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Biotic and Abiotic Elicitors Induce Biosynthesis and Accumulation of Some Withanolide Compounds in the Callus Culture of *Withania somnifera* L.

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WITHANIA somnifera L. is widely used as a medicinal plant for its therapeutic properties. In this study, the impact of Chitosan as a biotic stimulus and exposure to light and UV rays as physical stimuli on cultured callus were investigated.

Compared to the control, the results showed that Chitosan significantly reduced callus fresh and dry weight (more than 70% decrease), while exposing the callus to UV rays for 60 minutes led to a significant increase in weights (18% increase). Six withanolide compounds were quantified in all treatments of callus cultures, using HPLC analysis. The production of these compounds was significantly enhanced through elicitation strategies. Substantially, Chitosan at 100 mg/l stimulated the biosynthesis of 27-OH-withanolide-A (52.0µg/mL, 160% increase) compared to control, while 150mg/L of Chitosan displayed optimal production of withanolide-B (96.3µg/mL, 255% increase), and 27-OH-withanolide-B (124.6µg/mL, 40% increase). Continued light conditions (24h/day) increased the production of Withaferin-A and Withanone compounds (179.5µg/mL, 82% increase, and 145.3µg/mL, 215% increase) respectively. While exposing the callus culture to UV rays for 60 minutes promoted the formation of withanolide-A (241.1µg/mL, 99% increase), exposure for 90 minutes resulted in a high content of 27-OH-withanolide-B (124.8µg/mL, 40% increase). In conclusion, the elicitors used in this study demonstrated their efficiency in stimulating the biosynthesis of withanolide compounds in the callus cultures of *W. somnifera*. These findings provide valuable insights for optimizing the production of therapeutic compounds in the *Withania* plant, which is widely used for its medicinal properties.

Keywords: Chitosan, Light elicitation, UV rays, *Withania somnifera*, Withanolides.

Introduction

For thousands of years, *Withania somnifera* (L.) «Ashwagandha», a member of the Solanaceae family, has been known for its considerable therapeutic uses (Gupta & Singh, 2014). So, it has been included in the World Health Organization's list of medicinal plants "WHO monographs on selected medicinal plants" (Cepae, 1999) https://apps.who.int/iris/handle/10665/42052. withanolides and alkaloids are responsible for the medicinal properties of ashwagandha leaves and roots (Atef et al., 2017; Durg et al., 2020). The plant is usually used as anti-cancer (Kaul & Wadhwa, 2017), hypnotic, anti-ageing (Kelgane et al., 2020) it is used for various ailments and general well-being, including the treatment of geriatric patients. Managing quality of life (QoL, and for immunomodulatory diseases (Muralikrishnan et al., 2010; Alanazi & Elfaki, 2023). withanolide compounds play a defense role in plant life and have a primary structure consisting of a steroidal ring and a lactone side chain (Gaurav & Kumar, 2019). withanolides are

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triterpenoid compounds that are synthesized in plant cells through two distinct pathways: 1- the mevalonate (MVA) pathway, which takes place in the cytosol, and 2- the methylerythritol phosphate (MEP) pathway, which takes place in the plastids. The synthesis of these compounds begins with the cholesterol compound through the action of acetyl coenzyme A (Gaurav et al., 2023).

In vitro cultures are useful methods to produce secondary metabolites for pharmaceutical purposes, agricultural and industrial applications due to their controlled environment, elimination of environmental variability, increased productivity, rapid production, constant supply and genetic and biochemical modification (Ochoa-Villarreal et al., 2016; Lashin et al., 2022).

Elicitors are stress, coming from abiotic and biotic sources that enhance secondary metabolite synthesis or produce novel natural products in plants (Ebel & Cosio, 1994). Chitosan, as biotic elicitor that is produced from shrimp shells, is a polysaccharide compound and more soluble than chitin (Ravi Kumar et al., 2004). It stimulates plants to manufacture defense-related secondary metabolites by mimicking the effects of some pathogenic microorganisms (Ferri & Tassoni, 2011). For example, chitosan improved withanolides productivity; reaching 100mg/L in cell suspension culture of *W. somnifera* (Sivanandhan et al., 2014). It also enhanced biosynthesis of withaferin-A by 69% in ashwagandha plant, compared to the control (Jonathan et al., 2015). Similarly, the concentration of phyllanthin and hypophyllanthin increased to 238% compared to the control sample in the cell culture of Phyllanthus amarus under chitosan stress (Thakur et al., 2012).

Light is an energy source and a relevant physical condition that can influence plant growth and metabolite synthesis (Murthy et al., 2014). For many secondary metabolites, light may act as a signal to activate the major enzymes in the biosynthetic processes (Wang & Wu, 2013) and plays an important role in regulating gene expression in plants (Hartmann et al., 2020). The accumulation of secondary metabolites is affected with light quality, intensity and photoperiod. In *W. somnifera* callus, red light increased biomass and withaferin-A accumulation (Adil et al., 2019a). Also, the total carotenoid content was increased in the callus of Artemisia absinthium under a white light source (Tariq et al., 2014) light is the key environmental factor that alters plant architectural development; however, the understanding that how light controls plant growth and developmental processes is still poor and needs further research. In this study, we monitored the effect of various monochromatic lights and plant growth regulators (PGRs. Furthermore, after exposing the callus tissue to light for 24h, the content of kaempferol, qurecetin and ricinin in Ricinus communis culture were significantly increased (Twaij & Taha, 2017).

Ultraviolet (UV) radiation is a physical stress which significantly influences the accumulation of many secondary metabolites to protect plant tissue from harm (Narayani & Srivastava, 2017) plant cell/tissue culture has emerged as an alternative platform for the in vitro production of value added bioactive secondary metabolites. Implementation of several productivity enhancement strategies, including elicitation, can overcome the limitations faced by plant cell technology that hampers its extensive commercialization. Elicitation is a technique that involves exogenous addition of elicitors (abiotic or biotic. In many in vitro cultures, UV rays stimulated the production of phenolic compounds like flavonoids and anthocyanin, along with terpenes and alkaloids (Singh et al., 2017). Moreover, Kalidhasan et al. (2013) showed that cultivated Withania plant increased anthocyanin content by 42% after 30 d of exposure to UV rays. In addition, UV rays increased the fresh and dry weights of Solanum nigrum and Hypericum triquetrifolium callus cultures, also, the production of several active chemicals was significantly stimulated (Twaij, 2011). Moreover, exposing the callus cultures to UV rays for 20 min considerably raised the content of salanin in Melia azedarach culture (Ahmed, 2014) and citrullol and cucurbitacin B in the Citrullus colocynthis culture (Mutasher, 2015). On the other hand, the UV radiation's negative impacts on cellular growth are generally linked to its toxicity, impact on active cell enzymes, breakdown of genetic material, or generation of gene mutations, especially after long term exposure (Singh et al., 2017). Due to the simplicity of controlling the maintenance of calluses, their multiplication and production efficiency of metabolites in their tissue; callus culture is regarded as one of the strategies utilized by numerous researchers in the advancement of in vitro withanolide production (Bhoyar & Suryawanshi, 2015). Chitosan, light stress, and UV illumination successfully

generated withanolides in various *W. somnifera* cultures (Pandey et al., 2017).

Through a variety of elicitations, this study aimed to use *in vitro* culture to raise the concentration of some substantial phytochemicals in *Withania somnifera* plant.

Materials and Methods

Plant material and callus induction

The dried seeds of Withania somnifera L. were soaked in 300mg/L of GA_3 for 24h to break the seed dormancy state. Surface seed sterilization was achieved via sodium hypochlorite at 2% for 10 minutes. The sterilized seeds were cultured on half strength of Murashige and Skoog MS medium (Murashige & Skoog, 1962) to initiate aseptic in vitro seedlings. The leaves were removed from the one-month-old seedlings, cut into suitable pieces, and planted on full-strength MS medium supplemented with 0.5mg/L of each Thidiazuron (TDZ) and Naphthaleneacetic acid (NAA) to induce the callus (Abed & Taha, 2022). The culture was kept under incubator conditions (25±1ºC in darkness) for 30 days. For two months, the initiated callus was maintained (every three weeks) by sub-culturing on the same medium that was used in induction callus cultures.

Elicitation strategies

Approximately 200mg of callus was cultured on MS growth medium having 0.5mg/L each of TDZ and BA with individual concentrations of chitosan (0, 50, 100 and 150) mg/L, as biotic elicitor. Chitosan was purchased by (Sigma-Aldrich, USA) and suspended according to Khan et al. (2019).

Similar cultures were exposed to the physical factors, including light and UV rays. Different light regimes were applied (continuous darkness, 16h light/day and continuous light for 24h/day) at intensity of 3000 Lux. While, the UV rays were also applied at different durations (0, 30, 60 and 90) min, then the incubation process was continued in dark conditions. All treatments were maintained at 25±1°C and harvested after 30d, then the fresh callus weight was determined before oven-drying at 45ºC for 24h to determine the dry weight (Soni et al., 2018). Changes in fresh and dry weight can reveal information on the growth, development and metabolite formation within a cell as a function of the culture medium, incubation conditions, or stress level (Pan et al., 2020).

Extraction of withanolide compounds

Equal weights (100mg) of dried elicited callus were extracted with the aid of reflux and sonication (fixed frequency at room temperature for 45min) in 50mL of 80% methanol. Each extract was vacuum-concentrated and the volume adjusted to 100mL using methanol. The aliquots were filtered via a 0.22µm Millipore filter (Dwivedi et al., 2017). Three replicates were extracted for each treatment.

Estimation of withanolide compounds using HPLC technique

The qualitative and quantitative analysis of withanolide compounds were conducted at 230nm using high-performance liquid chromatography (HPLC): Shimadzu-10 system, with UV and diode array detector (DAD). The column has 3µm particle size, C-8 (50 x 4.6mm) l.D. The flow rate was 1mL/min. The mobile phase has two constituents, including solvent A (0.01M Orthophosphoric acid) and solvent B (acetonitrile). The gradient program was achieved for chromatographic separation with a linear gradient B% (from 0-100% in 15min.). The concentrations of withanolide compounds in the samples were estimated according to (Mohammed, 2019) as formula below:

$$\text{Concentration of sample}\left(\frac{\mu g}{ml}\right) = \frac{\text{Area of sample}}{\text{Area of standard}} \text{x Conc. of standard x dilution factor}$$

Six standards of withanolide compounds (withanolide-A, withanolide-B, withaferin-A, withanone, 27-OH-withanolide-A, and (f) 27-OH-withanolide-B) were purchased from (Sigma-Aldrich Company, USA) and dissolved individually in methanol to obtain 25mg/L as a final concentration.

Statistical analysis

Twenty replicates were applied for each treatment of elicitation, while the extraction method and HPLC analysis were carried out for three replicates. The "Complete Randomized Design (CRD)" was applied as experimental design using one-way ANOVA test with Duncan's Multiple Comparisons test at ($P< 0.05$) (Seltman, 2018). All data were analyzed by SPSS software (version 23).

Results

Influence of the kind of elicitors on the weight of callus

The impact of various elicitors and their concentrations on the fresh and dry weight of Withania callus cultures is shown in Fig. 1 (a and b). In brief, all chitosan doses caused more than 70% decrease in callus weights, compared to control. It is worth noting that treating callus with chitosan caused a watery texture with an unhealthy appearance Fig. 2 (b).

Exposure callus cultures to a 16h. of light regime or continuous light did not significantly alter callus growth, compared with darkness-grown control (Fig. 1, a and b). In contrast, the UV rays significantly increased callus growth, especially exposing for 60 min where fresh and dry weight reached 843.9 and 81.7mg, respectively; that reflected about 18% increase, compared to the control.

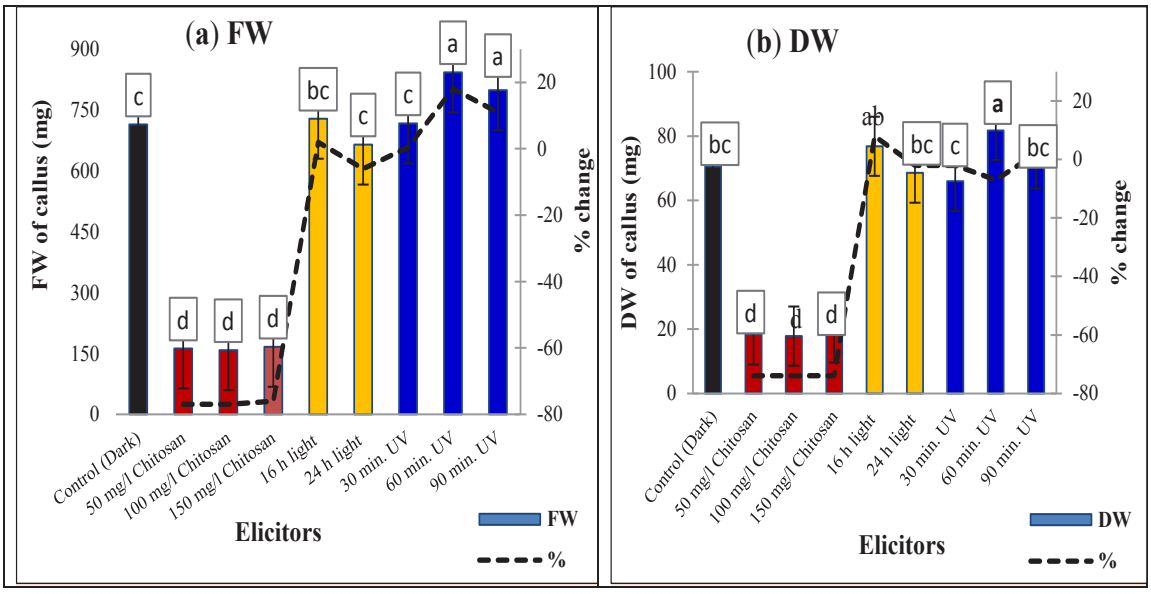

Fig.1. Effect of different concentrations of chitosan and different exposure times to light and UV rays on the (a) fresh weight (FW) and (b) Dry weight (DW) of *W. somnifera* callus culture, and percentage (%) of change compared to control [Bars with the same letter are not significantly different, using Duncan's Multiple Range Test at 5% level]

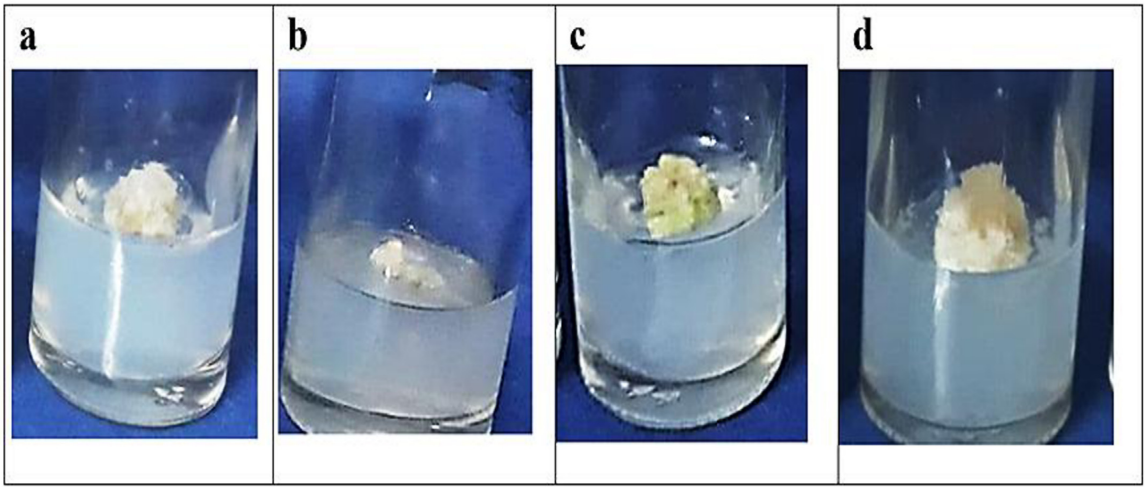

Fig. 2. Morphology of callus culture under biotic and abiotic stress [(a) control, (b) 150mg/L chitosan, (c) 24h/day light, (d) 60min. UV rays}

Influence of the kind of elicitors on concentration of some withanolide compounds in callus extract using HPLC technique

Influence of chitosan

The HPLC analysis showed that adding different concentrations of chitosan (50, 100, 150) mg/L to the growth medium exhibited various stimulation of withanolide compounds in the Withania callus culture (Table 1, and Fig. 3) Significantly, 100mg/L enhanced biosynthesis of 27-OH-withanolide-A to give (52.0µg/mL, with 160 increase) (Fig. 3, e), while 150mg/L gave a high concentration of withanolide-B (96.3µg/mL), at 255% increases compared to control (Fig. 3, b) in comparison to the control. Furthermore, both treatments (100 and 150) mg/L of Chitosan achieved significant values of 27-OH-withanolide-B (124.2, 124.6) µg/mL, respectively, at 40% increase (Fig. 3, f). In conclusion, when compared to the control and other treatments, high concentrations of chitosan promoted the formation of three withanolide compounds.

TABLE 1. Percentage changes of withanolide compounds compared to control treatment in *W. somnifera* callus cultures after being treated with different elicitors

Withanolide compounds	Percentage (%) change compared to control							
	Chitosan (mg/L)			Light (h/day)		UV exposure (min)		
	50	100	150	16	24	30	60	90
Withanolide A	+ 26	+ 25	+ 55	+ 25	+ 54	+ 31	**+ 99**	+ 60
Withanolide B	+ 144	+ 129	**+ 255**	+ 125	+ 107	- 11	+ 29	+ 151
Withaferin A	+ 6	+ 25	+ 62	+ 58	**+ 82**	+ 15	+ 34	+ 35
Withanone	+ 19	+ 58	+ 32	+ 41	**+ 215**	+ 6	+ 2	+ 71
27-OH-withanolide A	+ 20	**+ 160**	+ 130	+ 30	+ 40	+ 50	+ 65	+ 65
27-OH-withanolide B	+ 9	**+ 40**	**+ 40**	+ 27	+ 38	+ 31	+ 29	**+ 40**

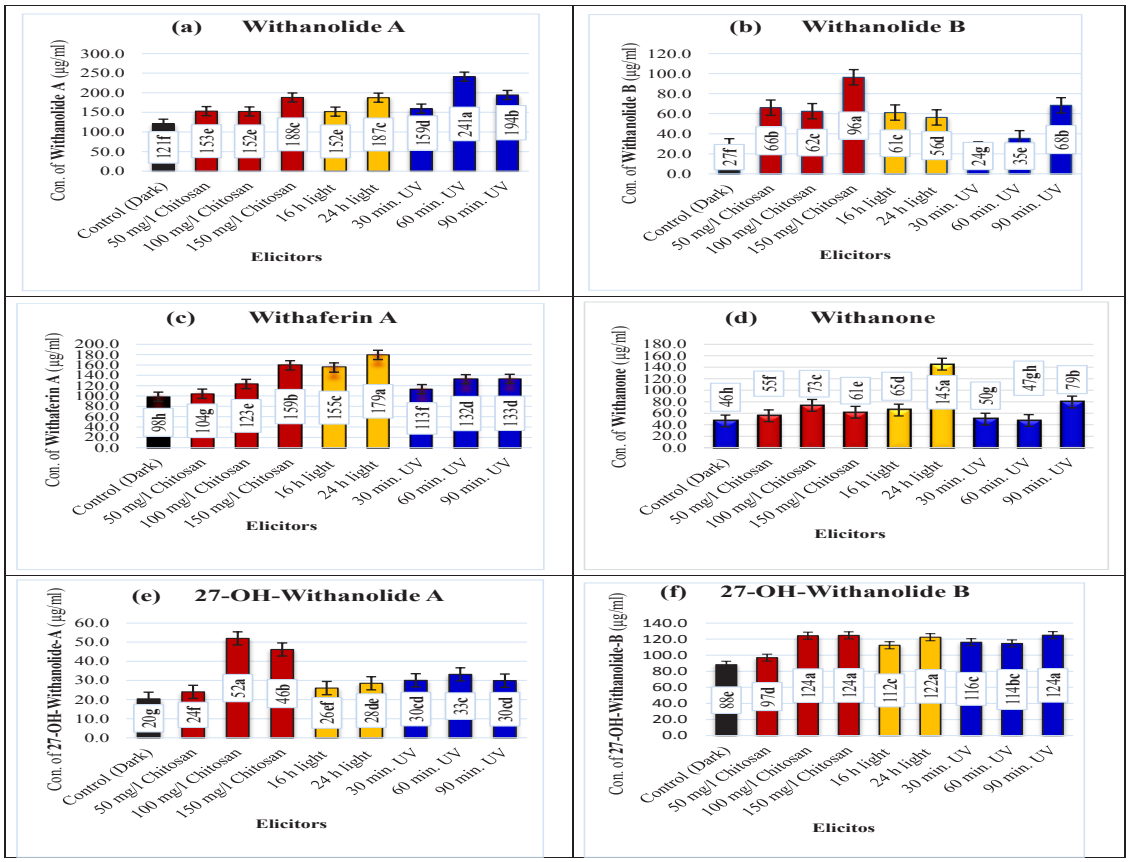

Fig. 3. Effect of different elicitors' treatments on concentration (Con.) of some withanolide compounds, (a) withanolide-A, (b) withanolide-B, (c) withaferin-A, (d) withanone, (e) 27-OH-withanolide-A, and (f) 27-OH-withanolide-B in *W. somnifera* callus culture [Bars with the same letter are not significantly different, using Duncan's Multiple Range Test at 5% level]

Influence of light

In callus cultures of *W. somnifera*, the current study evaluated elicitation techniques using light conditions under various photoperiods. The results in Table 1, and Fig. (3, c and d) show that continuous light conditions (24h/day) significantly promoted biosynthesis of withaferin-A and withanone (179.5, 145.3) µg/mL, respectively, in the callus compared to the control treatment (darkness), led to increase (82% and 215%), respectively. Additionally, this treatment achieved a significantly higher concentration of 27-OH-withanolide B (122.3 µg/ml, 40% increase) than the control (Fig. 3, f).

Influence of UV rays

The impact of exposing callus cultures to UV radiation at different intervals is shown in Table 1 and Fig. 3. The 60 minute-exposure callus to UV rays has the highest significant mean of concentration withanolide-A compound compared to control (241.1µg/mL, 99% increase) (Fig. 3, a). Moreover, the high exposing time (90min) recorded a high significant mean of 27-OH-withanolide B content (124.8µg/mL, 40% increase) (Fig. 3, f).

Discussion

Most of biotic and physical stimuli have demonstrated their inhibitory effect on the growth of biomass of plant cultures and the degree of the effect often varies according to the type of stimulus, its intensity and exposure time. Biotic and abiotic stresses can vigorously alter ion homeostasis, water balance, osmolyte regulation, programmed cell death and gene expression (Pareek et al., 2019; Loutfy et al., 2022; Budran et al., 2023; Drwish et al., 2023). In order to achieve sustainable production of withanolide compounds from tissue cultures, it is desirable to employ stimuli that do not exert a severe impact on plant cell growth, including their weights. Instead, a moderate stimulus is preferred to achieve balanced and synchronized cell growth while enhancing the production of the targeted metabolic compounds.

Chitosan is a chelating agent, that may decline callus biomass because it selectively binds water molecules and inhibits the activities of several enzymes (Ferri & Tassoni, 2011). It may also interfere with mRNA and bind to DNA strands, potentially leading to the formation of complex molecules and a reduction in protein synthesis (Yin et al., 2006). Our observations agree with previous studies that applied similar concentrations of chitosan. According to Wiktorowska et al. (2010), treating Calendula officinalis suspension culture with (50, 100 and 150) mg/L chitosan altered culture from yellowish to light brownish, reducing the growth and cell viability. Furthermore, the decrease in callus weight and tending to brown colour were demonstrated by Khan et al. (2019) through in vitro treatment of *Fagonia indica* callus culture with chitosan.

In our current study, exposing the callus to different periods of light led to a relative increase in the weight of the callus, and these results are consistent with some studies and differ with others because of the different effects of light and darkness on callus growth according to the type of plant and its genetic makeup as well as the brooding environment, as these factors often, outwardly, reflect significant influences (Cioć et al., 2018). In this regard, Usman et al. (2020) reported that the different sources of light could have a considerable impact on cell activity, division, and callus formation. Via different receptors; like phytochrome, phototropin and cryptochrome, plant cells are specialized to detect individual spectra in a broader range (Batista et al., 2018). Accordingly, the transcription, translation and the stability of mRNA in plant cells are affected by light signals (Adil et al., 2019b). Moreover, the content of total proteins and many enzymatic reactions were enhanced under various light conditions (Tariq et al., 2014) light is the key environmental factor that alters plant architectural development; however, the understanding that how light controls plant growth and developmental processes is still poor and needs further research. In this study, we monitored the effect of various monochromatic lights and plant growth regulators (PGRs. Additionally, several modifications in gene expression have been found by using transcription factors or enzymes that are activated by various spectral lights (Hartmann et al., 2020).

However, plant tissues respond differently to the applied photoperiod (Siddique & Islam, 2018). According to Twaij & Taha (2017), sequential light periods led to an enhanced fresh and dry weight of callus tissue compared to constant light or dark conditions in Ricinus communis culture. An increase in different phytochemicals was recorded in various cultures of plants (Victório et al., 2015).

Moreover, many research groups have indicated

the importance of short-term exposure to UV rays in cellular growth. For example, increasing the weights of Verbascum Thapsus callus when exposed to UV radiation for 20min (Abed et al., 2020), 20-40min in Fagonia indicaculture (Abbasi et al., 2021) the inadequate biosynthesis of such metabolites in intact plants has hampered scalable production. Thus, herein, we have established an in vitro based elicitation strategy to enhance such metabolites in callus culture of *F. indica.* Cultures were exposed to various doses of UV radiation (UV-C, and for 60min in *Lepidium sativum* culture (Asad Ullah et al., 2019). According to Singh et al. (2017), the low doses of UV rays are considered an environmental regulator that manage gene expression, cells and metabolic activity, as well as growth and development; through their effects as a signaling molecule on photoreceptors (Heijde & Ulm, 2012). Moreover, several investigators have suggested that exposure to UV rays frequently boosts formation of certain secondary metabolites; like Flavonoids, which have effective radical scavenging abilities and can directly contribute to enhancing formation of normal photosynthetic activity and the photosynthetic pigments, thus promote cell growth (Ibañez et al., 2008).-

Although different stresses have diverse impacts on the biomass growth of plant cultures, the majority of these stresses increase the activity of secondary metabolism in these cultures, which results in the production of metabolic phytochemicals in significant amounts. Sivanandhan et al. (2012) proved that the application of 100 mg/l chitosan stimulated the formation of withanolides in adventitious root cultures of *W. somnifera*. Also, treatment of Phyllanthus amarus cell culture with chitosan induced phyllanthin and hypophyllanthin production (Thakur et al., 2012). Further, chitosan treatment increased flavonoids in the callus of Rumex vesicarius (Sayed et al., 2017) and Fagonia indica (Khan et al., 2019). When chitosan oligosaccharides are released and bind to cell membrane receptors, many cell signaling and systemic acquired immunity are generated like arising of cytosolic H^+ and Ca^{2+}, MAP-kinases stimulation, reactive oxygen species (ROS), jasmonate and phytoalexins production (Ferri & Tassoni, 2011). These reactions often stimulate the production many secondary compounds (Shanker & Shanker, 2016), and it is believed that withanolides metabolism could be linked to these cellular reactions. The previous investigations demonstrated that withanolide compounds; like withaferin A, could mitigate ROS formation through various pathways, such as by modifying the redox potential and targeting the cytoskeletal proteins, heat shock proteins, and chaperones (Hassannia et al., 2020). In addition, Mishra & Sangwan (2019) described how withanolides stimulate the production of antioxidant compounds like glutathione and ascorbate.

Conclusively, many previous studies have demonstrated the efficiency of using light as a physical abiotic factor in stimulating the biosynthesis of primary and secondary plant compounds through increased activity of the phenylalanine ammonia-lyase (PAL) enzyme (Abbasi et al., 2007), enhancement of chlorophyll biosynthesis and regulation of gene expression responsible for the synthesis of terpenes (Kawoosa et al., 2010), improvement of the accumulation of phenolic and flavonoids contents (Adil et al., 2019a) and formation of anthocyanins and carotene pigments (Li & Kubota, 2009). According to Twaij & Taha (2017), the incubation of callus culture of *Ricinus communis* under continuous light conditions was more effective for the stimulation of quercetin, kaempferol and ricinin compounds. In *Eclipta alba* callus culture, the long light periods also induce the biosynthesis of several secondary metabolites (Khurshid et al., 2020). Reichel et al. (2022) mentioned that light intensity promotes the MEP pathway, which increases terpenoid biosynthesis (like withanolides) in plant tissue. Limited data is available on how light impacts the production of withanolides. However, Adil et al. (2019a) have reported that violet light conditions are beneficial for the production of phenols and flavonoids in *W. somnifera* calli. Conversely, callus cultures grown under red light showed a notably higher concentration of chlorogenic acid and withaferin A and greater antioxidant activity compared to other wavelengths. These observations may indicate the augmentation of withanolide compounds in our current study when the *Withania* callus tissue was exposed to continuous illumination.

Furthermore, several studies have found that using UV rays to the callus cultures of higher plants increases the production of many secondary metabolites and reported that its effects vary depending on the exposure time and type of plant or tissue that was exposed to radiation (Nazir et al., 2020; Abbasi et al., 2021). The low and moderate doses of UV radiation lead to the

production of reactive oxygen species (ROS) and the development of oxidative stress that act production of phenolic, terpenes, alkaloids and other compounds, while high doses of radiation may induce negative effects on macromolecules, such as lipids, proteins and amino acids; which cause cell death by reducing cell viability (Katerova et al., 2012). According to Nazir et al. (2020), the exposure to UV rays for 10min enhanced rosmarinic acid, chichoric acid, and anthocyanin production, while 50min increased Peonidin biosynthesis in cultured callus of *Ocimum basilicum* plant. Also, in the callus of *Althaea officinali*, the exposure to UV rays at 60min was significantly elevated salicylic acid, scopolotein and coumarin concentration (Al-Oubaidi et al., 2014). It's worth mentioning that the maximum amounts of many phytochemicals were found in *Fagonia indica* callus culture after 30min of UV rays (Abbasi et al., 2021).

Conclusion

Biotic and abiotic stimuli had different impacts on *W. somnifera* callus culture growth and development. When chitosan inhibited callus growth, physical stress encouraged it. In terms of the effect of these stimuli on the withanolide compounds accumulation, all types studied resulted in a significant increase in their content depending on the stress type, concentration, or exposure period. Chitosan exhibited greater efficacy compared to other treatments in promoting the accumulation of most of the tested withanolide compounds. The subsequent treatments of light stress and UV rays followed in their effectiveness, respectively. High concentrations of chitosan were more effective than lower dosage. Additionally, the long exposure time to lighting and UV rays were more efficient than short-term. Therefore, it is safe to say that the use of chitosan, continuous light and UV stress can stimulate the production of beneficial phytochemicals in the *Withania* culture. The current study has shown that elicitation techniques hold promise for increasing the accumulation of withanolide compounds in callus tissue cultures. Further investigations can focus on refining the application of these elicitors, as well as examining the potential synergistic effects of combining different biotic and abiotic elicitors. Such studies may facilitate the development of novel approaches to enhance the production of these compounds in callus tissue cultures, which could have important implications for the pharmaceutical and nutraceutical sectors.

Competing interests The authors report no conflicts of interest regarding this work.

Authors' contributions: A.J.T. contributed to the design of the study and supervised the research. A.S.A. performed the experiments and wrote the paper.

Ethics approval: Not applicable

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المستحثات الحيوية والفيزيائية تحفز التخليق الحيوي وتراكم بعض مركبات الوذانوليدية في مزرعة كالس *Withania somnifera* L.

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تُستخدَم *Withania somnifera* L. على نطاق واسع كنبات طبي لخصائصه العلاجية. في هذه الدراسة، تمت دراسة تأثير الكيتوزان كمحفز حيوي وتعريض الكالس المستنبت للضوء و الأشعة فوق البنفسجية كمحفزات فيزيائية. وأظهرت النتائج أن الكيتوزان أدى إلى تقليل كبير في الوزن الطازج والجاف للكالس المستنبت بنسبة أكثر من 70% مقارنةً بالمجموعة الضابطة، بينما أدى تعريض الكالس المستنبت للأشعة فوق البنفسجية لمدة 60 دقيقة إلى زيادة ملحوظة في الوزن (زيادة بنسبة 18%). تم تحديد ستة مركبات ويذانولايد في جميع معاملات زراعة الخلايا باستخدام تحليل HPLC. وتم تعزيز إنتاج هذه المركبات بشكل معنوي من خلال استراتيجيات التحفيز. على سبيل المثال، أثر الكيتوزان بتركيز 100 ملغم/لتر حفز بشكل كبير تخليق مركب 27-OH-Withanolide A (52.0ميكروغرام/مل، زيادة بنسبة 160%) مقارنة بالمجموعة الضابطة، بينما أدى تركيز الكيتوزان 150 ملغم/لتر إلى إنتاج مثلى لمركب Withanolide-B (96.3 ميكروغرام/مل، زيادة بنسبة 255%) ومركب 27-OH-Withanolide B (124.6 ميكروغرام/مل، زيادة بنسبة 40%). كما زادت ظروف الإضاءة المستمرة (24 ساعة/يوم) إنتاج مركبي Withaferin-A و Withanone بشكل معنوي (179.5 ميكروغرام/مل، زيادة بنسبة 82% و145.3 ميكروغرام/مل، زيادة بنسبة 215% على التوالي). بينما ادى تعريض الكالس للأشعة فوق البنفسجية لمدة 60 دقيقة أدى إلى تكوين مركب Withanolide-A بكمية ملحوظة (241.1 ميكروغرام/مل، زيادة بنسبة 99%)، في حين أدى التعرض لمدة 90 دقيقة إلى زيادة معنوية في محتوى مركب 27-OH-Withanolide B (124.8 ميكروغرام/مل، زيادة بنسبة 40%). نستنتج ان المحفزات المستخدمة في هذه الدراسة قد أثبتت فعاليتها في تحفيز تخليق مركبات ويذانولايد في مزارع كالس *W. somnifera*. كما تقدم هذه النتائج رؤى قيمة لتحسين إنتاج المركبات العلاجية في نبات *Withania* والذي يستخدَم على نطاق واسع لخصائصه الطبية.