 mg/l/h) was 0.4 g/l. Hence it was selected to be used in the following experiments.

Table (11): Effect of different ammonium sulfate concentrations on red pigment production by *M. purpureus* grown on MPI medium (growth conditions as in table 10).

$(NH_4)_2SO_4$ (g/l, as N_2)	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
0.2	11.60	5.41	0.42 ±0.001	46.63	3.62	4.37
0.4	15.23	7.80	**0.77 ±0.002	51.21	5.06	8.02
0.6	17.82	8.23	0.36 ±0.001	46.18	2.02	3.75
0.8	13.41	6.52	0.19 ±0.003	48.62	1.41	1.97
1.0	10.23	5.13	0.15 ±0.002	50.14	1.07	1.14

* Mean ± SE

** Significant from all values ($P < 0.05$)



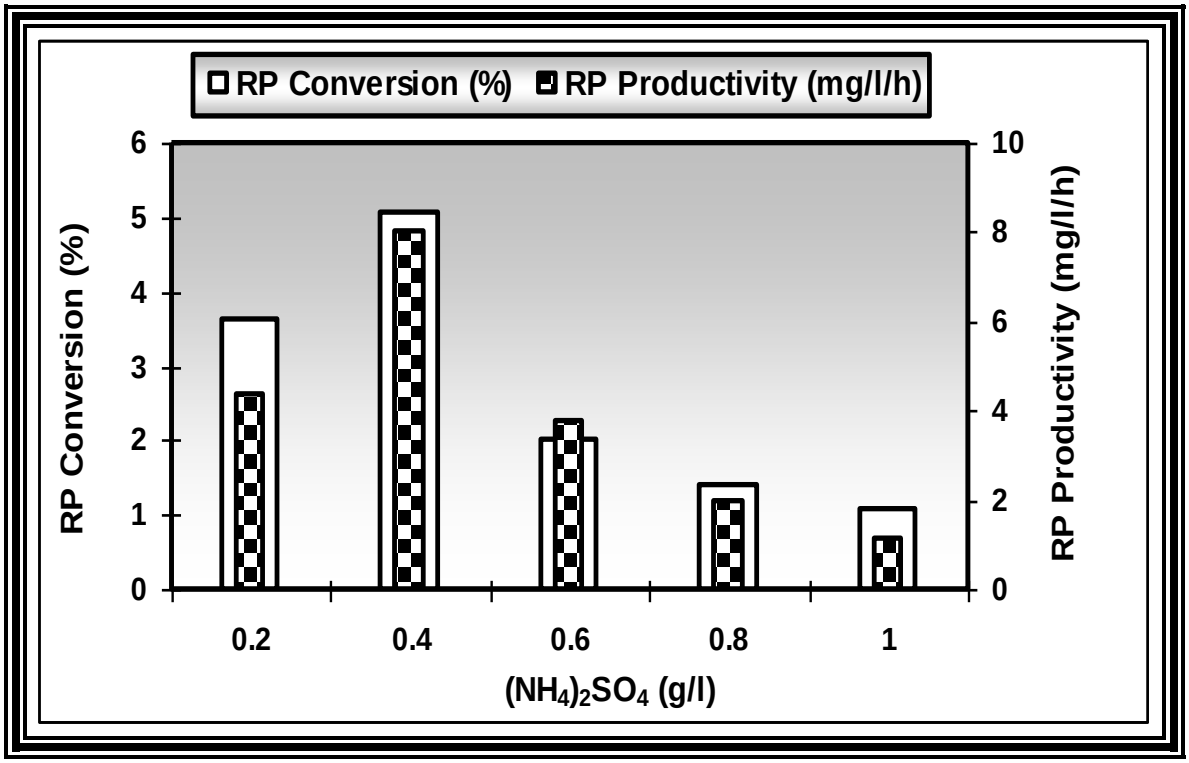


Fig. (13): Effect of different ammonium sulfate concentrations on red pigment production by *M. purpureus* grown on MPI medium (Growth conditions as in table 10).

4.5.10. Effect of bio-elements:

In this experiment, *M. purpureus* was cultured on production medium containing starch (2 % w/v) and ammonium sulfate (0.04 %, N_2) only as a sole source of carbon and nitrogen, respectively (CN medium), plus one of the following bio-element ($CaCO_3$, $MgSO_4$, $FeSO_4$, $MnSO_4$, K_2HPO_4 and NaCl) at concentration of 0.1% (w/v). The inoculated flasks (pH 5.5) were incubated at 30 °C for 4 days under shaking (150 rpm).

The results in table (12) and figure (14) showed decreasing of biomass content to 5.81 g/l and red pigment production to 0.50 g/l, when the fungus was grown on medium containing starch and ammonium sulfate only (CN medium) comparing with control medium (modified MPI medium, MMPI), which containing 2% (w/v) starch and 0.04% (w/v) ammonium sulfate as carbon and nitrogen sources, respectively. On the other hand, addition of bio-element Ca^{2+} in the form of $CaCO_3$ at concentration 0.1 % to the above medium (CN) improved the biomass content (6.42 g/l) and red pigment production (0.61 g/l) with productivity rate 6.35 mg/l/h. The other bio-elements appeared to have no notable or detrimental effect on either mycelial growth or pigment production. So, $CaCO_3$ was the most essential mineral salt for red pigment production, and it was used in the further investigation.

Table (12): Effect of different bio-elements (0.1 % w/v) on red pigment production by *M. purpureus* (growth conditions as in table 8).

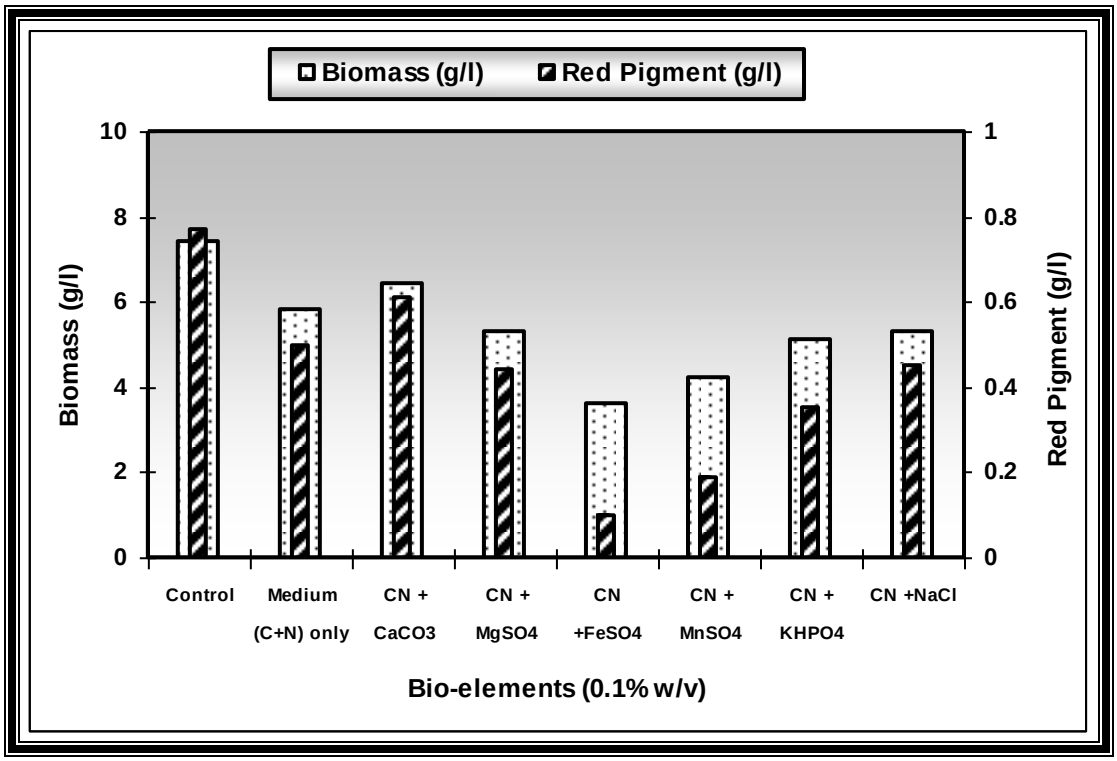
Bio-elements added	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
Control (MMPI medium*)**	15.51	7.40	0.77 ±0.002	47.71	4.96	8.02
CN medium****	13.24	5.81	0.50 ±0.003	43.88	3.77	5.20
CN + $CaCO_3$	16.40	6.42	**0.61 ±0.004	39.14	3.71	6.35
CN + $MgSO_4$	12.53	5.31	0.44 ±0.002	42.37	3.51	4.58
CN + $FeSO_4$	7.31	3.60	0.10 ±0.002	49.24	1.36	1.04
CN + $MnSO_4$	9.60	4.22	0.19 ±0.002	43.95	1.97	1.97
CN + $KHPO_4$	11.82	5.10	0.35 ±0.002	43.14	2.96	3.64
CN + NaCl	13.42	5.30	0.45 ±0.003	39.49	3.35	4.68

* Mean ± SE

** Significant from all values ($P < 0.05$) except control value

***MMPI medium: MPI, containing 2% w/v starch and 0.04% w/v ammonium sulfate, as carbon and nitrogen sources, respectively.

****CN medium: starch (2 % w/v) and ammonium sulfate (0.04 % w/v), as carbon and nitrogen sources, respectively .



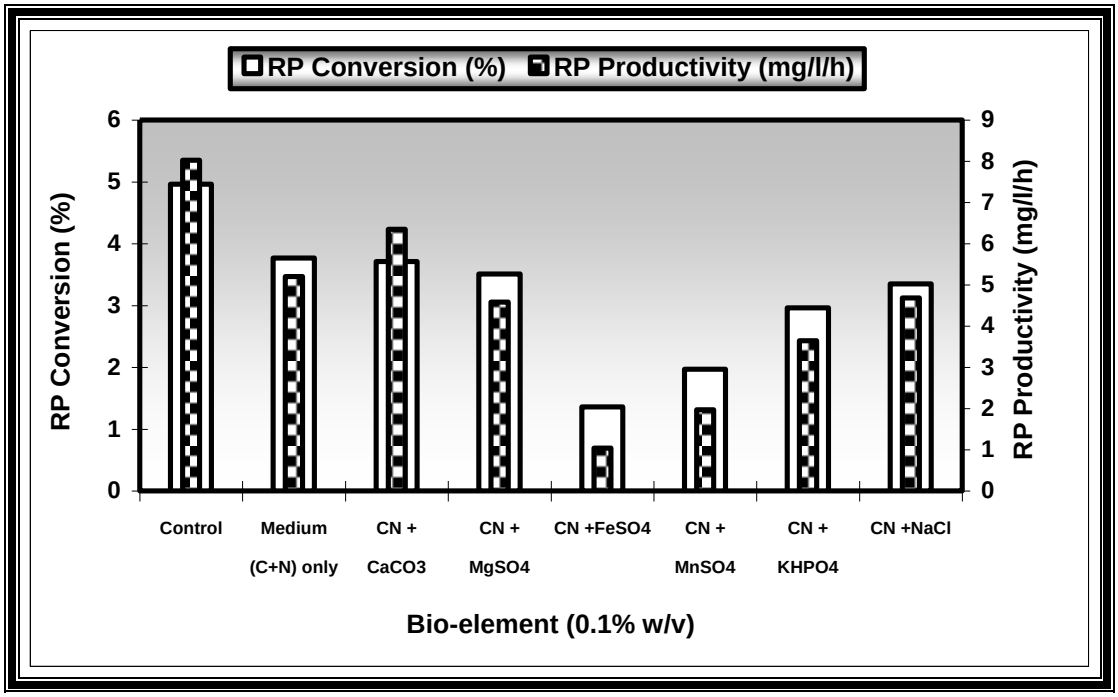


Fig. (14) : Effect of different bio-elements (0.1 % w/v) on red pigment production by *M. purpureus* (growth conditions as in table 8). MMPI medium: MPI, containing 2% w/v starch and 0.04% w/v ammonium sulfate, as carbon and nitrogen sources, respectively. CN medium: starch (2 % w/v) and ammonium sulfate (0.04 % w/v), as carbon and nitrogen sources, respectively.

4.5.11. Effect of $CaCO_3$ concentrations:

The effect of $CaCO_3$ concentrations on growth and red pigment production by *M. purpureus* grown on modified MPI medium (which containing starch and ammonium sulfate, as carbon and nitrogen sources, MMPI) over range from 0.0 to 0.3 % w/v was studied.

A shown in table (13) and figure (15), the biomass content increased by increasing $CaCO_3$ concentrations, and the maximum content (7.61 g/l) was recorded at 0.15 % $CaCO_3$ concentration. Similarly, increasing $CaCO_3$ concentration lead to enhancement red pigment production, and the maximum pigment content (0.91 g/l) was recorded at 0.1 % $CaCO_3$ concentration. Also, the highest values of red pigment conversion (5.61 g/l) and productivity (9.47 mg/l/h) were recorded when the fermentation process carried out in MMPI medium containing 0.1% $CaCO_3$ concentration.

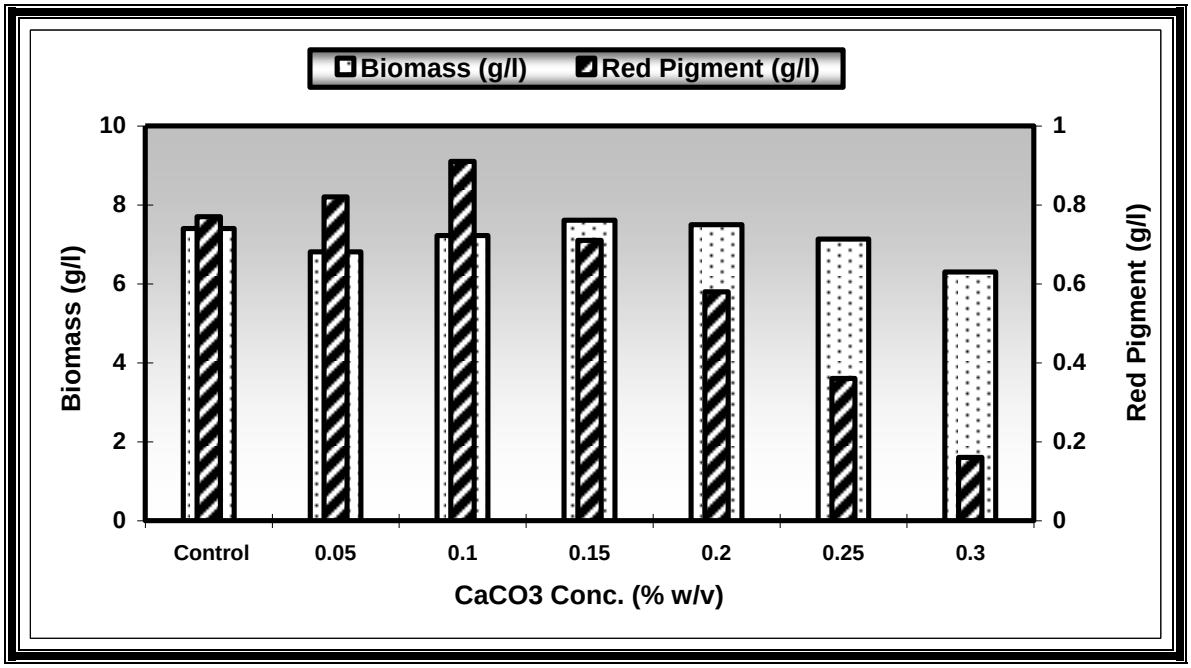
Worthily, the MMPI medium (which containing starch, 2% w/v and ammonium sulfate, 0.04% w/v, as a sole source of carbon and nitrogen, respectively) plus 0.1 % w/v $CaCO_3$ was used in the further investigations as optimized modified MPI medium (OMMPI).

Table (13): Effect of different $CaCO_3$ concentrations (w/v) on red pigment production by *M. purpureus* grown on MMPI medium (growth conditions as in table 8).

$CaCO_3$ conc. (% w/v)	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
0.0 (Control, MMPI)	15.52	7.40	0.77 ±0.004	47.68	4.96	8.02
0.05	15.61	6.81	0.82 ±0.001	43.62	5.25	8.54
0.1	16.20	7.22	**0.91 ±0.002	44.56	5.61	9.47
0.15	15.13	7.61	0.71 ±0.002	50.29	4.69	7.39
0.2	14.21	7.50	0.58 ±0.002	52.77	4.08	6.04
0.25	13.60	7.13	0.36 ±0.002	52.42	2.79	3.95
0.3	12.42	6.30	0.16 ±0.002	50.72	1.28	1.66

* Mean ± SE

** Significant from all values ($P < 0.05$)



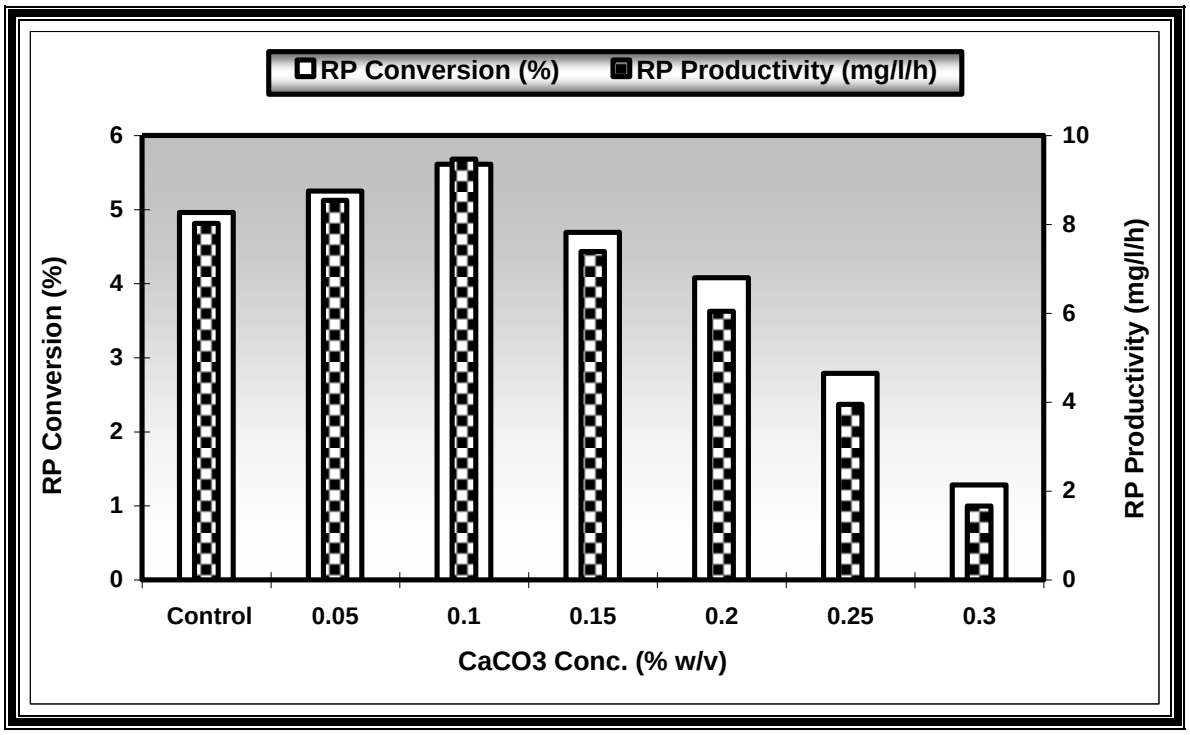


Fig. (15): Effect of different $CaCO_3$ concentrations (w/v) on red pigment production by *M. purpureus* grown on MMPI medium (Growth conditions as in table 8).

4.5.12. Effect of inoculum type:

In this experiment the effect of different types of *M. purpureus* inocula (spore suspension, seed culture and mycelium disc) on the biomass and red pigment production from OMMPI medium was studied.

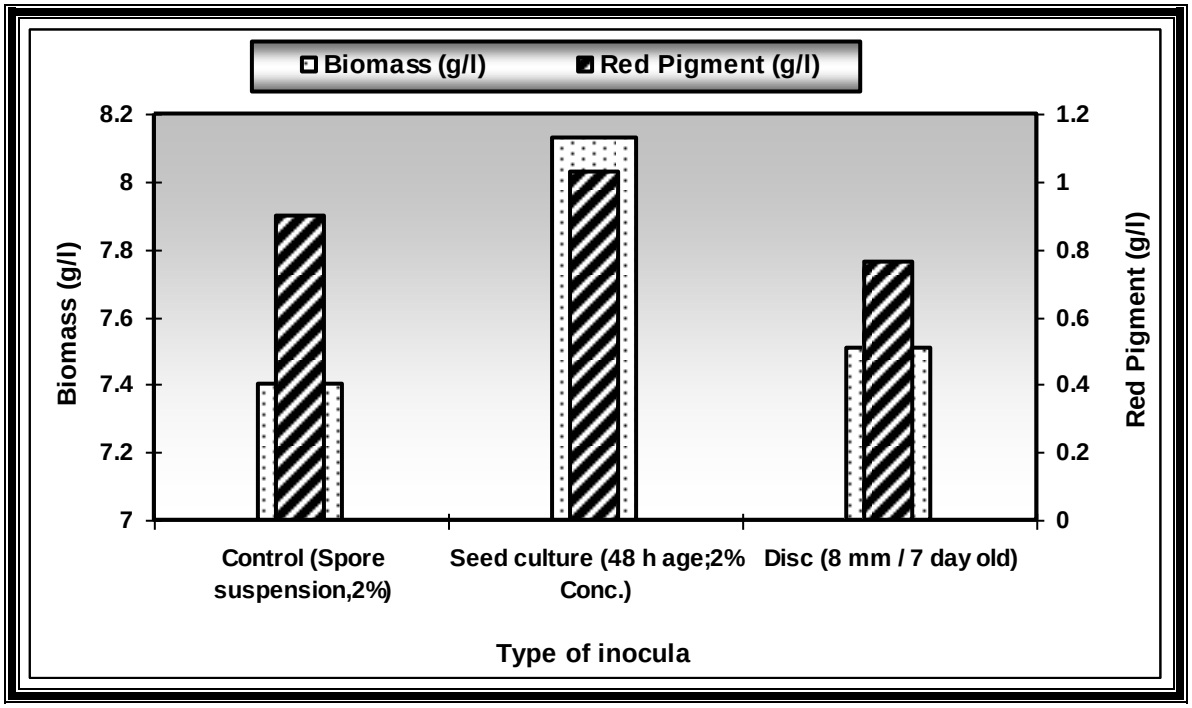
The results presented in table (14) and figure (16) showed that the seed culture inoculum (spore suspension incubated for 48 h before used as inoculum) was found to be the best inocula type for fungus growth (8.13 g/l) and red pigment production (1.03 g/l). Whereas, reduction in biomass content and pigment production (7.51 g/l and 0.75 g/l, respectively) was obtained when vegetative mycelium disc was used as inoculum comparing with seed culture inoculum. Also, maximum red pigment conversion (6.12%) and productivity (10.72 mg/l/h) were recorded when seed culture was used as inoculum.

Table (14): Effect of type of *M. purpureus* inocula on red pigment production from OMMPI medium (MMPI + 0.1 % w/v $CaCO_3$) Growth conditions as in table 8.

Type of inocula	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
Control (Spore suspension,2%)	16.53	7.40	0.90 ±0.007	44.76	5.44	9.37
Seed culture (Age,48 h;2%)	16.81	8.13	**1.03 ±0.005	48.36	6.12	10.72
Disc 8 mm 7 day old	14.20	7.51	0.76 ±0.004	52.88	5.35	7.91

* Mean ± SE

** Significant from all values ($P < 0.05$)



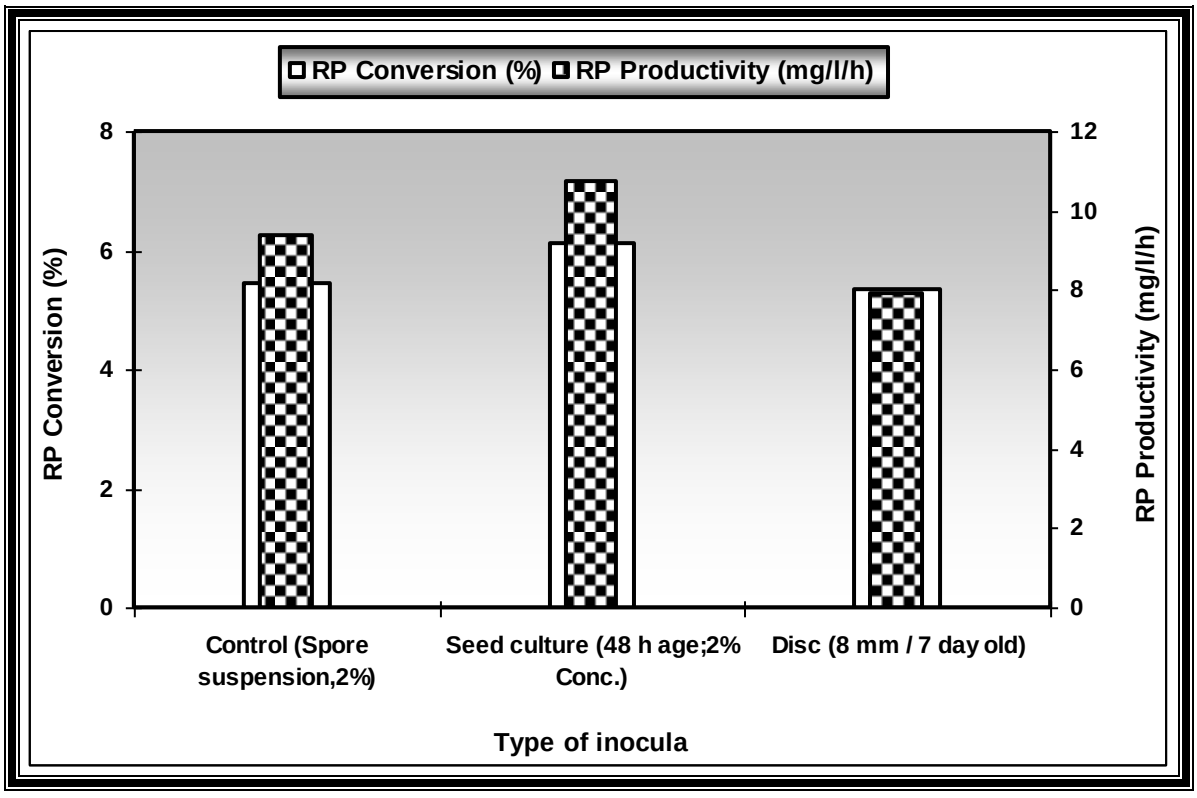


Fig. (16): Effect of type of *M. purpureus* inocula on red pigment production from OMMPI medium (MMPI + 0.1 % w/v $CaCO_3$) (Growth conditions as in table 8).

4.5.13. Effect of inoculum age:

The effect of inoculum (seed culture) age on growth and red pigment production of *M. purpureus* was studied. The OMMPI media (initial pH 5.5) were inoculated with different inoculum ages (0, 12, 24, 36 and 48 h) and incubated at 150 rpm and 30 °C for 4 days.

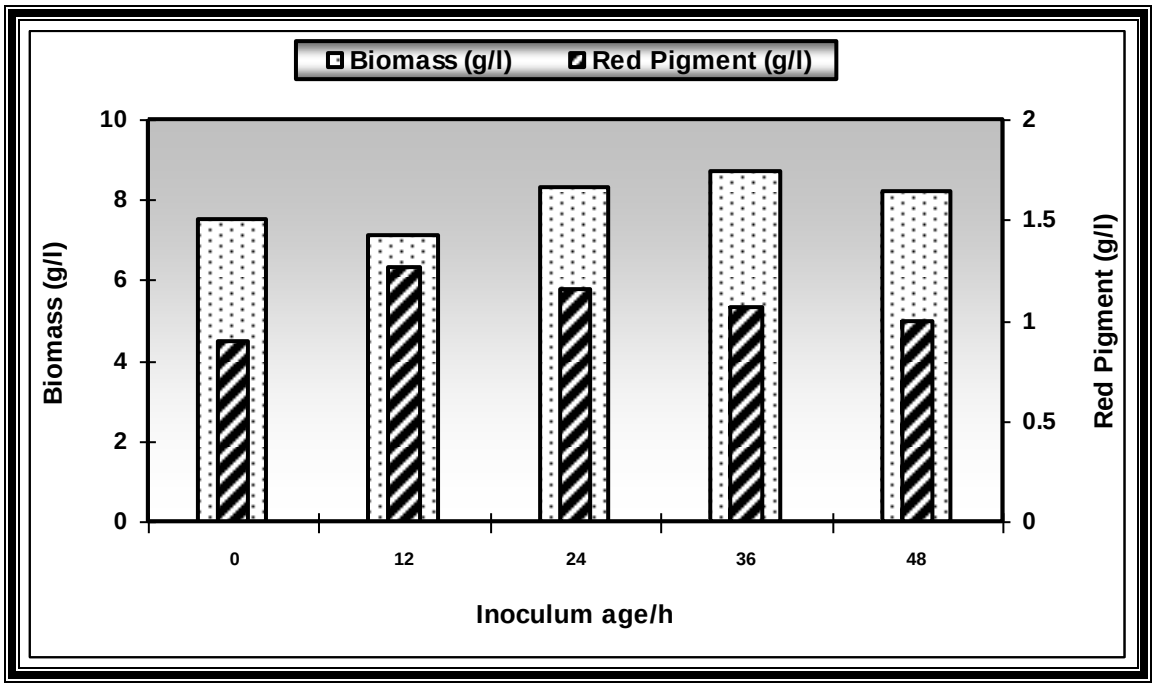
The data represented in table (15) and figure (17) indicated that the inoculum age (24, 36 and 48 h) resulted in nearly similar cell concentration ranging from (8.3, 8.71 and 8.2 g/l, respectively). While, the inoculum age (12 h) gave the lowest growth (7.13 g/l) compared with the spore suspension inoculum (0 h) which recorded 7.51 g/l biomass content. On the other hand, the highest red pigment production (1.26 g/l) was obtained by (12 h) inoculum age with high pigment conversion and productivity (7.63 % and 13.12 mg/l/h, respectively). Whereas, the inoculum age (0 h) recorded lower pigment concentration (0.9 g/l). Meanwhile, it was found that the inoculum age (36 h and 48 h) resulted in relatively similar pigment concentration (1.06 and 1.0 g/l, respectively). As the inoculum age 12h was the best for pigment production, subsequently it was selected to carried out the next experiments.

Table (15): Effect of inoculum (seed culture) age of *M. purpureus* on red pigment production from OMMPI medium (Growth conditions as in table 8).

Inoculum age/h	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
0 (Spore suspension)	16.42	7.51	0.90 ±0.002	45.73	5.5	9.46
12	16.50	7.13	**1.26 ±0.015	43.21	7.63	13.12
24	17.21	8.30	1.15 ±0.005	0.482	6.68	11.97
36	17.63	8.71	1.06 ±0.005	0.494	6.01	11.04
48	17.12	8.20	1.00 ± 0.004	0.479	5.84	10.41

* Mean ± SE

** Significant from all values ($P < 0.05$)



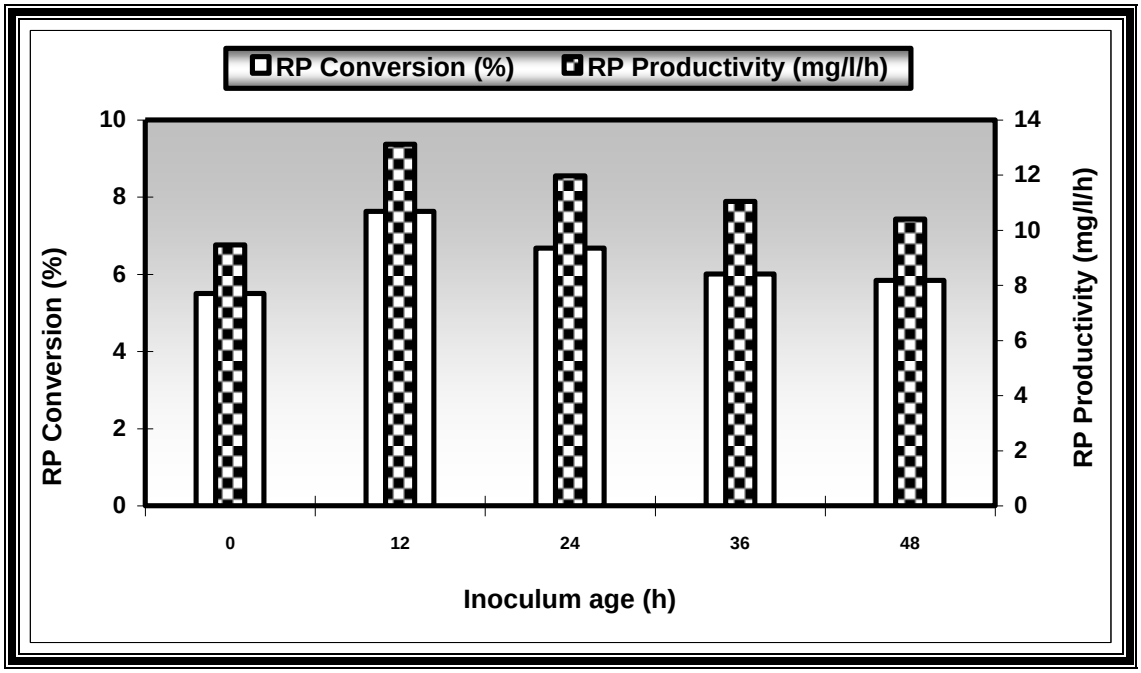


Fig. (17): Effect of inoculum (seed culture) age of *M. purpureus* on red pigment production from OMMPI medium (Growth conditions as in table 8).

4.5.14. Effect of inoculum density:

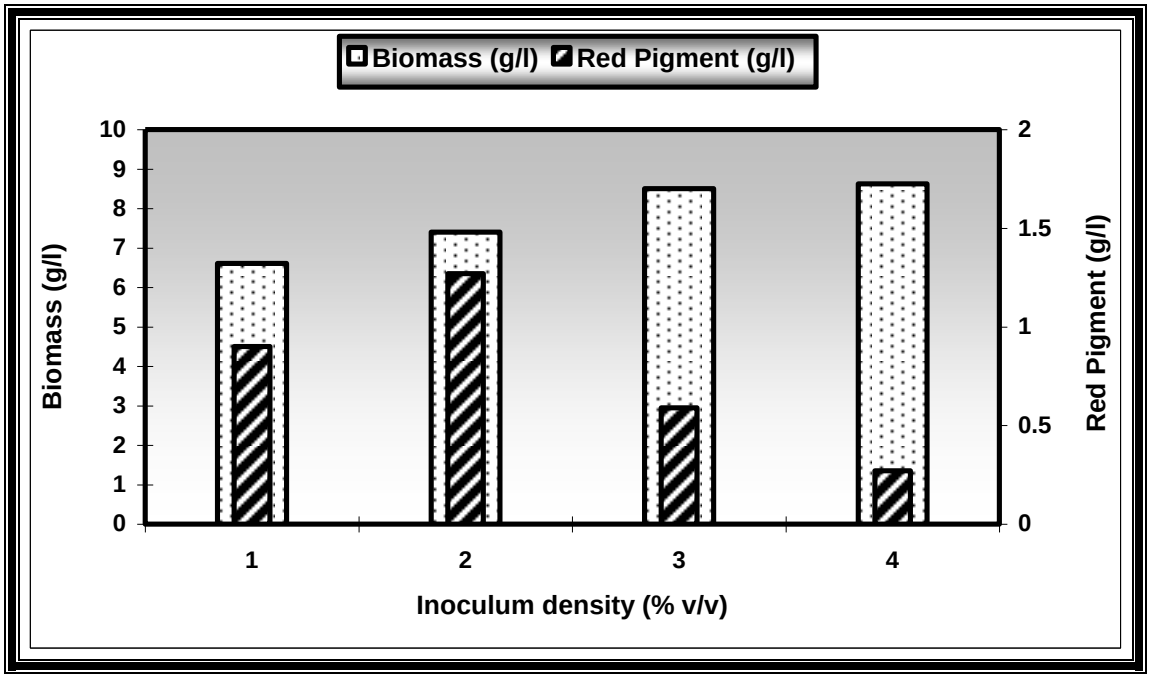
As the effect of inoculum age was studied, the inoculum density on growth and pigment production have to be studied as well. The OMMPI media were inoculated by inoculum age 12 h with different concentration (0.5, 1.0, 1.5 and 2.0 % v/v).

As shown in table (16) and figure (18) the mycelium growth increased from (6.61 to 8.62 g/l) by increasing the inoculum density from (0.5 to 2.0 %) with maximum value of biomass conversion(46.44%) at 1.5 % inoculum density. Regarding red pigment production, gradually increasing in its content from (0.90 to 1.27 g/l) was recorded by increasing inoculum density from (0.5 to 1.0%, respectively). Then pigment synthesis begin to decrease sharply by further increase of inoculum density reaching to 0.27 g/l at 2% inoculum density. Also, the highest conversion of red pigment production (7.54 %) and productivity (13.22 mg/l/h) were recorded at inoculum density 2 %.

Table (16): Effect of inoculum density of *M. purpureus* on red pigment production from OMMPI medium (Growth conditions as in table 8).

Inoculum density (% v/v)	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
1	15.24	6.61	0.90 ±0.002	43.37	5.90	9.37
2	16.83	7.40	**1.27 ±0.018	43.96	7.54	13.22
3	18.30	8.50	0.59 ±0.005	46.44	3.22	6.14
4	19.41	8.62	0.27 ±0.004	44.41	1.39	2.81

* Mean ± SE
** Significant from all values (P < 0.001)



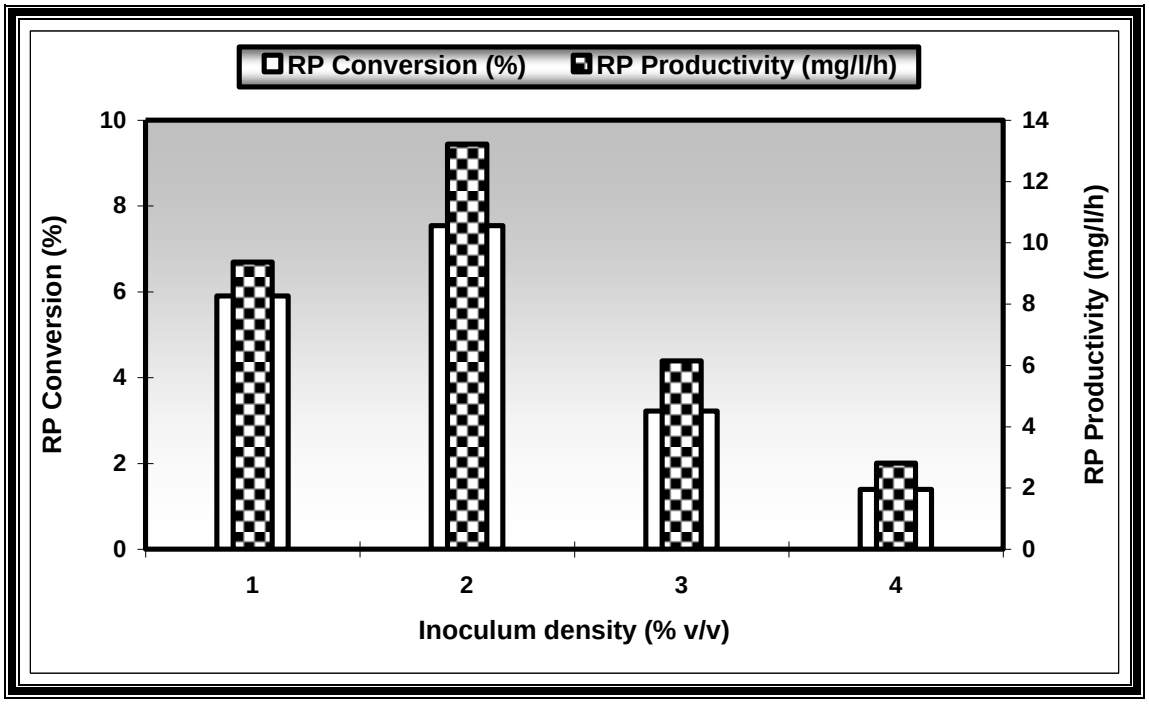


Fig. (18): Effect of inoculum density of *M. purpureus* on red pigment production from OMMPI medium (Growth conditions as in table 8).

4.5.15. Effect of working volume:

The effect of different working volume of OMMPI medium (25, 50, 75, 100, 125 and 150 ml) on growth and red pigment production was studied in 250 ml Erlenmeyer flask at initial pH 5.5 and 30 °C for 4 days, under shaking (150 rpm).

The data represented in table (17) and figure (19) indicated that the maximum growth (8.14 g/l) and biomass conversion were obtained at working volume 75 ml, then it is going to decrease gradually by increasing the volume of the medium reaching (3.72 g/l) at 150 ml. Whereas, the maximum red pigment concentration (1.28 g/l) was obtained at 50 ml medium, with conversion and

productivity content (7.84 % and 13.33 mg/l/h, respectively). On the other hand, sharp decrease of pigment production (0.40 and 0.25 g/l) was obtained at working volumes 125 and 150 ml, respectively.

Table (17): Effect of working volume of OMMPI medium on red pigment production by *M. purpureus* (Growth conditions as in table 8).

Working volume /ml	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
25	19.31	6.31	0.55 ±0.005	32.67	2.84	5.72
50	16.32	7.24	**1.28 ±0.002	44.36	7.84	13.33
75	17.80	8.14	1.13 ±0.004	45.73	6.34	11.77
100	14.24	6.50	0.82 ±0.003	45.64	5.75	8.54
125	11.60	4.41	0.40 ±0.005	38.01	3.44	4.16
150	9.33	3.72	0.25 ±0.005	39.87	2.67	2.60

* Mean ± SE

** Significant from all values ($P < 0.05$)

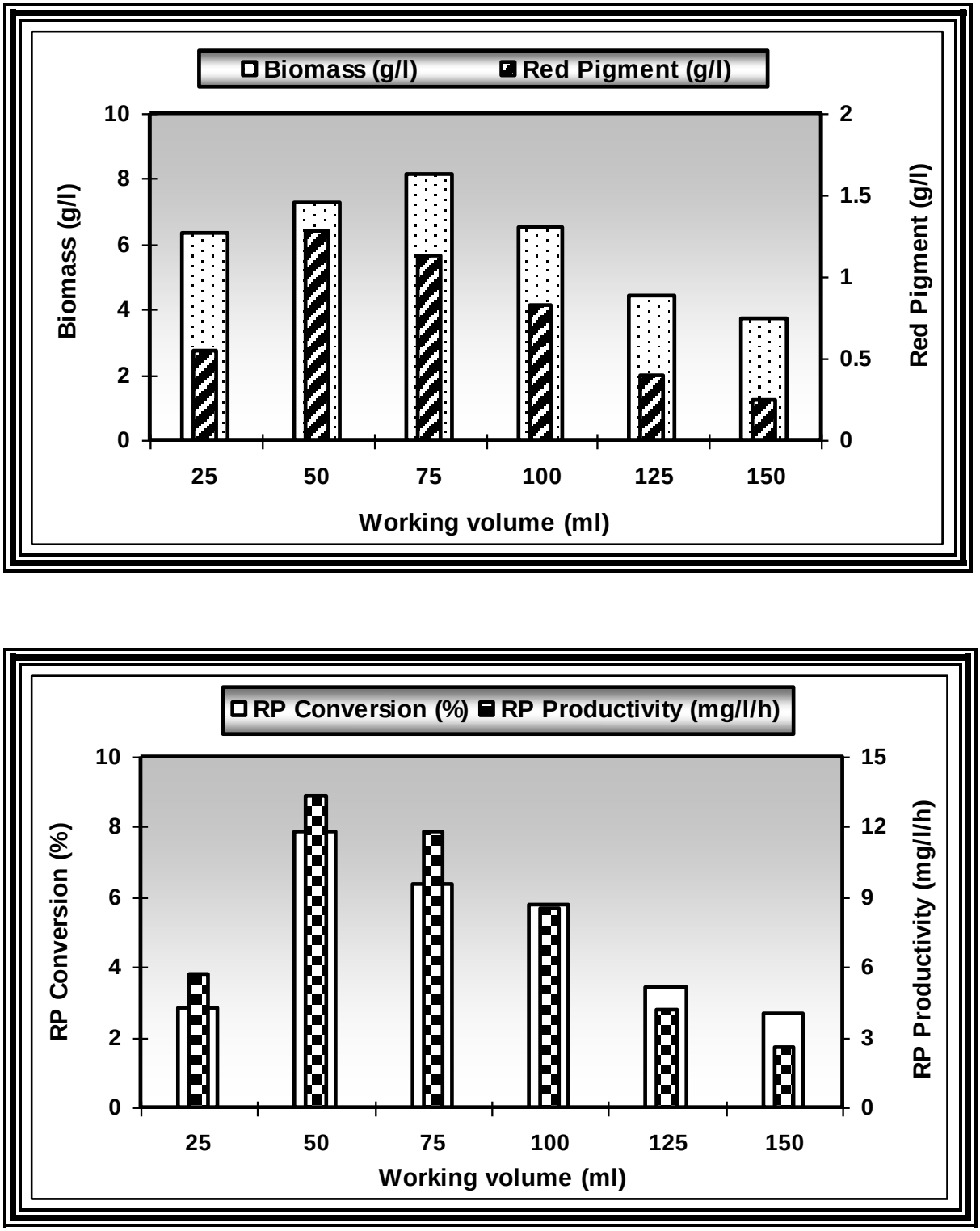


Fig. (19): Effect of working volume of OMMPI medium on red pigment production by *M. purpureus* (Growth conditions as in table 8).

4.6. Gamma irradiation study:

4.6.1. Effect of gamma irradiation on the survival of *M. purpureus*:

The spore suspension (2 x 10^7 CFU /ml) of the higher producer red pigment strain, *M. purpureus*, was exposed to increasing doses of gamma irradiation (0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0 and 4.0 kGy), using ^{60}CO gamma source at dose rate of 0.83 kGy /min. The dose response curve of the survivors were drawn, and the D_{10}-values (the dose required to reduce the initial population by 90% or by one loge cycle) was determined.

Table (18) and figure (20) showed the effect of different doses of gamma-radiation on survival of *M. purpureus*. From these results, it was noticed that the number of viable cells was greatly decreased with increasing the irradiation doses. In addition, dose survival curve for *M. purpureus* illustrated in figure (20) followed a straight line relationship. The D_{10}-value was calculated from the regression line equation as illustrated before,

$$D_{10}\text{-value} = \frac{-1}{b}$$

$$b = \frac{\sum xy - nx`y`}{\sum x^2 - nx`^2}$$

The D_{10}-value of *M. purpureus* obtained from this equation was 0.54 kGy, with correlation coefficient value, -0.991. This means that the correlation between x and y in this study was very strong.

Table (18): Effect of different doses of gamma radiation on the surviving of *M. purpureus* spores.

Doses/kGy	Average count	Log count
0	2×10^7	7.30
0.25	8.3×10^6	6.92
0.50	3×10^6	6.47
0.75	3×10^5	5.47
1.00	6×10^4	4.77
1.50	9.3×10^3	3.96
2.00	1.6×10^3	3.2
2.50	2×10^2	2.3
3.00	30	1.47
4.00	No growth	-

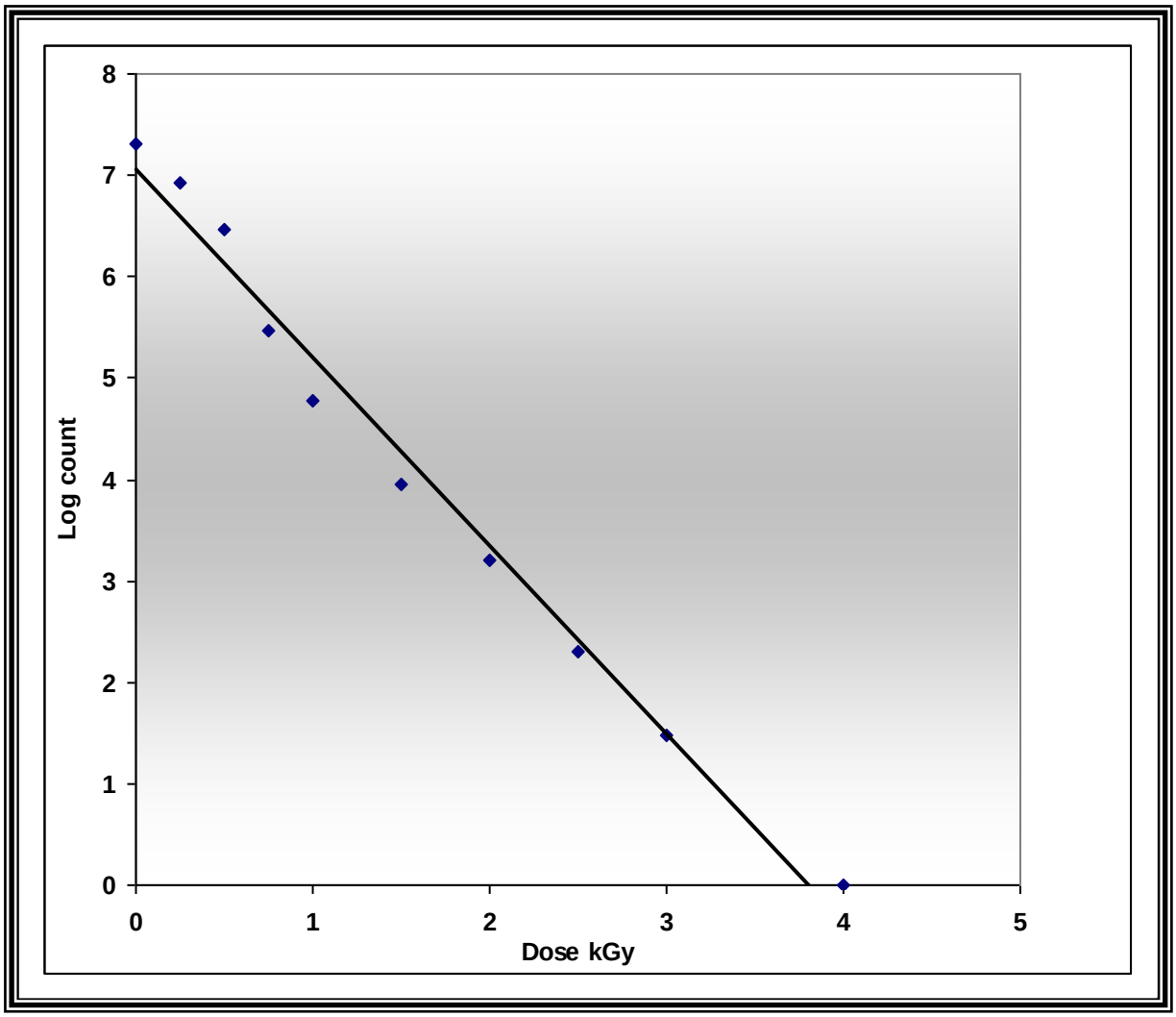


Fig.(20): Effect of different doses of gamma radiation on the surviving of *M. purpureus* spores.

4.6.2. Effect of gamma irradiation on the production of red pigment:

The present experiment was carried out to investigate the effect of gamma radiation on the activity of *M. purpureus* towards red pigment production. The irradiated spore suspensions (3.6.1) were used for preparation of seed culture inocula (12 h-old). The inoculated OMMPI medium (pH 5.5) was incubated at 30 °C for 4 days under shaking (150 rpm), the cell dry weight and red pigment concentrations were investigated.

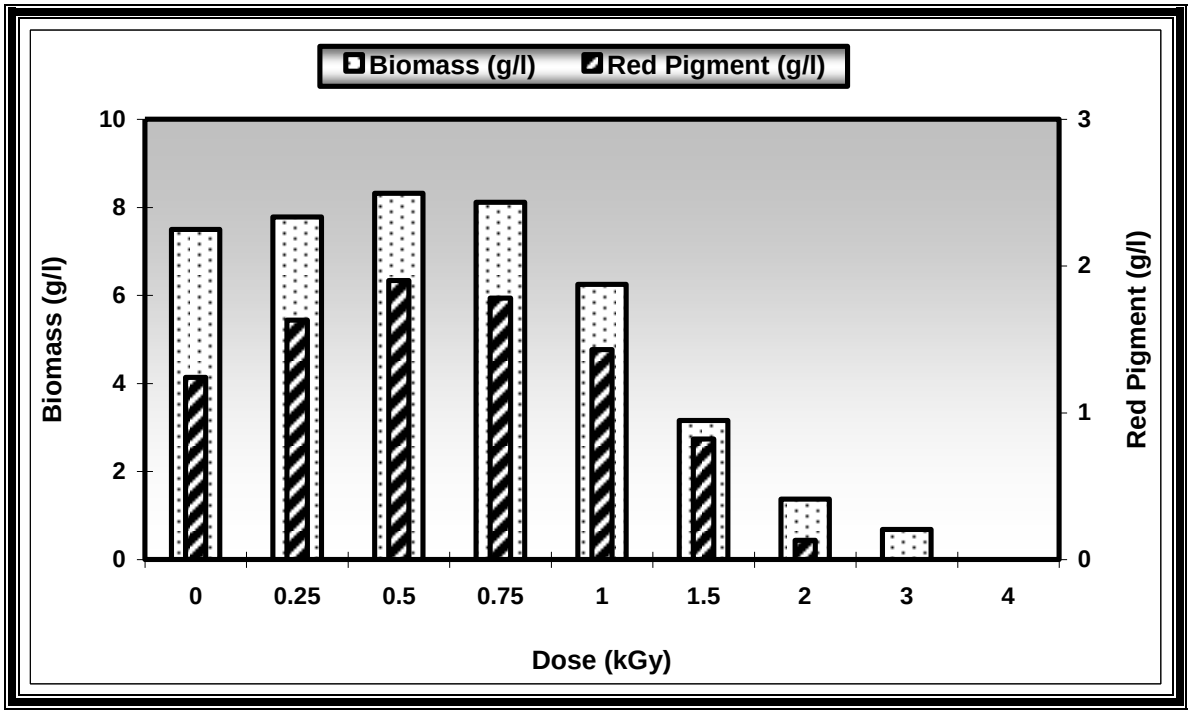
The data in table (19) and figure (21) showed that the low doses of gamma radiation from 0.25 to 0.75 kGy stimulate both growth content and red pigment production in comparison with the control (non irradiated inoculum). The highest cell content and pigment concentration 8.32 and 1.90 g/l, respectively, were achieved at the dose 0.50 kGy with pigment conversion of 10.32 % and productivity of 19.79 mg/l/h. While, a decrease in the biomass was recorded by the increasing doses of gamma radiation (>0.75 kGy) compared with control. Also, decreasing in red pigment production was achieved by the increasing doses (> 1.0 kGy) in comparison with control. Furthermore, the data revealed that little amount of biomass content and no production of pigment was obtained at a dose 3.0 kGy. In addition, no growth of fungus was obtained at a dose 4.0 kGy.

Table (19): Effect of different doses of gamma radiation on the activity of *M. purpureus* for red pigment production from OMMPI medium (Growth conditions as in table 8).

Dose/ kGy	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
Zero	16.23	7.50	1.24 ±0.005	46.21	7.64	12.91
0.25	17.30	7.78	1.63 ±0.004	44.97	9.42	16.97
0.50	18.40	8.32	**1.9 ±0.004	45.21	10.32	19.79
0.75	18.24	8.11	1.78 ±0.002	44.46	9.75	18.54
1.00	14.91	6.25	1.43 ±0.004	41.91	9.69	14.89
1.50	7.50	3.16	0.82 ±0.002	42.13	10.93	8.54
2.00	2.80	1.37	0.13 ±0.005	48.92	4.64	1.35
3.00	1.62	0.68	-	41.97	-	-
4.00	**No growth**					

* Mean ± SE

** Significant from all values ($P < 0.05$)



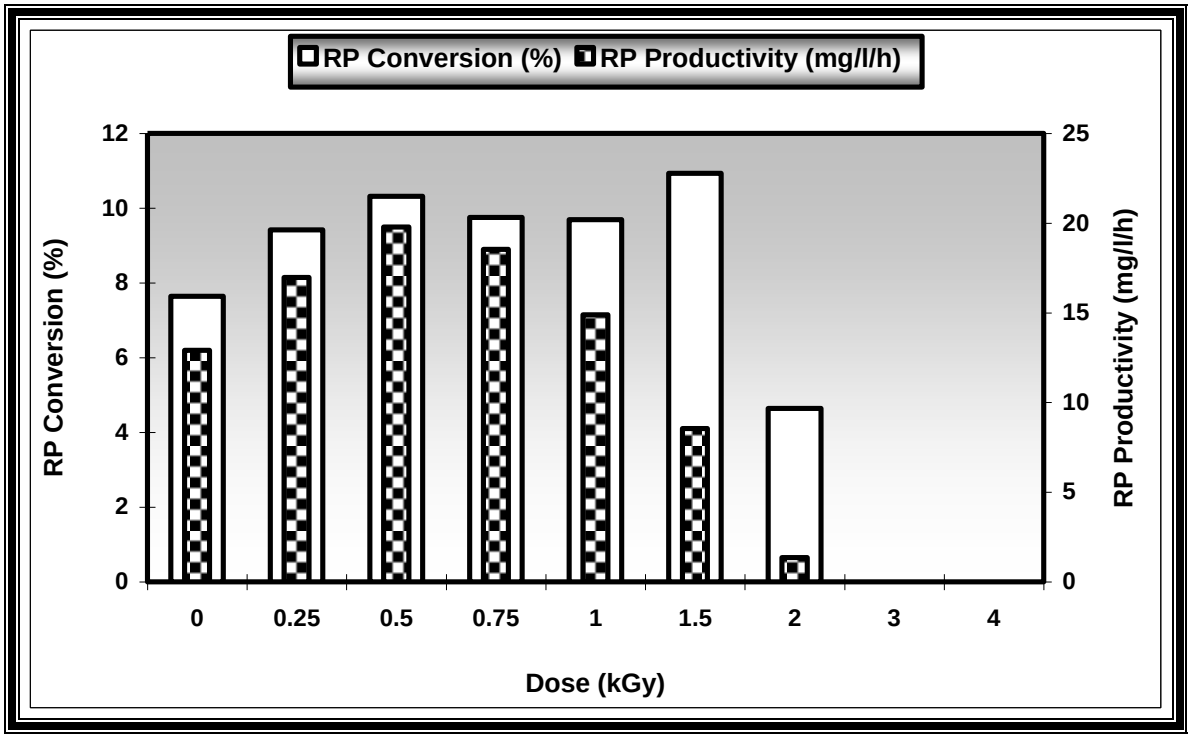


Fig. (21): Effect of different doses of gamma radiation on the activity of *M. purpureus* for red pigment production from OMMPI medium (Growth conditions as in table 8).

4.7. Immobilization study:

Conventional methods of fermentation that use free cells in batch processes have several limitations, the use of immobilized cells offers several advantages over free cells, such as its higher production and stability for long fermentation time.

4.7.1. Red pigment production by sponge-immobilized cells of gamma irradiated *M. purpureus*:

In this experiment, production of red pigment by irradiated cells entrapped in sponge cubes was investigated. The irradiated spores (0.5 kGy) of *M. purpureus* were immobilized in sponge cubes (for 12 h) and the batch cultures (50 ml) of OMMPI medium (pH 5.5) with sponge cubes (0.5 g) containing irradiated cells were incubated under obtained optimize cultural conditions.

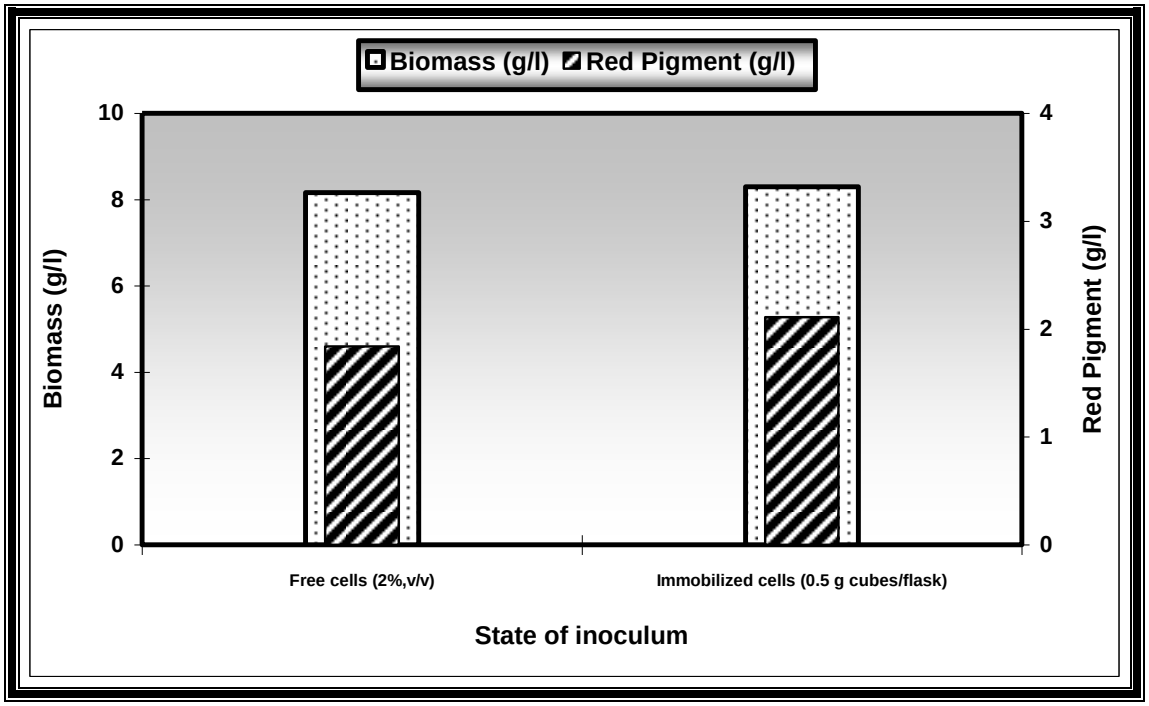
The results present in table (20) and fig. (22) showed that using of immobilized cells as inoculum lead to increasing of biomass and red pigment production (8.3 and 2.11 g/l, respectively) comparing with free cells inoculum (8.16 g/l and 1.84 g/l, respectively). Also, the conversion of pigment production and its productivity reached to their maximum values (11.57 % and 21.97 mg/l/h, respectively) with immobilized cells.

Table (20): Red pigment production from OMMPI medium by immobilized gamma irradiated (0.5 kGy) cells (12 h age) of *M. purpureus* (Growth conditions as in table 8).

State of inoculum	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
Free cells (2%,v/v)	17.44	8.16	1.84 ±0.004	46.78	10.55	19.16
Immobilized cells (0.5 g cubes/flask)	18.23	8.30	**2.11 ±0.006	45.52	11.57	21.97

* Mean ± SE

** Significant from all values ($P < 0.05$)



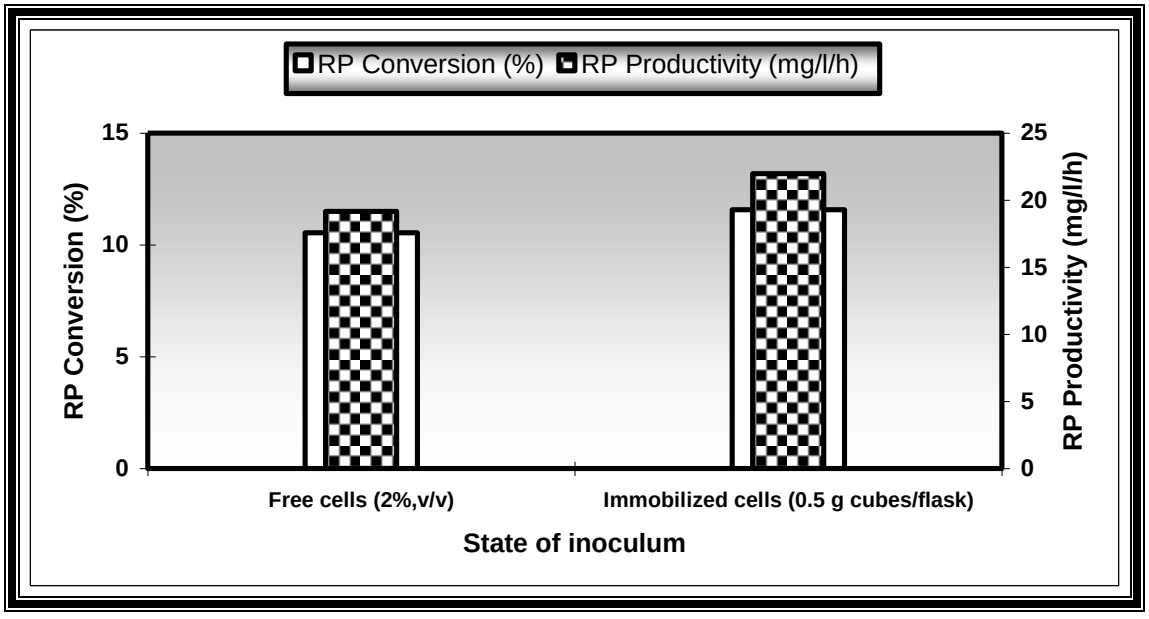


Fig. (22): Red pigment production from OMMPI medium by immobilized gamma irradiated (0.5 kGy) cells of *M. purpureus* (Growth conditions as in table 8).

4.7.2. Effect of immobilized inoculum age:

To investigate the effect of the age of immobilized cells on growth and red pigment production from OMMPI medium, irradiated spore suspension with 0.5 kGy, was immobilized on 0.5 g sponge cubes for different time ranging from 12 to 48 h, and used as inocula.

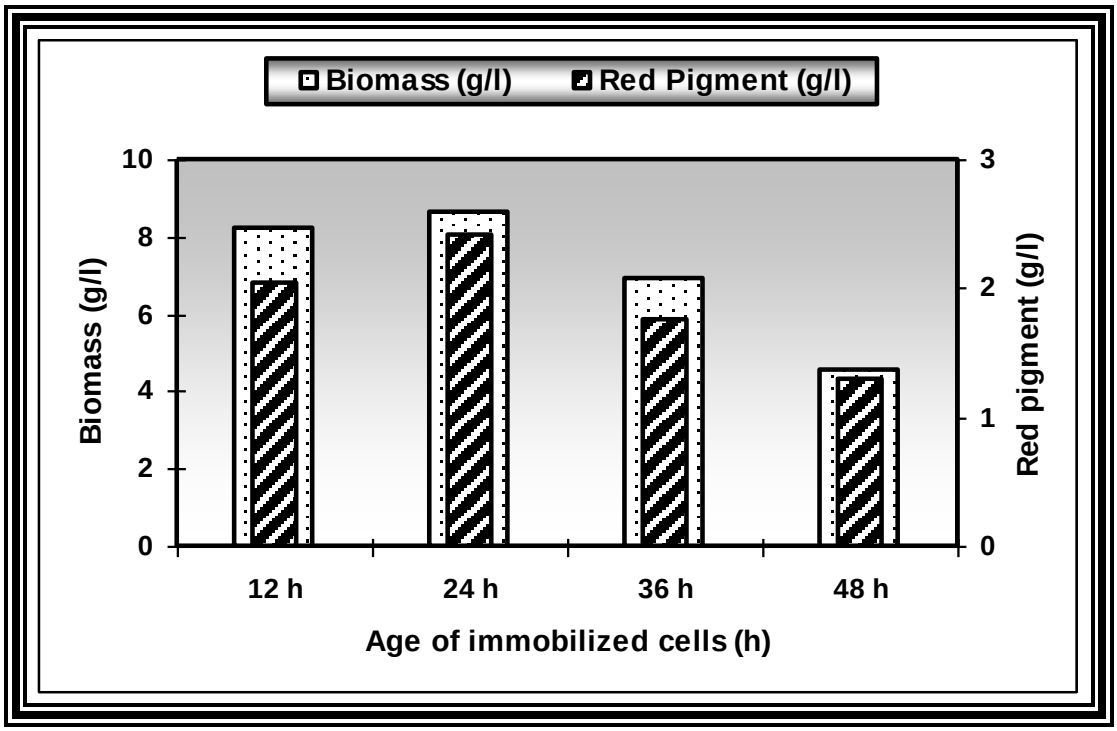
The data represented in table (21) and figure (23) indicated that the immobilized cells age (24 h) gave the highest biomass (8.64 g/l) and red pigment (2.41 g/l) production. While, the lowest growth (4.55 g/l) and pigment (1.29 g/l) amounts were recorded with immobilized cells age (48 h). Also, red pigment conversion (12.80 %) and its productivity rate (25.10 mg/l/h) were recorded with immobilized cell age (24 h).

Table (21): Effect of inoculum age of gamma irradiated (0.5 kGy) immobilized cells of *M. purpureus* (0.5 g cubes/flask) on red pigment production from OMMPI medium (Growth conditions as in table 8).

Age of immobilized cells/h	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
12 h	18.15	8.20	2.05 ±0.004	45.17	11.29	21.35
24 h	18.82	8.64	**2.41 ±0.003	45.90	12.80	25.10
36 h	16.30	6.90	1.76 ±0.004	42.33	10.79	18.33
48 h	11.45	4.55	1.29 ±0.004	39.73	11.26	13.43

* Mean ± SE

** Significant from all values ($P < 0.05$)



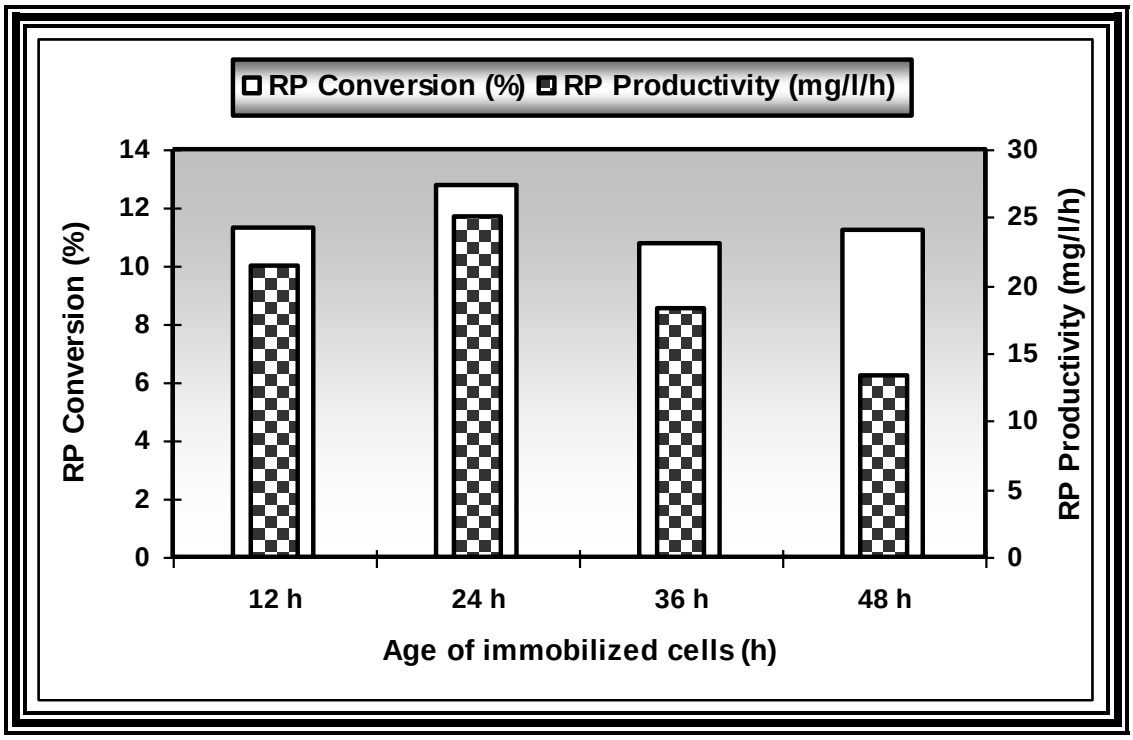


Fig. (23): Effect of inoculum age of gamma irradiated (0.5 kGy) immobilized cells of *M. purpureus* (0.5 g cubes/flask) on red pigment production from OMMPI medium (Growth conditions as in table 8).

4.7.3. Effect of immobilized inoculum density

The aim of this experiment is to determine the best inoculum density of immobilized cells for growth and red pigment production. Erlenmeyer flasks containing 50 ml of OMMPI medium (pH 5.5) were inoculated with different weight of immobilized sponge cubes (24 h age) ranging from 0.25 to 1.0 g/ flask, and incubated under optimized fermentation conditions.

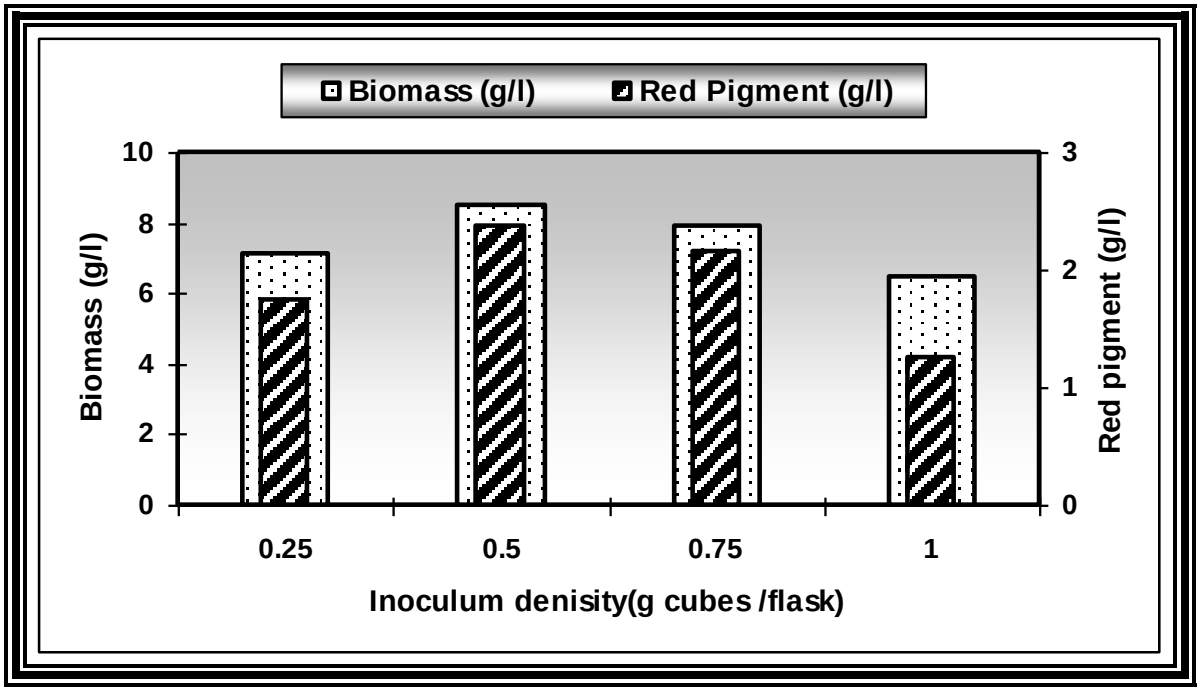
The data represented in table (22) and figure (24) illustrated that the red pigment concentrations ranged from 1.74 to 1.25 g/l with immobilized cells densities from 0.25 to 1.0 g (% w/v). The maximum red pigment production (2.38 g/l) was obtained at inoculum size of 0.5 g (% w/v) with conversion value (12.72 g/l) and productivity rate (24.78 mg/l/h). Regarding microbial growth the maximum biomass (8.50 g/l) was obtained at immobilized cells density 0.5g (% w/v). Meanwhile, increasing inoculum size lead to decreasing in biomass content.

Table (22): Effect of inoculum density of gamma irradiated (0.5 kGy) immobilized cells of *M. purpureus* on red pigment production from OMMPI medium (Growth conditions as in table 8).

Inoculum density (g cubes/flask)	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
0.25	15.63	7.13	1.74 ±0.005	45.61	11.13	18.12
0.50	18.71	8.50	**2.38 ±0.003	45.43	12.72	24.79
0.75	19.24	7.92	2.15 ±0.001	41.16	11.17	22.39
1.00	18.80	6.44	1.25 ±0.004	34.25	6.64	13.02

* Mean ± SE

** Significant from all values ($P < 0.05$)



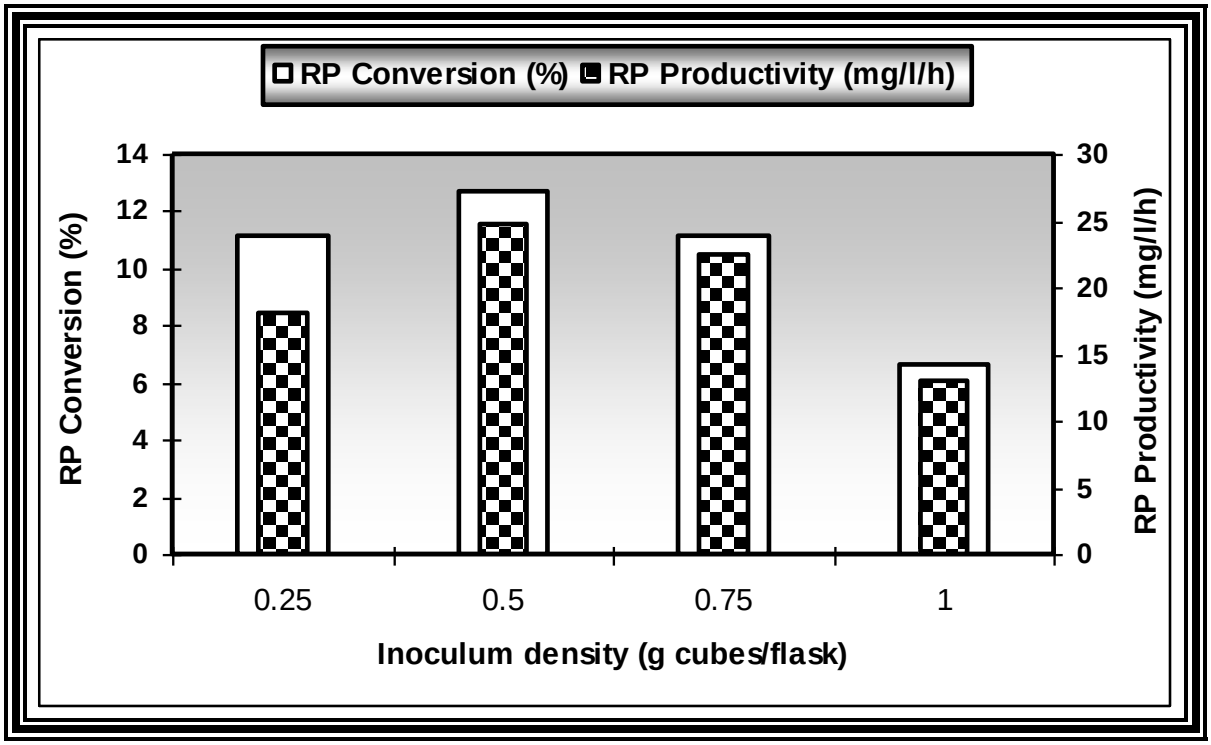


Fig. (24): Effect of inoculum density of gamma irradiated (0.5 kGy) immobilized cells of *M. purpureus* on red pigment production from OMMPI medium (Growth conditions as in table 8).

4.7.4. Time profile:

In this experiment, the inoculated flasks containing 50 ml OMMPI medium (pH 5.5) and 0.5 g of immobilized sponge cubes (24 h age) were incubated at various cultivation times under optimized fermentation conditions.

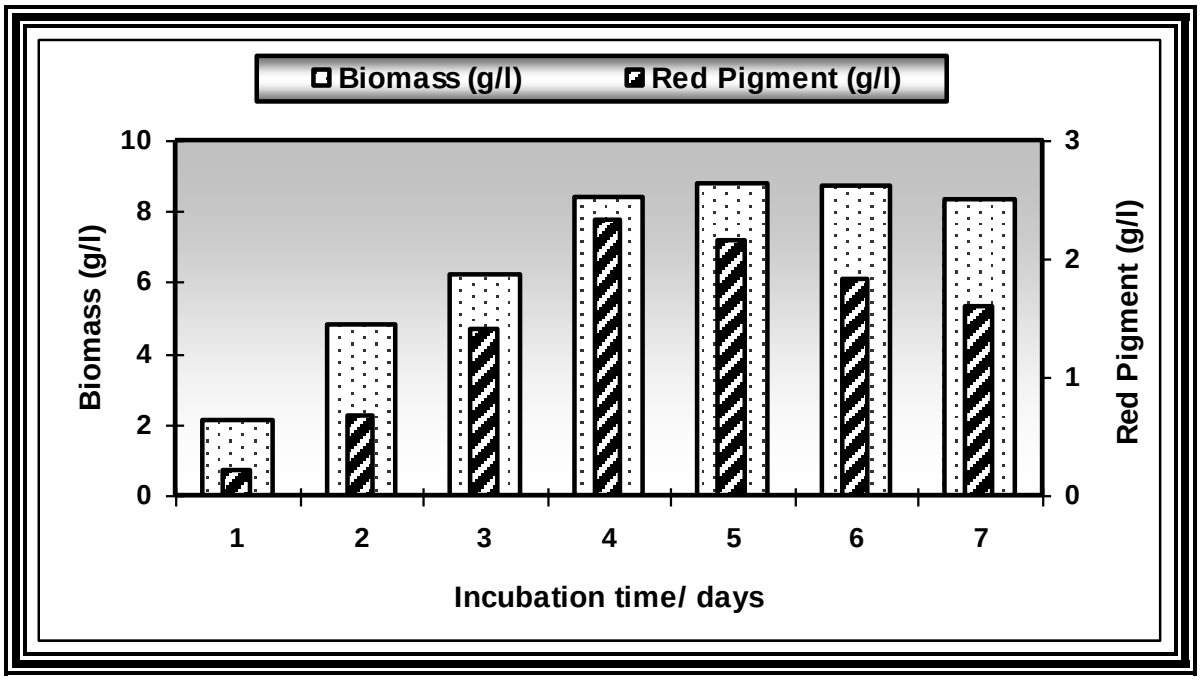
From the results in table (23) and figure (25), it is clear that the red pigment production was increased rapidly for the first 4 days then decreased. The amount of pigment production reached its maximum concentration (2.32) g/l after 4 days of cultivation, comparing with (1.84 g/l) for pigment produced by free irradiated cells at the same time (Table-20). Thereafter, the amount of produced pigment decreased to be (1.60 g/l) after 7 days of incubation. In addition, the maximum values of red pigment conversion and productivity (12.53 % and 24.16 mg/l/h, respectively) were recorded after 4 days of incubation. On the other hand, the mycelium growth was increased rapidly with increasing incubation period till reached its maximum (8.76 g/l) after 5 days of cultivation. In addition, little decrease of biomass production was occurred with increasing incubation time after 5 days.

Table (23): Time course of red pigment production by gamma irradiated (0.5 kGy) immobilized cells of *M. purpureus* from OMMPI medium (Growth conditions as in table 8).

Incubation time/days	Consumed sugar, CS (g/l)	Biomass, B (g/l)	Red pigment, RP (g/l) *	B Conversion (%)	RP Conversion (%)	RP Productivity (mg/l/h)
1	4.82	2.13	0.21 ±0.003	44.19	4.35	8.75
2	9.13	4.80	0.68 ±0.004	52.57	7.44	14.16
3	14.70	6.25	1.40 ±0.003	42.51	9.52	19.44
4	18.51	8.42	**2.32 ±0.002	45.48	12.53	24.16
5	19.32	8.76	2.15 ±0.003	45.34	11.12	17.91
6	19.50	8.74	1.82 ±0.002	44.82	9.33	12.63
7	19.62	8.32	1.60 ±0.002	42.40	8.15	9.52

* Mean ± SE

** Significant from all values ($P < 0.05$)



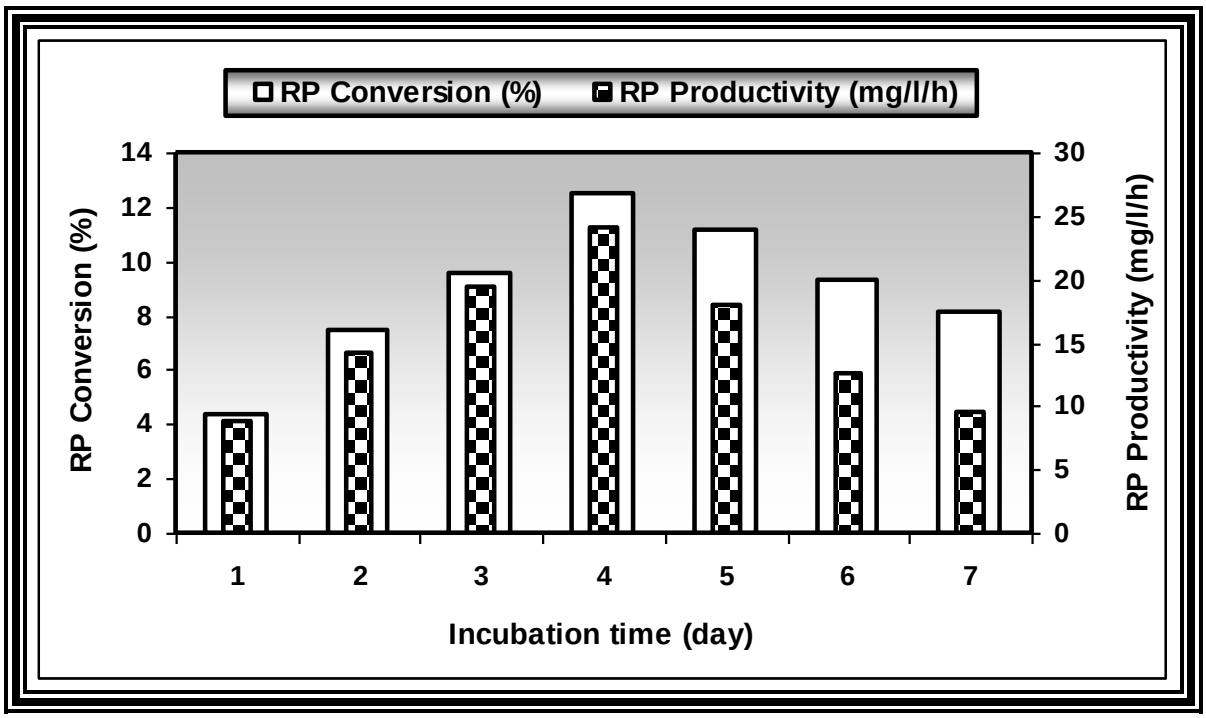


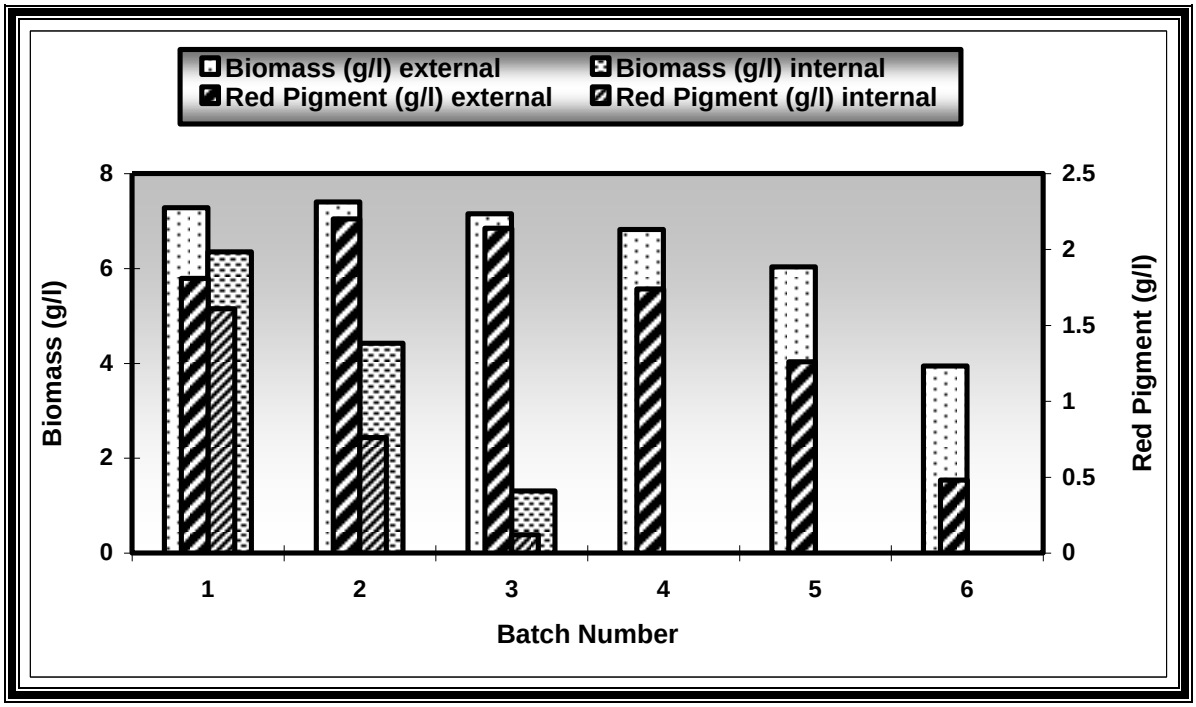
Fig. (25): Time course of red pigment production by gamma irradiated (0.5 kGy) immobilized cells of *M. purpureus* from OMMPI medium (Growth conditions as in table 8).

4.7.5. Effect of repeated batch fermentation:

The present study was directed to use PPW (cheap agro-industrial waste), which containing total sugar 21.8 g/l and nitrogen content 0.46 g/l, as the main medium for red pigment production to substitute the OMMPI medium under repeated batch fermentation. Each 50 ml of prepared PPW (pH 5.5) were inoculated by 0.5 g of immobilized sponge cubes (24 h age) and incubated at 30°C and 150 rpm (optimized fermentation conditions) for 6 cycles, (each cycle consisting of 4 days).

As shown in table (24) and figure (26), the amount of red pigment produced by free cells reached its maximum (1.61 g/l) in the first batch and decreased markedly in the subsequent batches. With immobilized cells, the red pigment production was increased from the first batch (1.81g/l) to the fourth (1.74 g/l), in comparison with maximum free cells production, with maximum concentration (1.96 g/l) at the second batch. In addition, the highest red pigment conversion and productivity (9.98 % and 20.41 mg/l/h, respectively) were observed by immobilized cells at the second batch. Similarly, biomass production by immobilized cells was fairly highly after 4 batches, in comparison with maximum free cells production, with maximum content (7.40 g/l) at the second batch. On the other hand, the maximum biomass produced by free cells (6.35 g/l) was obtained in the first batch, then sharp decrease was occurred.

(Table 24): Red pigment production from PPW in repeated batch process (4 days for each run) by gamma irradiated (0.5 kGy) free (1%,v/v, 12 h age) or immobilized (0.5 g cubes/flask, 24 h age) cells of *M. purpureus* under optimized fermentation conditions (initial pH: 5.5, incubation temp. : 30 °C, working volume medium: 50 ml/flask and agitation rate:150rpm).



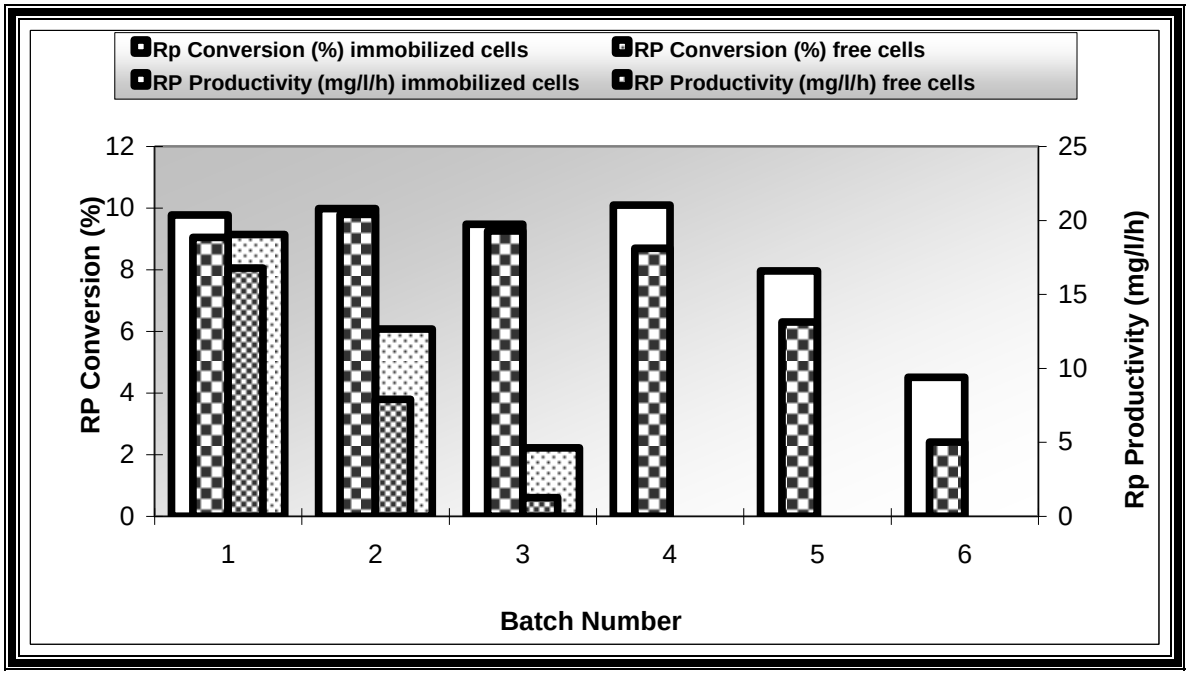


Fig. (26): Red pigment production from PPW in repeated batch process (4 days for each run) by gamma irradiated (0.5 kGy) free (1%,v/v, 12 h age) or immobilized (0.5 g cubes/flask, 24 h age) cells of *M. purpureus* under optimized fermentation conditions (initial pH:5.5, incubation temp.:30°C, working volume medium: 50ml/flask and agitation rate:150rpm).

Furthermore, Table (24) and Fig. (27) shows the reduction of BOD profiles of the operated runs (6 batches). The results revealed that the major reduction of BOD was occurred by immobilized cells during the first 4 batches (80.2 to 76.5 %), but the sharp reduction (82.6%) was recorded in the second batch and it reached 34.40 % after 24 days (6 batches) of runs.

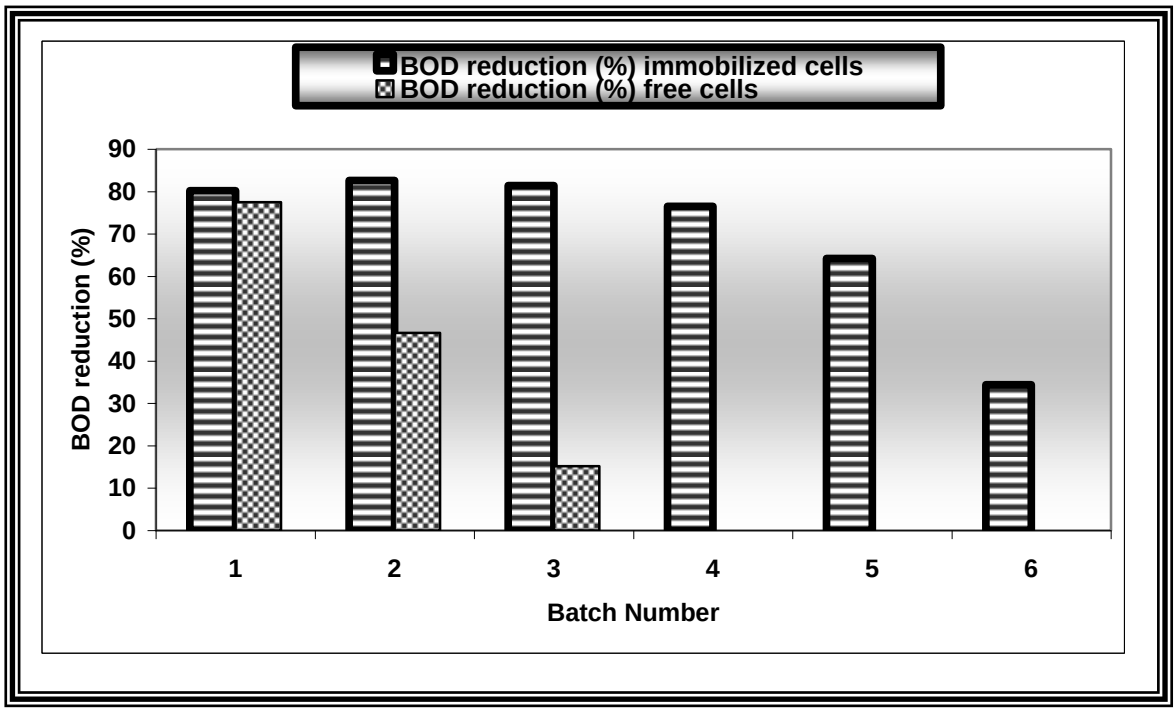


Fig. (27): Reduction of BOD for PPW in repeated batch process (4 days for each run) by gamma irradiated (0.5 kGy) free (1%,v/v, 12 h age) or immobilized (0.5 g cubes/flask, 24 h age) cells of *M. purpureus* under optimized fermentation conditions (initial pH:5.5, incubation temp.:30°C, working volume medium:50ml/flask and agitation rate:150rpm).

4.8. Antimicrobial effects:

The antimicrobial activity of the ethanol extracted red pigment produced by *M. purpureus* (0.1 %) was demonstrated on *B. subtilis, E. coli, Asp. flavus* and *Asp. ochraceus.* As shown in Plate (2), the red pigment produced by this strain was found to be without any antibacterial and antifungal activities and had no influence on the growth rate.

4.9. Toxicological evaluation:

Fertile eggs were employed in testing for toxins, especially mycotoxins, since chicken embryos are very sensitive to aflatoxin and other mycotoxins. In this regard, the same method was used to determine the possible toxicity of the *M. purpureus* culture filterate and evaluate the presence of toxic substances contaminating the red pigment recovered from ethanol extraction of the culture filtrate.

As shown in table (25) and figure (28), the data revealed that survival of chicken embryos inoculated with red pigment dissolved in ethanol indicated that the trace amount of soluble toxic substance in the sample lead to only 8 % death of chicken embryo. However, little toxic substance in the culture filtrate of PPW medium cultivated with immobilized cells of gamma irradiated *M. purpureus* caused a moderate death rate (12 %) among tested chicken embryos, compared with the control treatment (sterile PPW) which caused 10 % death.

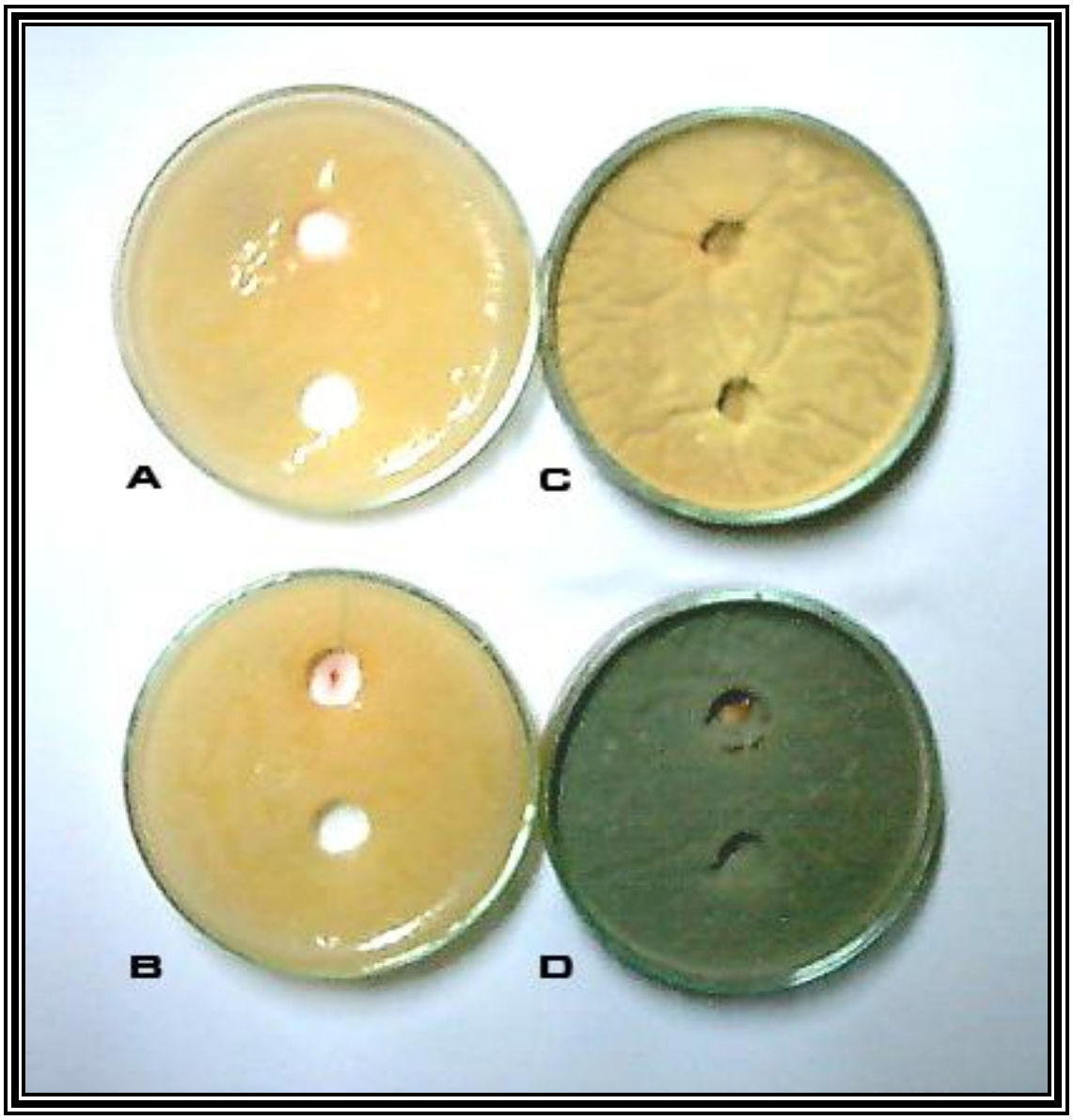


Plate (2) Antimicrobial activity of ethanolic crude red pigment extract (0.1% v/v) from *M. purpureus* (the extract in the upper holes and the control, ethanol, in the lower holes). A= *Bacillus subtilis*, B= *Escherichia coli*, C= *Asp. ochraceus* and D= *Asp. flavus*.

Table (25) Effect of red pigment produced by gamma irradiated (0.5 kGy) immobilized cells of *M. purpureus* on chicken embryos.

Treatment	Dead chicken embryos/total inoculated eggs	Dead (%)
Control eggs inoculated with:		
Sterile distilled water	0 / 16	0
Sterile PPW medium	2 / 20	10
Sterile ethanol	1 / 20	5
Culture filtrate of PPW medium cultivated with *M. purpureus*	3 / 25	12
Red pigment dissolved in ethanol (0.1%v/v)	2 / 25	8

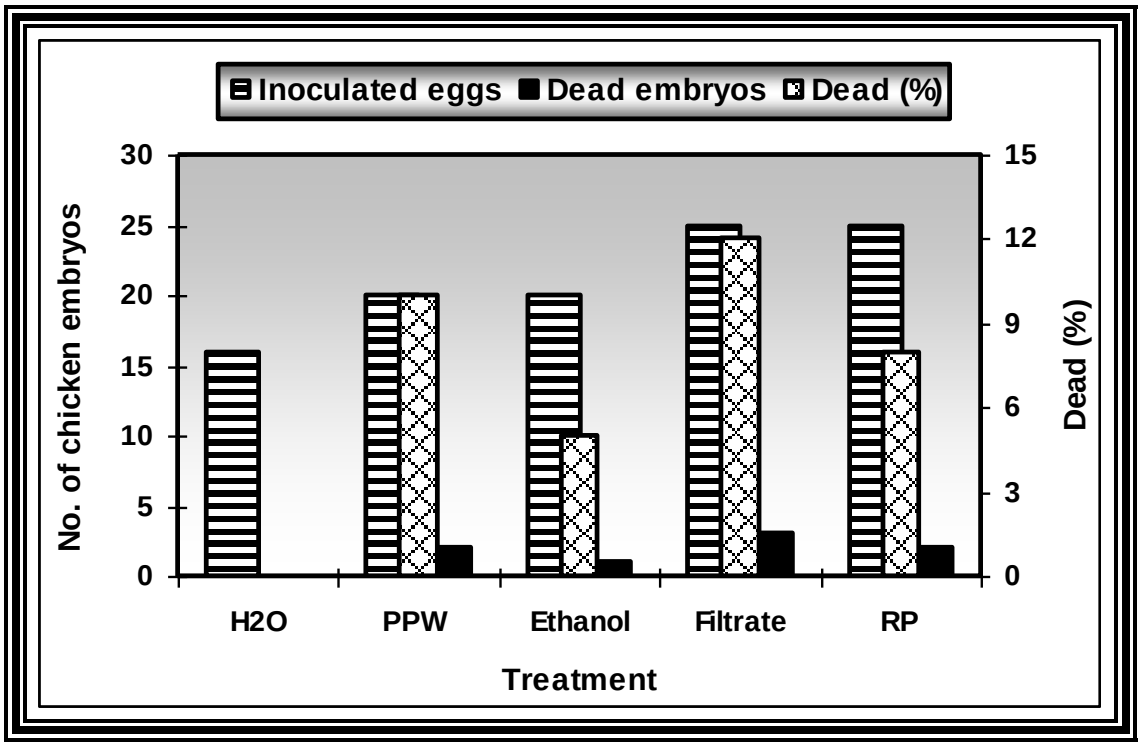


Fig (28): Effect of red pigment produced by gamma irradiated (0.5 kGy) immobilized cells of *M. purpureus* on chicken embryos.