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Effect of Mannitol and PEG on the Accumulation of Rutin in Callus culture of *Ruta Graveolens*

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ABSTRACT

Rutin, a secondary metabolite contained in Ruta graveolens, has been used in medicine for a variety of applications. Elicitation of mannitol and polyethylene glycol (PEG) for Rutin development enhancement in Ruta graveolens callus culture would be a viable alternative. Cell growth and Rutin production were also affected by mannitol and PEG. The most effective elicitation method was to treat cell cultures with 10 mg/l PEG, that resulting in the high Rutin output (0.70 mg/g DW). PEG elicitation is a useful method for growing Rutin production in Ruta graveolens callus culture.

Keywords
Mannitol, *R. graveolens*, Callus culture, Rutin

Introduction

Ruta graveolens, also known as rue, is really a herbal shrub in the rutaceae family that is used in gardening as an ornate and therapeutic herb. The plant's product is used to treat ulcers and inflammatorys (Ratheesh *et al*., 2009). This plant extract has cytotoxic, antihypertensive, and antibacterial properties(Farah *et al*., 2013), antihelminthic and phytotoxic effects (Asgarpanah and Khoshkam, 2012).Bioactive compounds are being used to treat problems with reproduction. *Ruta graveolens* decoction helps with menstruation. *R. graveolens* is a medicinal plant that is high in flavonoids and has a wide variety of biological properties (Pirouzpanah *et al*., 2006). Many plant cell cultures were formed, but only a small percentage of them produce enough secondary metabolites. Environmental stresses affect secondary metabolite biosynthesis in plants, and elicitors may improve their accumulation (Zhao *et al*., 2010 and Wang *et al*., 2015). Elicitors are classified based on their nature (Fig. 1), which can be biological (polylisaccharides and components of microbial cells), chemical (heavy metals, minerals, and salts), or physical (ultraviolet, osmotic stress, salinity, drought, and thermal stress factors) and cause enzymatic activity in response to stress. Signaling molecules such as Mannitol and polyethylene glycol have been proposed (Ahmad *et al*., 2020). They've already been seen to boost the production of natural products in cultured cells as well as play a pivotal role in plant immune response gene regulation factors (Moreno-Perez *et al*., 2020). This study looked at the influence of Mannitol and PEG on cell growth and Rutin biosynthesis in *Ruta graveolens* callus culture. In terms of callus growth and Rutin development, some parameters, such as Mannitol and PEG concentration, were studied in depth.

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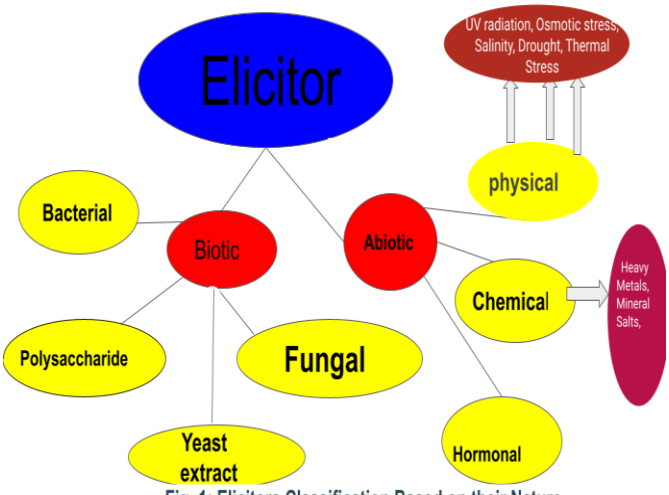

Fig. 1: Elicitors Classification Based on their Nature

Materials and Methods

Plant material and callus induction

R. graveolens leaves are cut into 2 cm lengths and cultured on MS complete medium with 3% sucrose and 8 g/l agar. The medium used for callus induction was supplemented with Naphthalene Acetic Acid (NAA) at a level of 0.2 mg/l, according to Zuraida (2014).

Mannitol as an elicitor in callus cultures

A total of 500 mg of callus was grown on the following combination:

1. MS medium complemented with 0.2 mg/l NAA (control)

2.MS medium complemented with 0.2 mg/l NAA + 50 mg/l Mannitol

3.MS medium complemented with 0.2 mg/l NAA + 100 mg/l Mannitol

4.MS medium complemented with 0.2 mg/l NAA + 150 mg/l Mannitol

5.MS medium complemented with 0.2 mg/l NAA + 200 mg/l Mannitol

6. MS medium complemented with 0.2 mg/l NAA + 250 mg/l Mannitol

After 4 weeks of culturing, samples were harvested, the fresh weight was registered, and the samples were then dried in an oven at 40°C for two days, and the dry weight was calculated.

PEG as an elicitor on callus cultures

A total of 500 mg of callus was grown on the following combination:
1.MS medium complemented with 0.2 mg/l NAA (control)

2. MS medium complemented with 0.2 mg/l NAA + 2.5 mg/l PEG

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3. MS medium complemented with 0.2 mg/l NAA + 5 mg/l PEG

4. MS medium complemented with 0.2 mg/l NAA + 7.5 mg/l PEG

5. MS medium complemented with 0.2 mg/l NAA + 10 mg/l PEG

After 4 weeks of culturing, samples were harvested, the fresh weight was registered, and the samples were then dried in an oven at 40°C for two days, and the dry weight was calculated.

Extraction and HPLC analysis of Flavonoid

Ruta graveolens extracts were made by soaking 500 mg of dried cells in 10 ml methanol and ultrasonically extracting them twice for 1 hour each time. After that, the extracts were centrifuged for 10 minutes at 10,000 rpm at 0°C. The precipitate was mixed, and it was subjected to HPLC analysis and flavonoid determination(Mahood, 2021In quantitative and qualitative analysis of Rutin in methanol extracts, the RP-HPLC (Sykum-German) method with C18 reversed-phase column (250×4.6mm) was used. Acetonitrile (A) and 0.1 percent trifluoroacetic acid were found to be effective in the mobile process (B). The collected samples were isolated using a gradient approach: 0-25 min, 15%-40% A; 25-40 min, 40%-100% A. The elution flow rate was set to 1.0 mL/min and the detection wavelength was set to 254 percent. HPLC verified the identification of the isolated peaks by normal injection, and UV spectroscopy was used to confirm the compound's identity (Mahood, 2018).

The experiments were carried out using Complete Random Desing (CRD), and the results were compared statistically against a test of the Least Significant difference (LSD) and the level of blindness. Probability (0.05).

Results and discussion

Callus induction

Culturing leaf segments excised from in vitro growing *Ruta graveolens* plantlets on MS medium complemented by 0.2 mg/l NAA resulted in callus induction. After 4 weeks of culture, the callus grew successfully upon this chosen medium (callus induction medium). Callus was a white and small (Fig.2 A).

Effect of Mannitol and PEG as elicitors in callus fresh and dry weights

It was found that Mannitol and PEG caused significant changes in the callus growth. Mannitol at levels of 50, 100, 150, 200, and 250 mg/l was added to the most preferred medium (MS medium + 0.2 mg/l NAA) for callus production as a control to show the effects on each of these parameters, fresh and dry weights.The measurements of callus growth are seen in (Fig 3). In contrast to the control (1.7 g), MS medium complemented by 50, 100, 150, 200, or 250 mg/l Mannitol increased callus fresh weight, reaching (1.9, 2.1, 2.7, 2.9, and 3.1g) respectively. The highest value (3.1g) was obtained with a medium containing 250 mg/l Mannitol (Fig. 3A). Mannitol also raised the dry weight of the callus. In comparison to the control (0.76 g), MS medium complemented by 50, 100, 150, 200, or 250 mg/l Mannitol increased callus dry weight (0.89, 0.93, 0.97, 1.6, and 1.8g). The highest value (1.8g) was recorded with 250 mg/l Mannitol containing medium,. Callus was large, nodular and white to pale yellow (Fig. 2B).The obtained

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results indicate that increasing PEG concentration caused gradually increasing of callus fresh and dry weight with increasing of PEG in culture medium (Fig. 3B). In comparison to the control (1.7 g), MS medium supplemented with 2.5, 5, 7.5, or 10 mg/l PEG increased callus fresh weight reaching (2.1, 2.2, 2.9, and 3. g) respectively. The highest value (3.3g) was obtained with a medium containing 10 mg/l PEG. PEG also raises the dry weight of the callus. As compared to the control (0.76 g), MS medium complemented by 2.5, 5, 7.5, or 10 mg/l PEG increased callus dry weight (0.9, 1.2, 1.8, and 2.1 g) respectively. The highest value (2.1g) was obtained with a medium containing 10 mg/l PEG. It was observed that callus grown on PEG medium turned a dark brown color (Fig. 2C).

Fig. 2: (A) Callus induction of *Ruta graveolens* from leaf explant grown on MS medium supplemented with 0.2 mg/l NAA. (B) Callus grown in MS medium supplemented with Mannitol © Callus grown in MS medium supplemented with PEG

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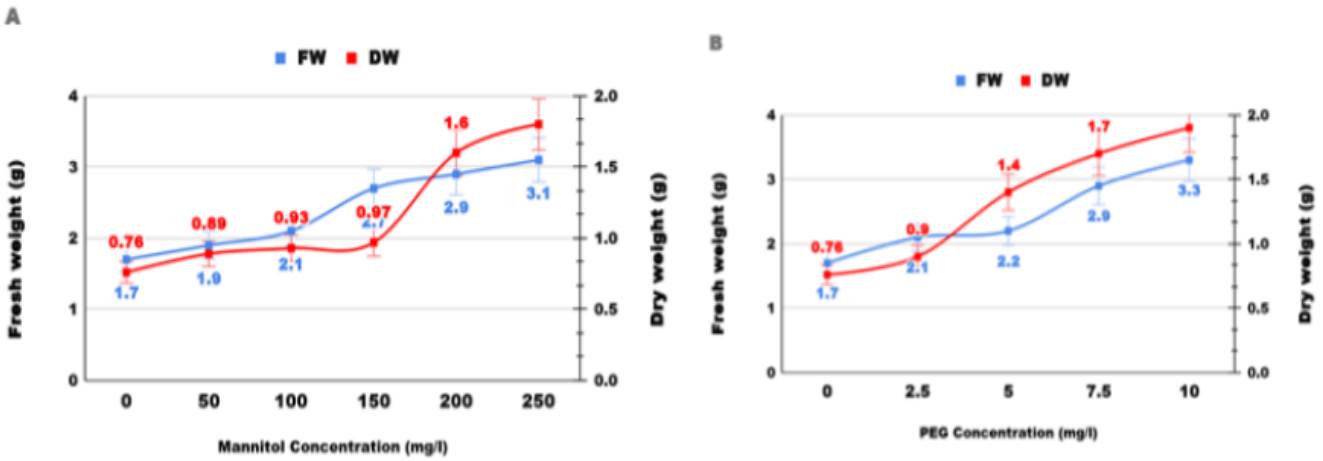

Fig 3: (A) Effect of Mannitol concentration in fresh and dry weights. (B) Effect of PEG concentration in fresh and dry weights.

Effect of Mannitol and PEG on callus accumulation of Rutin

The estimated Rutin content in callus grown on MS medium complemented by 50, 100, 150, 200, or 250 mg/l Mannitol was shown in Fig.4A. Rutin content was measured in mg/g of callus dry weight. When different Mannitol concentrations were used, it was discovered that the Rutin content increased as the Mannitol concentrations increased. Mannitol at concentrations ranging from 50 to 200 mg/l increased Rutin accumulation more than the control. Rutin was found to have the highest concentration (0.60 mg/g DW) in a medium containing 200 mg/l Mannitol (Fig 5C). The addition of 200 mg/l Mannitol to a Ruta graveolens callus culture increased Rutin production by about twofold over the control. Bekheet (2015) discovered that increasing the concentration of Mannitol increased the dry weight of the callus and milk thistle silymarin content. In contrast to the control, Ebad et al. (2017) found that lower concentrations of Mannitol increased growth while higher concentrations increased total flavonoid content in Solanum nigrum callus. Sorbitol treatment increased phenolic and flavonoids in sweat potato calluses, according to El-Far and Taie (2009). These findings are in conflict with those of Shaaban and Maher (2016) and Mahood (2018). They discovered that as the concentration of Mannitol increased, the fresh and dry weights gradually decreased. The average values of Rutin content in callus grown on MS medium supplemented with 2.5, 5, 7.5, or 10 mg/l PEG were shown in Fig.4B. Rutin content was measured in mg/g of callus dry weight. When different PEG concentrations were used, the gradual increase in Rutin content with increasing PEG concentrations was observed. In general, adding PEG at different concentrations from 2.5 to 10 mg/l raised Rutin accumulation more than the control. Maximum value of Rutin (0.70 mg/g DW) was registered with medium containing 10 mg/l (Fig 5D). Otherwise, the Rutin component was the most responsive to the presence of PEG in culture medium. It is important here to notice that the enhancement of Rutin accumulation in callus cultures of *Ruta graveolens* by PEG was higher

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compared with Mannitol. In this respect, polyethylen glycol (PEG) has been used as an elicitor in tissue cultures of many plant species in order to enhancement their active compounds. In this study, different PEG concentrations were tested to see if they could improve Rutin production in *Ruta graveolens* callus as well as callus growth. Similar results were recorded by Ahmad *et al*. (2020) for direct treatment in Stevia rebaudiana, indicating that growing PEG in the culture medium was very effective in increasing shoot organogenesis and secondary metabolite content in *S. rebaudiana* shoots grown through tissue culture technique. Sarmadi *et al*. (2018) found that raising PEG concentration increased the production of taxanes in a Taxus baccata callus culture. According to Khashan and Karrar (2018), raising PEG concentration in the medium induces an increase in Lycopene content and growth in *Lycopersicon esculentum* callus culture.

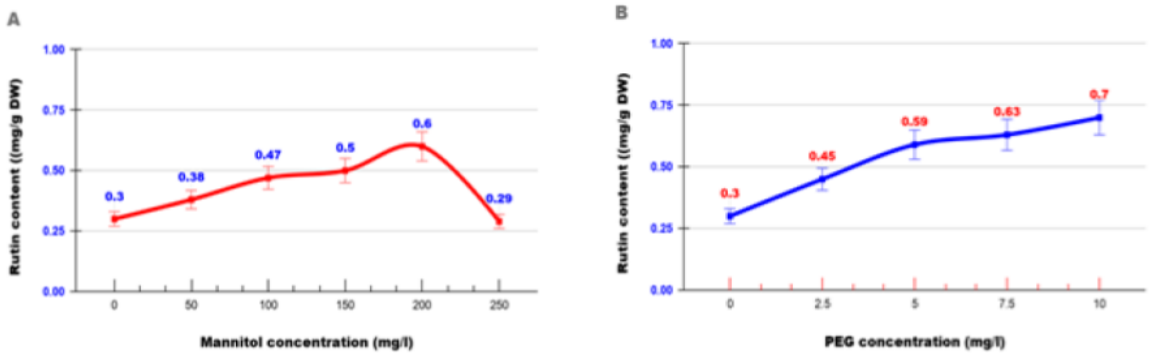
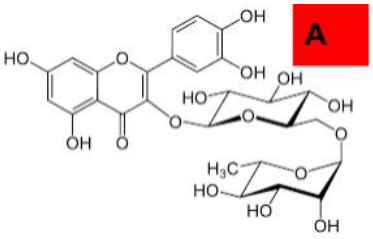
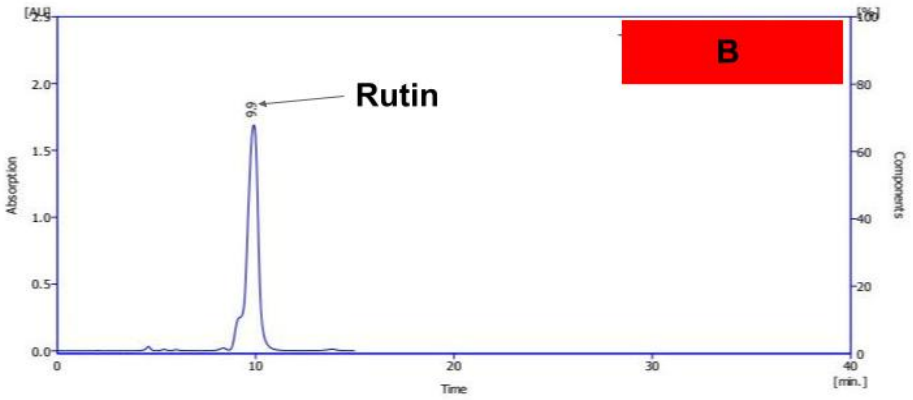


Fig 4: (A) Effect of Mannitol concentration on Rutin Content.(B) Effect of PEG concentration on Rutin Content





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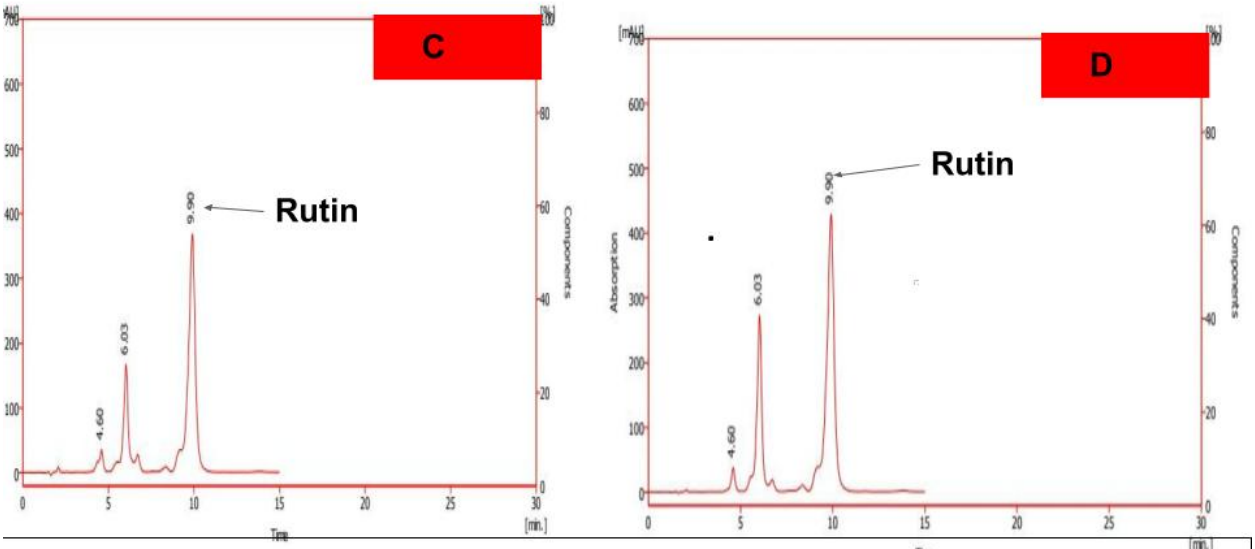

Fig. 5: (A) Chemical structure of Rutin. (B) Standard Rutin © Rutin produced by callus treated with 200 mg/l Mannitol .(D) Rutin produced by callus treated with 10 mg/l PEG.

Conclusions

Plant callus culture is a feasible alternative to conventional biochemical processing methods. Mannitol and PEG-elicited callus cultures of *Ruta graveolens* were used to improve Rutin production. After the optimum elicitation conditions, rutin output reached 0.70 mg/l DW. These findings could help with the hyper production of Rutin from *Ruta graveolens* and also the development of compounds from several other plant cell cultures.

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