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Improvement of Phytoremediation Potentiality of Artificial Polluted Water by Increasing Coagulating Protein Production from Syrian Rue (*Peganum Harmala* L.) under *in Vitro* Conditions

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Abstract : Harmal (*Peganum harmala* L.) is an abundant flowering plant in the Middle East and North Africa which grows in semi-arid conditions and is of Central Asian origin. In this study, *P. harmala* shoots were regenerated on MS medium supplemented with 3 mg L^{-1} BAP, 30 gL^{-1} sucrose and 0.8% agar. The effect of different additives with different concentrations on protein production *in vitro* was investigated with the aim of further utilization in precipitating lead (Pb) and cadmium (Cd) pollutants/ flocculants in artificial contaminated water. Results showed that the media supplemented with 200 mg L^{-1} tryptophan, 200 mg L^{-1} tyrosine or 20 mg L^{-1} pyruvic acid enhanced protein production. 0.2 ppm Pd and 20 ppm Cd added to double distilled water were flocculated using NaOH and turbidity was measured after protein samples were added to determine coagulation efficiency of proteins extracted from plants of each treatment. Protein coagulation was induced on pH 6, 5, 4 and 3, subsequently and extracted proteins were quantified and analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The gels were showed 63 bands that were of high polymorphism among the accessions with noticeable differences observed in the 90.94 KDa area which is responsible to precipitate heavy metals. Results showed that adding 200 mgL^{-1} tryptophan to the culture medium resulted in the highest value of this protein (96.7 KD protein) which afterwards precipitated heavy metals in water more efficiently compared to the other additives.
Keywords: Phytoremediation Potantialty of Artificial Polluted Water, Coagulating Protein Production, Syrian Rue (*Peganum Harmala* L.).

Introduction

Harnal (*Peganum harmala*) is a flowering plant which belongs to the Zygophyllaceae family. It is usually propagated from seeds[1] and has multi-branched stems that rarely grow over two feet tall and generally appears round and bushy[2]. Plants have antibacterial, antifungal and antiviral effects[3] and seeds contain the highest proportion of alkaloids which proved to be toxic and affect central nervous and respiratory systems[4] however, it possesses strong antioxidant activity compared to vitamin C[5]. Moreover, *P. harmala* has numerous pharmaceutical applications which are not limited to anti-proliferation and anti-HIV-1 reverse transcriptase activities[6], insecticidal activity[7], anti-spasmodic and anti-histaminic effects (Asghari and Lockwood, 2002), analgesic and anti-inflammatory properties[8]. Many of those reported pharmacological effects may probably be

attributed to its β-carboline alkaloids, among which, harmaline, harmine, harmalol, harmol and tetrahydroharmine are identified and quantified as the main alkaloids in *P. harmala* extracts[2].

Protein level in protein isolate or concentrations is conditioned by many factors. One potential limiting factor is protein concentration in raw material; witch is dependent on plant genus, preliminary processing and method of protein isolation and coagulation[9]. Kind of extraction system used for protein isolation from harmal seeds significantly influenced on the usefulness polyelectrolytes in a protein coagulation process. Conventional propagation of *P. harmala* is from seed and it has several limitations including germination. In vitro propagation of *P.harmala* from cotyledonary node and hypocotyl explants were reported by[10,11].

This study was aimed at to identify a suitable explant and a protocol for *in vitro* multiplication of *P. harmala* for improve protein production and select the type of KDa protein which responsible for participate the heavy metals.

Material and Methods

1.1. Plant materials

Seeds of *P. harmala* were extracted from mature capsules collected from plants growing wild in a Saudi Arabian Research Station. Uniform, healthy seeds were selected and surface sterilized for thirty minutes with 100 ppm Savlon solution to which a few drops of Tween-20 surfactant were added. After gentle rinsing under tap water for an hour, seeds were sterilized with 70 % ethanol alcohol for two minutes under sterile conditions. Afterwards, they were treated with 15 % (w/v) aqueous sodium hypochlorite solution (Clorox) for ten minutes and finally were thoroughly washed three times with sterilized distilled water to remove traces of sodium hypochlorite. The micronodes were used as explants and were collected from 28 days old *in vitro* germinated seedlings grown on growth regulators- free MS medium[12] supplemented with 30 gL^{-1} sucrose and 0.8 % agar.

1.2. Protein influenced treatments

Explants produced under sterile conditions were transplanted on MS medium supplemented with 3 mg/l benzylaminopurine (BAP), 30 gL^{-1} sucrose and 0.8% agar and its pH was adjusted to 5.6–5.8 before autoclaving. One of the following additives/ treatments was added to the medium:

- No additives (control).
- Tryptophan at 50, 100 and 200 mg L^{-1}.
- Tyrosine at 50, 100 and 200 mg L^{-1}.
- Pyruvic acid at 20, 40 and 80 mg L^{-1}.
- Jasmonic acid at 15, 50 and 100 µM.
- Silver nitrate ($AgNO_3$) at 25, 50 and 100 µM.

For each of the previous treatments, 40 ml of the medium was poured into 250 sterile glass jars under sterile conditions. In each jar, four explants were incubated in a growth chamber under controlled conditions at a temperature of 25 ± 2 °C with a 16 hours photoperiod controlled automatically by an electric timer, under cool white fluorescent tubes with light intensity of 3000 lux. 4 weeks before they were subcultured twice and harvested for protein extraction.

1.3. Sedimentation process

All samples were grounded and sieved by 18- mesh screen. The samples macerated were used for the extraction of protein

- **Coagulation protein extraction**

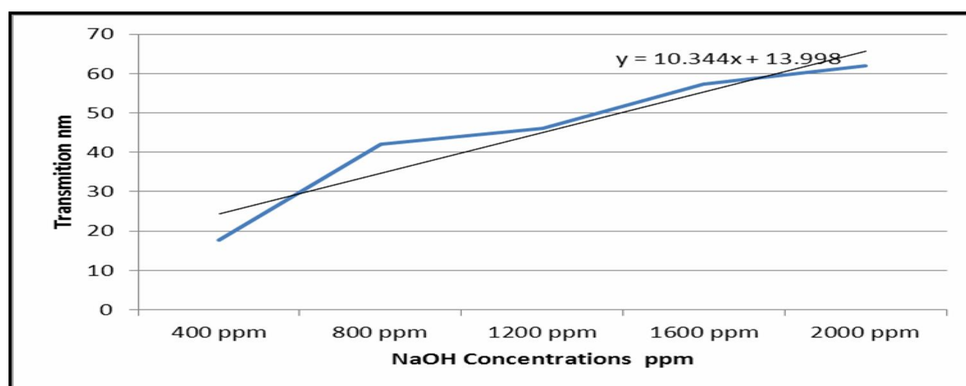
For spectrophotometric preparation protein was extracted from the explants of *P. harmala* according to the protocol reported by[13]**.** From the fresh samples, 5% (w/v) solutions were prepared using distilled water. The solution was mixed for 30 minutes and filtered through Watmann paper no. 3 and 0.45 µm fiberglass filters. The filtrate was termed as a crude protein extract (CPE).

For electrophoresis preparation protein extraction was conducted by mixing 0.1 g of fresh leaves or dried precipitant protein. The sample were then ground to fine powder using a mortar and pestle and homogenized with 1 M Tris-HCl buffer, pH 6.8 in clean eppendorf tube and left in refrigerator overnight then centrifuged at 10.000 rpm for 10 min. Then boil for 5 minutes in water bath before loading in the gel[14].

- **Water pollution**

Double distilled water was polluted with (0.2 ppm Pb or 20 ppm Cd) and NaOH 0.1N was added afterwards to precipitate the added heavy metal traces. NaOH concentrations used for standard curve construction were 400, 800, 1200, 1600 and 2000 ppm, samples were left for an hour and transmittance was determined after centrifugation 5000xg /20 min by absorption measurement using a spectrophotometer at a wave length equal to 290 nm[15]. Calculation was used by the following equation:

Y = 10.344X+13.998



Standard curve of NaOH concentrations with transmittance at 290 nm

- **Coagulation process**

The successive explants cultured on MS medium containing protein elicitors and produced high amounts of protein were used as source of coagulation protein. The protein extraction adjusted pH at four levels (6, 5, 4, and 3) by decreasing acidity with 2M HCl. The contaminated water was added to the protein extracts at equal volume for each. Coagulated protein were removed by centrifugation (6000x g; 20 min), dried in oven at 45˚C for 3 hrs and used for further analysis. Protein from remaining supernatants was subsequently coagulated in the pH 6, 5, 4 and 3, with contaminated water follow this protocol. Proteins were dissolved in 1mM NaOH and their levels were determined follow Lowry method with bovine albumin as a standard[16].

- **Electrophoretic coagulation protein**

A volume of 80 µl of the protein extract was loaded on the gels. Control wells were loaded with standard protein marker Medium range from 10 KDa to 140 KDa (Fermentas.Com). Lower and upper buffer tanks were filled with the running buffer (electrode buffer). This buffer was prepared by adding 15.0 g Tris, 72.0 g glycine and 5g SDS to 1 liters distilled water and shacked well with magnetic stirrer. Then the volume was completed to 5 liter with distilled water and kept at 4˚C.

The polyacrylamide gels were fixed between the two tanks in a suitable position. The electrodes were connected to the power supply. The run was performed at 100 volt until the tracing dye (bromophenol blue) entered the separating gel. Then the voltage was increased to 200 volt until the bromophenol blue dye reached the bottom of the separating gel. Gels were removed from the apparatus and placed in plastic tanks, then covered with the staining solution. Gels were agitated gently overnight. Then the staining solution was removed and the gels were covered with distaining solution. The distaining solution was changed several times until the gel background became clear. Gels were photographed scanned, analyzed using Gel Doc VILBER LOURMAT system.

- **Transmittance association polluted water**

Transmittance of solutions means increasing precipitation processing by proteins, 100 and 10 mg/100 ml ddw of Cd and Ld were prepared to associating the precipitation process by NaOH 0.1N as an artificial precipitant standard[15]**.**

1 ml of contaminated water by Cd and Ld + 1 ml NaOH 1N or protein extraction and start incubation time T1 at 37˚C after T2 incubation time and centrifugation at 4000x for 20 min. Transmission at 290 nm of supernatant on spectrophotometric was determined after centrifugation for supernatant.

$T = [\Delta P / \Delta T]$, $T = [10^{\varepsilon CL}]$, $A = [-\log T] = [\log(1/T)]$, $A = [\varepsilon C L]$

A/ε = molar concentration, $(A/\varepsilon_{percent})10 = C$ mg/ml

Where: T: transmittance, L cuvette volume 1cm3, ε: molar absorptivity, C: molar concentration,

$\varepsilon_{percent}$ for albumin = 6.67 [17].

Using NaOH in calibration curve gave the next equation: Y=10.344X + 13.998

After protein extraction one ml of sol. was taken and added to 1 ml of ld and Cd sol. and reading transmission at 290 nm[18].

- **Total protein**

1ml of plant extract was added to 1 ml phenyl blue and incubation for 2 h then reading absorbance at 570 nm, albumin was reading at same wave length as a standard material.

- **Growth morphology**

Three replicates and fifteen explants were inoculated for one month. Subculture of these treatments for two times and finally, survival %, shoot no, shoot length, leaves no, fresh and dry weights of callus were recorded and extraction of fresh explants was carried out to determine total protein (mg/g); transmittance % and protein electrophoresis.

Results and Discussion

1. Survival and morphological characteristics

As shown in Table (1), all investigated pyruvic acid concentrations and 100 and 200 mgL^{-1} tryptophan applications significantly reduced survival rate compared to the control. While other treatments either increased or decreased survival rate, insignificantly. Meanwhile, 50 µM AgNO3 and 100 µM Jasmonic acid additives resulted in a maximum survival (100%). As for the number of shoots, media supplemented with 10 mgL^{-1} pyruvic acid and 100 µM jasmonic acid significantly increased numbers of regenerated shoots compared to the control. Other supplements were of negative or positive trivial effects in this regard. Meanwhile, unlike its negative effect on survival rate, pyruvic acid generally increased shoot length compared to the control, though statistical significance was recorded for the 5 and 10 mg L^{-1} concentrations only. Similarly, 100 and 200 mg L^{-1} tyrosine supplements resulted in significant increases in shoot length compared to control. Although none of the investigated supplements significantly increased number of leaves or callus fresh and dry weights compared to control, but adding 200 mg L^{-1} tryptophan to the medium led to the production of the highest number of leaves (5.39) and resulted in the highest values of callus fresh (11.43 mg) and dry (0.47 mg) weights. Contrarily, plantlets grown on media supplemented with 75 µM AgNO3, 15 µM jasmonic acid and 50 mg L^{-1} tryptophan recorded significantly reduced numbers of leaves compared to control. As for the callus FW, all AgNO3, jasmonic and pyruvic acid concentrations along with the 50 and 100 mg L^{-1} tryptophan and 200 mg L^{-1} tyrosine significantly reduced callus FW. On the other hand, only 50 and 75 µM AgNO3 and 50 and 100 µM jasmonic acid and 50 µM tryptophan significantly reduced callus DW compared to control.

In plants, aromatic amino acids tryptophan and tyrosine are incorporated in proteins synthesis and are precursors to a variety of specialized metabolites. Trp-derived indole- 3- acetic acid (IAA) is an essential growth regulator[19] and is the primary auxin in plants which functions in determination of leaf patterning[20], and initiation of lateral roots and shoots[21,22]. Maximum survival % in response to $AgNO_3$ found in this trial is in harmony with the 100% shoot proliferation [23]reported for *P. lentiscus* L. in response to the same elicitor.

The unsignificantly affected shoot length recorded in this trial in response to AgNO3 and reduced shoot length in response to jasmonic acid is confirmed by the results of [23]recorded for *P. lentiscus* L.. Meanwhile, it contravenes increased nodes and internode length found on *Vitis vinifera* L. stems in tissue culture when 0.5 µM jasmonic acid was added to the culture medium[24]. Our results are in agreement with results obtained by[25] who reported a stimulating effect for AgNO3 on the number of shoots of *Vitex negundo*. Contrarily, it disagrees with results of [23]who reported no significant effect for AgNO3 and a negative effect for jasmonic acid on multiple shoot formation in P. lentiscus L.

Previous experiment showed the role of tryptophan in plant growth, in this concern, mutations that disrupt tryptophan biosynthesis result in various developmental defects in plant organs, but how tryptophan affects organ growth and development remains unclear.

Table 1. Effect of some protein induction elicitors on morphological characterization of *Peganum harmala*

Treatments	survival	Shoot No.	Shoot length	Leaves No	F.W. mg	D.W. mg
Control	96.29 ab	00.94 c	1.87 def	4.53 abc	10.65 ab	0.35 a-c
$AgNO_3$ 25µM	74.07 a-d	2.22 bc	1.62 ef	3.05 cde	4.70 ef	0.22 c-g
$AgNO_3$ 50 µM	100.0 a	1.00 bc	1.42 f	3.03 cde	1.81 g	0.12 fg
$AgNO_3$ 75 µM	66.66 a-d	1.66 bc	2.62 a-f	2.11 de	2.29 g	0.13 efg
Jas. acid 15 µM	88.89 ab	2.21 bc	1.24 f	1.40 e	2.16 g	0.23 b-g
Jas. Acid 50 µM	66.66 a-d	2.22 bc	1.40 f	3.45 cd	2.41 g	0.17 d-g
Jas. acid 100 µM	100 a	2.44 b	2.67 a-d	3.93 cd	2.18 g	0.11 g
Tryp. 50mgl^{-1}	96.29 ab	0.93 c	1.48 f	2.05 de	3.51 fg	0.16 d-g
Tryp. 100 mgl^{-1}	44.44 de	1.07 bc	2.19 b-f	4.18 abc	4.68 ef	0.26 b-f
Tryp. 200 mgl^{-1}	44.44 de	1.00 bc	2.07 c-f	5.39 a	11.43 a	0.47 a
Tyrosin 50mgl^{-1}	88.89 ab	1.11 bc	1.28 f	3.64 bcd	7.00 d	0.23 b-g
Tyrosin 100 mgl^{-1}	85.18 abc	1.14 bc	3.11 ab	4.47 abc	9.09 bc	0.31 bcd
Tyrosin 200 mgl^{-1}	77.78 a-d	2.10 bc	3.17 ab	3.68 bcd	7.72 cd	0.37 ab
Pyruvic 5 mgl^{-1}	29.62 e	0.77 c	2.98 abc	5.25 ab	4.47 ef	0.22 c-g
Pyruvic 10 mgl^{-1}	51.83 cde	4.24 a	3.62 a	4.47 abc	6.01 de	0.30 bcd
Pyruvic 20 mgl^{-1}	62.92 b-e	2.22 bc	2.69 a-d	3.00 cde	4.44 ef	0.27 b-e
LSD 5 %	34.4	1.468	1.025	1.671	1.873	0.1491

Fig. 1 Left : *Pegonium harmala* explants of *in vitro* culture. Right : water contaminated with Cd and Ld after adding NaOH to participation heavy metals .

2. Protein production

As presented in Table (2), all elicitors investigated in this trial increased total protein production, however, increases were not always significant. 50 µM jasmonic acid, 100 and 200 mg L^{-1} tryptophan, 200 mg L^{-1} tyrosine and 20 mg L^{-1} pyruvic acid additives led to the production of significantly high quantities of proteins compared to the control medium (additive free- medium). On the other hand, results reflect that higher

protein production was not always correlated with enhanced turbidity reduction. Although none of the treatments led to the production of protein that significantly reduced turbidity compared to protein produced from control medium, but some treatments seemed promising in this regard. Among those, 200 mgl^{-1} tryptophan, 200 mg L^{-1} tyrosine and 20 mgl^{-1} pyruvic acid additives enhanced protein production that was most efficient in reducing turbidity. This is most probably attributed to their role in protein synthesis in plants. According to fig (3) the electrophoresis of explant protein is a method to investigate protein pattern and classify plant coagulation protein because these proteins are highly produced after expose the explants to protein elicitors. The gels showed 99 bands that were high polymorphism among the accessions (Fig. 3). The noticeable differences were observed in area with 90.94 KDa. In this area there were not any bond in the accession numbers 2, 11, 15 and 16 but were exist in other accessions (it is illustrated by arrows in Fig. 3).

Table (2) Effect of some protein induction treatments on protein contents and turbidity on contaminated water by lead and cadmium by using harmal protein extract

Treatments	**Total protein mg/g**	Transmittance %
Control	0.05 e	3.31 fg
$AgNO_3$ 25µM	0.11de	10.99 c
$AgNO_3$ 50 µM	0.18 b-e	25.4 a
$AgNO_3$ 75 µM	0.19 b-e	6.87 de
Jas. acid 15 µM	0.12 cde	2.07 g
Jas. Acid 50 µM	0.38 abc	2.96 fg
Jas. acid 100 µM	0.20 a-e	4.17 efg
Tryp. 50mgl^{-1}	0.13 cde	5.57 def
Tryp. 100 mgl^{-1}	0.33 a-d	15.67 b
Tryp. 200 mgl^{-1}	0.40 ab	1.67 g
Tyrosin 50mgl^{-1}	0.10 de	4.70 efg
Tyrosin 100 mgl^{-1}	0.28 a-e	8.68 cd
Tyrosin 200 mgl^{-1}	0.41 ab	1.44 g
Pyruvic 5 mgl^{-1}	0.14 cde	1.68 g
Pyruvic 10 mgl^{-1}	0.09 de	2.147 fg
Pyruvic 20 mgl^{-1}	0.46 a	1.85 g
LSD 5 %	0.2637	3.456

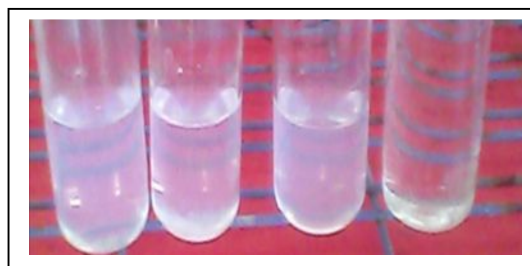


Fig. 2 Polluted water after NaOH additive

3. Water transmittance

Data observed in Table (2) remained that the quantity of protein gave the best results of transmittance which indicate to chelating the heavy metals and participate, these results included tryptophan at 200 ppm and tyrosine at 200 mg L^{-1} which gave more clearing water that containing both of Ld and Cd, they recorded 1.67 and 1.44 % translucent, respectively. The untreated water (control) is more turbidity but using $AgNO_3$ at 50 uM inhibition participate heavy metals protein synthesis, it was more turbid than all treatments 25.4 %.

4. Effect of pH

a. Total protein

Protein level in protein isolates or concentrates is conditioned by many factors. One potential limiting factor is protein concentration in raw material, which is dependent on plant genus, preliminary processing and method of protein isolation and coagulation[26].

Data reported in Table (3) shows that the protein bind with heavy metals was precipitate at various pH levels. In this concern, applying pH at 3, 4, 5 and 6 are gave a various response of agglutination process whereas, protein synthesis are increase to the maximum process of synthesis 0.63 mg/100 g fw for tryptophan 200 ppm followed by tyrosine 200 mg/l 0.53 mg/100 g fw compared with control (0.46 and 0.16) at the same pH level (4 and 5).

b. Coagulant protein

Coagulant protein is bind with heavy metals at high quantity in pH 4 for protein content with tryptophan 200 mg L^{-1} (50.52 ug/ml) compared with control (Table 3). In this case, the coagulate protein was migrated in the gel as shown in the fig (4-a and b) the result of analysis showed that the accessions shows one band 96.67 KDa protein which responsible for participate led and cadmium. This band was absent in pyrvic acid extraction with pH4 and pH3.

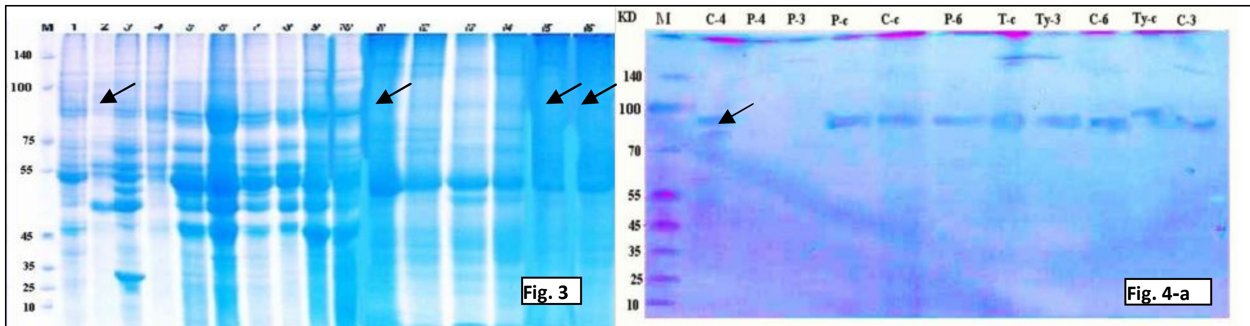
c. Polluted water transmittance

It is cleared that protein participation heavy metal by pH treatments was affected by quantity of total protein and band which responsible to bind with heavy metal. It is recorded that (0.63 mg/g) of total protein produced from explants treated with tryptophan at 200 mg L^{-1} for pH 4 as shown in Table (3).

Table (3) Effect of protein extraction by water on protein contents in harmal explants treated by some protein induction.

Treatments		Total protein mg/g	Coagulant protein ug/ml	Transmittance%
NaOH 0.5 %		0.0	0.0	> 7
Control	pH 3.0	0.24	19.49	> 7
	pH 4.0	0.46	36.28	0.9665
	pH 5.0	0.16	12.74	1.131
	pH 6.0	0.02	1.65	1.361
Tryp. 200 mgl^{-1}	pH 3.0	0.14	10.94	> 7
	pH 4.0	0.63	50.52	0.6201
	pH 5.0	0.07	5.25	1.05
	pH 6.0	0.22	17.24	1.229
Tyr. 200 mgl^{-1}	pH 3.0	0.29	23.31	2.23
	pH 4.0	0.32	41.08	0.633
	pH 5.0	0.52	25.86	1.31
	pH 6.0	0.35	27.74	> 7
Pyr. 20 mgl^{-1}	pH 3.0	0.27	3.45	0.82
	pH 4.0	0.04	21.89	0.711
	pH 5.0	0.14	11.24	1.6711
	pH 6.0	0.18	14.09	0.7834
LSD 5 %		0.1932	2.071	----

Acidic precipitation at pH 4.0 for protein produced by explants treated with tryptophan 200 mg/l allowed obtaining slightly higher amounts of precipitated protein(50.52 ug/ml) than those obtained by acidic precipitation of control (1.62 ug/ml) at pH 6.0.

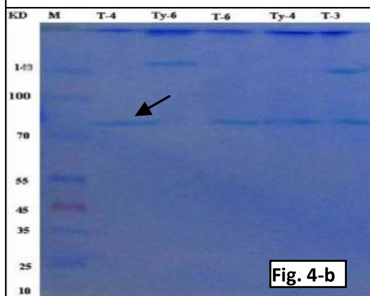


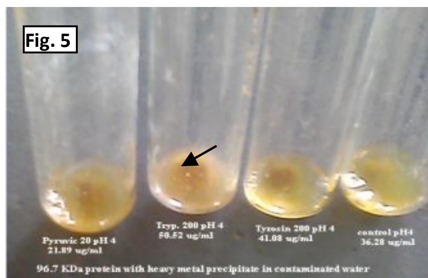
M Kda	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
139.554	125.49	114.59	114.59	90.94	114.59	115.54	114.59	116.50	116.50	115.54	127.22	121.74	124.45	123.77	51.45	112.10
108.829	90.94	66.27	92.20	90.94	90.94	90.94	90.94	90.94	90.94	90.94	88.6	96.62	95.82	90.94	12.47	53.03
72.593	53.76	60.18	65.91	58.39	66.27	67.93	65.91	67.37	68.87	69.44	50.33	80.13	69.83	68.31	-----	15.03
55.420	42.90	54.66	58.39	34.80	57.91	57.59	57.59	57.59	51.45	50.60	13.03	71.97	50.60	51.45	-----	12.74
33.786	34.52	42.67	53.03	-----	49.50	49.91	51.02	51.45	42.31	41.97	-----	49.91	44.59	27.62	-----	-----
25.987	28.86	-----	49.09	-----	41.62	42.31	41.97	41.97	35.58	35.09	-----	34.80	31.61	15.24	-----	-----
21.384	23.54	-----	41.97	-----	35.09	35.09	35.09	36.17	-----	-----	-----	12.09	16.06	13.32	-----	-----
17.205	-----	-----	36.37	-----	-----	-----	-----	-----	-----	-----	-----	-----	14.39	12.26	-----	-----
-----	-----	-----	23.94	-----	-----	-----	-----	-----	-----	-----	-----	-----	12.26	-----	-----	-----

Fig. 3 **Left** electrophoretic profiles of protein obtained from Harmal extracts after protein induction. Lanes 1 control., lannes 2-4 AgNO3, Lans 5- 7, tryptophan, Lans 8-10 jasmonic acid, lans 11- 13 tyrosin and lans 14-16 pyruvic. M – molecu-lar mass markers [kDa]

Fig. 4a-b Right electrophoretic profiles of protein precipitated with heavy metals from Harmal extracts. By pH treatments on protein contents. M – molecu-lar mass markers [kDa].
C-c: control, Number 3,4,5,6 mean pH 3,pH4, pH4, pH6
T: tryptophan, Ty: tyrosine, P: pyruvic

M	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
139.55	96.67	-----	177.03	96.67	154.23	96.67	178.03	178.03	161.33	96.673	96.67	---	96.67	96.67	96.67	149.7
108.82	-----	-----	-----	-----	96.673	-----	96.673	96.673	96.673	-----	-----	---	-----	-----	-----	96.67
72.59			77.99									84.1				





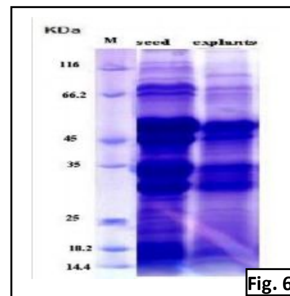


Fig. 6 Electrophoretic profiles of protein contents on seeds and explants of *Pegonium harmala*

Similar results have been obtained by[27] who precipitated pea proteins with polyelectrolytes Magnafloc M-22S and Superfloc A-150. Concentration of protein in the obtained preparations was about 8% lower than in control (precipitation by acidification). [28]performed isolation of protein from lupinus seeds using polymers of N-isopropyl acrylamide and methyl bisacrylamide. Yield of extraction was lower up to 50% in the comparison to the isolate obtained by precipitation in isoelectric points. Also methods based on the dialysis and ultrafiltration was less effective, protein loss 9 and 16%, respectively.

Additionally, these studies clearly show that type and levels of precipitated proteins were depended on the kind of flocculant materials (heavy metals) used. Protein agglomeration should be preceded by changes in the secondary structure of protein, which facilitate forming of new bonds. This process is the most effective when protein compound are not charged. In the conditions different than pI (Isoelectric point), proteins are charged and can be neutralized by oppositely charged flocculants compounds. At the time, those interactions disappear and aggregation of compounds ensues[29]. Therefore, colloids aggregation takes place more effectively in low acidic conditions when polyelectrolytes can easily absorb on the proteins. Finally, precipitation

efficiency depends on polyelectrolyte concentration (heavy metals) and type of protein band (KDa). Too high concentrations of coagulants cause formation of additional layer on the protein surface that protects colloid against precipitation[30]. Concentrations of flocculants (Ld & Cd), used in this work, did not influence negatively on the amounts of precipitated protein. Better extraction yields were obtained with explants treated with tryptophan at 200 mg L^{-1} followed by pH 4 and tyrosin 200 mg L^{-1} at pH 4 can suggest that colloids particles were in the majority positively charged. These observations confirm results obtained by [31]who found pea proteins were more effectively precipitated with anionic polyelectrolyte Superfloc A-150. It should be noted that coagulation conditions significantly influence chemical composition and functional properties of protein preparations[32].

Electrophoretic profiles of protein precipitated with heavy metals (lead and cadmium) show that amounts and molecular masses of protein were closely bound with coagulation conditions (pH) and protein induction treatments.

Irrespectively of the concentration and kind of heavy metals in the electrophoretic profiles of proteins precipitated from pH preparations only one main band was visible, corresponding to protein with molecular mass about 96.67 kDa (Fig. 4 a&b). In this concern, [33]observed the vivcilin, trimeric protein of lentil seeds that is composed of subunits with molecular mass about 48 kDa and belongs to globulin fraction. Similar results obtained [27]during precipitation of proteins from pea protein isolates. They also explain flocculants played key role in the precipitation of protein from pea protein preparations. It is noteworthy that protein subunits interactions are modified during preparation of protein isolates. Electrophoretic studies of cowpea flour and its protein isolates performed by[9] clearly confirmed these relationships.

Based on results of these studies, amount and kind of precipitated protein is conditioned by many factors that can influence on the process condition. Between them the most important seems to be a kind and concentration of heavy metals and pH of precipitation.

Finally, we can concluded that using protein extraction of *P. harmala* as coagulating factor allows obtaining a high amount of heavy metals sediment in raw water, however the yields of coagulation protein can obtained by applying tryptophan at 200 mg L^{-1} in tissue culture. And we can determine the quantity of protein and type by different electrophoreses systems, and increasing protein precipitablity by acidity pH condition of process. Bases on electrophoretic studies is visible that precipitation of proteins with heavy metals can be a good tool for protein fractionation although still needs further studies concerning selection of better conditions for protein isolation and precipitation.

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