# IMPACT OF BIOTIC AND ABIOTIC ELICITATION ON MORPHOLOGY, GROWTH, ACTIVE CONSTITUENTS AND ANTIBACTERIAL ACTIVITY OF *SOLANUM NIGRUM* (L.) CALLI INDUCED *IN VITRO*

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*Solanum nigrum* (L.) is an important herbaceous medicinal plant belonging to family Solanaceae. It is antiseptic, antibacterial, antidiuretic and anti-inflammatory. In the present study, stem nodal segments of *Solanum nigrum* were used as explants to induce callus formation on Murashige and Skoog basal medium fortified with 30 g/l sucrose, solidified with 6 g/l agar and supplemented with BA and 2,4-D under biotic (autoclaved *Fusarium oxysporum* culture filtrate) and abiotic elicitation (mannitol induced osmotic stress). Results revealed that the callus vigor of growth, color, texture and the nature of the surface of fresh and dry weight, size of callus and contents of some secondary metabolites were affected by the action and interaction and concentration of the two applied elicitors. Elicitation, in general, enhanced the biosynthesis and accumulation of secondary metabolites in callus tissues. Alkaloids were increased from 30 mg/g in dry callus powder (control) to 200 mg/g in calli treated with 1% fungal filtrate and 100 mM mannitol. Flavonoids increased from 20 mg/g in dry callus powder (control) to 410 mg/g in calli treated with 1% fungal filtrate without mannitol. Tannins increased from 10 mg/g in dry callus powder (control) to 310 mg/g in calli treated with 0.5% fungal filtrate and 50 mM mannitol or 1% fungal filtrate with or without 50 mM mannitol. The obtained results indicated also that elicited calli exhibited in general much more pronounced antibacterial effect against the four tested pathogenic bacterial strains (*Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Escherichia coli*). The obtained results indicate the possibility of maximizing the production of valuable phytopharmaceutics *in vitro* using biotic and abiotic elicitation.

**Keywords**: *Solanum nigrum*, callus, elicitation, *in vitro*, secondary metabolites

Higher plants are sources of a lot of important pharmaceutics, which can't be replaced by synthetic substitutes in many cases. Rates (2001) mentioned that 11% of the 252 drugs considered as basic and essential by WHO are exclusively derived from flowering plants but, unfortunately, many plants containing high-value compounds are difficult to cultivate or are becoming endangered because of overharvesting. Furthermore, Namdeo (2007) stated that the chemical synthesis of plant derived compounds is often not economically feasible because of their highly complex structures and the specific stereochemical requirements of the compounds. Although callus, cell suspension and hairy root cultures are regarded as modern biotechnological alternatives for the production of phytopharmaceutics, the lack of understanding of how secondary metabolites biosynthesis is regulated and the difficulties in selecting high-yielding plant cells and stable cultures (Verpoorte and Memelink, 2002) makes it difficult to use such biotechnological methods for the production of phytopharmaceutics on the commercial level. However, elicitation is now regarded as one of the most elite approaches, which can be used to trigger the plant cells and elevate their biosynthetic potential of bioactive substances *in vitro* and makes such systems closer to commercial or semi commercial production. Shikonin, ginsenosides, berberine and taxol are already good examples of important phytopharmaceutics, which could be produced commercially on large scale in bioreactors as described Malik et al. (2016).

Elicitation is the induced or enhanced biosynthesis of defense secondary metabolites by plant cell culture systems *in vitro* due to addition of trace amounts of elicitors (Radman et al., 2003). These substances (elicitors) can be of biological origin like microbial enzymes or cell wall fragments (biotic elicitors) or of non-biological origin like stress inducing chemicals including mannitol or sodium chloride (abiotic elicitors). The exact mechanism of elicitation is not fully understood.

The main objectives of the present study were to induce callus formation from stem nodal explants of *Solanum nigrum* (L.) and study the effect of biotic elicitation (autoclaved *Fusarium oxysporum* culture filtrate) and abiotic elicitation (mannitol induced osmotic stress) on callus growth, morphology, biosynthesis of some active constituents and antibacterial activity.

## MATERIALS AND METHODS

### 1. Explant Preparation

Surface sterilized seeds of *S. nigrum* were allowed to germinate and grow on Murashige and Skoog (1962) culture medium (MS medium) for three weeks. The seedlings were then used as sources of stem nodal explants (1 $cm^2$).

**2. Treatments**

For callus induction, explants were separately cultured on MS culture medium supplemented with BA (1 mg/l) in combination with 2,4-D (0.75 mg/l). Media were supplemented with biotic elicitor represented by fungal filtrate (*Fusarium oxysporum*) and abiotic elicitor represented by mannitol to create 15 different formulations in addition to the control treatment as illustrated in table (1).

**Table (1).** Amounts added of fungal filtrate and mannitol to MS culture medium to create biotic and abiotic stresses, separately and interacted.

| Mannitol (mM) | Fungal filtrate (%) | | | |
|---|---|---|---|---|
| | **0.0** | **0.5** | **1.0** | **1.5** |
| **0** | 1 | 2 | 3 | 4 |
| **50** | 5 | 6 | 7 | 8 |
| **100** | 9 | 10 | 11 | 12 |
| **150** | 13 | 14 | 15 | 16 |

**3. Determination of Secondary Metabolites**

**3.1. Total alkaloids**

Total alkaloids were determined according to Harborne (1984). Five grams of the sample were weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for four hours. This was filtered and the extract was concentrated in a water bath to one – quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

**3.2. Total tannins**

Total tannins were determined gravimetrically according to Ali et al. (1991). One gram fresh callus was boiled for one hour with two successive quantities, each of 100 ml of acetone: water (1:1). Filtration was carried out and the combined filtrates were completed to 250 ml using distilled water and heated again till boiling. Tannins were then precipitated from the extract as copper tannate by the addition of 30 ml of 15% aqueous solution of copper acetate. The precipitate was collected on ashless filter paper, washed with water till being free from copper acetate, ignited in porcelain crucible that was previously ignited to a constant weight. A few drops of nitric acid were added to the residue and reignited to a constant weight. The weight of

the resulting copper oxide was determined and the amount of tannins was calculated.

**3.3. Total flavonoids**

Total flavonoids were determined according to Bohm and Kocipai-Abyazan (1974). Two grams of the plant material were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through a filter paper number 42 (125 mm) and the filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

**4. Assay of Antibacterial Activity**

Assay of antibacterial activity was carried out by the disk-diffusion method described by Salie et al. (1996). Five mm (diameter) filter paper disks were allowed to imbibe different aliquots of ethanolic extract in order to have an exact amount of dry callus extract equal to 30 mg per disc. After the organic solvent was completely evaporated, the discs were put on the surface of nutrient agar seeded with test bacteria in 9 cm diameter Petri-dishes. All the plates were incubated at 37°C for 24 h. The experiment was performed three times under strict aseptic conditions. Antibacterial activity was determined by measuring the diameter of the inhibition zone and the mean values were calculated.

**5. Experimental Design and Statistical Analysis**

Experiments were repeated three times following a completely randomized block system. Statistical analysis (Anova- one way completely randomized, significance level "0.05") was performed using CoStat statistical software, CoHort info@cohort, www.cohort.com.

## RESULTS AND DISCUSSION

The effect of biotic elicitation (represented by the addition of autoclaved fungal filtrates to the callus inducing culture media) and abiotic elicitation (represented by the addition of mannitol to the callus inducing medium) on callus morphology (vigor of growth, color and texture) can be seen in table (2, 3 and 4) and fig. (1). The results obtained showed that the incorporation of fungal filtrate reduced the vigor of growth of *S. nigrum* callus, while on the other hand mannitol treatment (100 mM and 150 mM) enhanced callus growth. Except for one case, the interaction of fungal filtrate and mannitol reduced the callus growth from medium to week or mild growth. The color of callus and its uniformity varied according to treatment, but it is difficult to correlate the color of callus to a specific treatment, however the color ranged from green to greenish and from creamy to brownish and the treatment with 1.5% fungal filtrate alone or combined with

mannitol generally resulted in patchy calli. The calli obtained were either soft with smooth surface or compact and nodular. It could be obviously observed that mannitol or fungal filtrate resulted in a soft smooth callus resembling the control one, but the interaction of mannitol and the fungal filtrate in some concentrations (in the great majority) resulted in the production of compact nodular calli. The observed changes in growth, color and texture of calli may have resulted from altered metabolism of the cultured cells under the effect of elicitors. Many of the secondary metabolites are colored substances.

**Table (2).** Effect of biotic and abiotic elicitation on vigor of growth of *S. nigrum* callus.

| **Mannitol (mM)** | **Fungal filtrate (%)** | | | |
|---|---|---|---|---|
| | **0.0** | **0.5** | **1.0** | **1.5** |
| **0** | ++ | + | + | + |
| **50** | ++ | + | + | + |
| **100** | +++ | + | + | ++ |
| **150** | +++ | + | + | + |

Note: + (Mild growth), ++ (Medium growth), +++ (Vigorous growth)

**Table (3).**Effect of biotic and abiotic elicitation on color *S. nigrum* callus.

| **Mannitol (mM)** | **Fungal filtrate (%)** | | | |
|---|---|---|---|---|
| | **0.0** | **0.5** | **1.0** | **1.5** |
| **0** | Bsh/P | Cr/U | Cr/U | Gsh/P |
| **50** | G/P | G/P | G/P | G/P |
| **100** | Cr/U | Cr/P | G/P | G/P |
| **150** | Cr/U | Gsh/P | Bsh/U | Gsh/P |

Colors: G (Green), Gsh (Greenish), Y (Yellow), Ysh (Yellowish), B (Brown), Bsh (Brownish),Cr (Creamy), Uniformity: U (uniform), P (Patchy)

**Table (4).** Effect of biotic and abiotic elicitation on texture and surface of callus of *S. nigrum*.

| **Mannitol (mM)** | **Fungal filtrate (%)** | | | |
|---|---|---|---|---|
| | **0.0** | **0.5** | **1.0** | **1.5** |
| **0** | S/Sm | S/Sm | S/Sm | S/Sm |
| **50** | S/Sm | C/Nr | C/Nr | S/Sm |
| **100** | S/SP | S/Sm | C/Nr | C/Nr |
| **150** | S/SP | C/Nr | S/Sm | C/Nr |

Texture: S (Soft), Sp (Spongy), C (Compact); Surface: Nr (Nodular), Sm (Smooth).

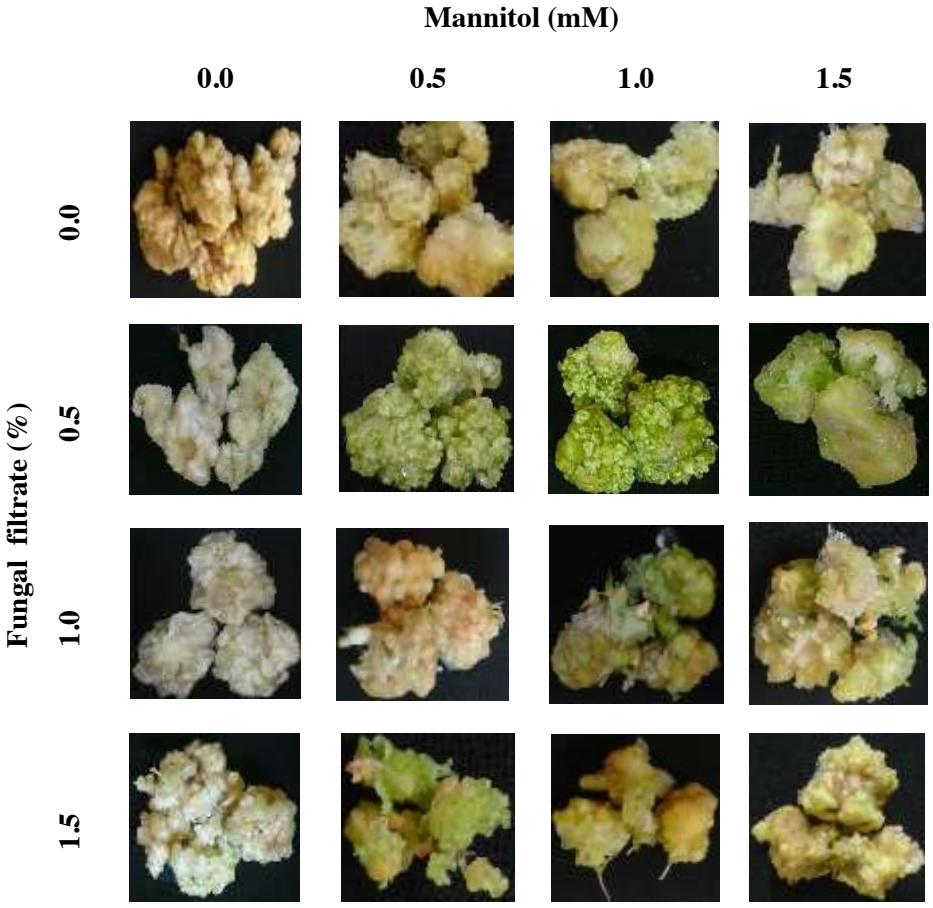


**Fig. (1).** Calli of *S. nigrum* produced under biotic and abiotic elicitation.

With respect to callus size, the results illustrated in table (5) show that the treatment with mannitol generally increased the callus size significantly. The lower concentration of the fungal filtrate (0.5%) exhibited no effect on callus size in comparison to the non-treated control calli. While the treatment with 1% fungal filtrate significantly increased the callus size, the higher concentration (1.5%) reduced the size of the callus. The interaction between mannitol and autoclaved fungal filtrate exhibited various effects on callus size and it is clear from the results that the effect depended on the concentration of the two factors applied. In general, the highest callus size reached up to 8.33 $cm^3$ in response to the treatment with 50 mM mannitol and 1% fungal filtrate. This value represents a significant increase over all the other treatments. The minimum callus size (1.0 $cm^3$) resulted from the treatment with 150 mM mannitol and 0.5% fungal filtrates. It is to be mentioned that the callus with the maximum size is not necessary to be

the one with the highest fresh or dry weight. The nature, morphology and growth of the callus may play a role.

**Table (5).** Effect of biotic and abiotic elicitation on size of callus of *S. nigrum*.

| Mannitol (mM) | Fungal filtrate (%) | | | |
|---|---|---|---|---|
| | **0.0** | **0.5** | **1.0** | **1.5** |
| **0** | 3.33 cd | 3.66 c | 5.66 b | 2.66 cd |
| **50** | 9.33 a | 2.66 cd | 8.33 a | 2.66 cd |
| **100** | 6.00 b | 2.00 cd | 2.66 cd | 2.33 cd |
| **150** | 6.33 b | 1.00 d | 2.00 cd | 1.33 cd |

Each value is a mean of three determinations. Different letters means significant value, LSD 0.05 = 1.499

The increase in callus size as a result of the treatment with mannitol is accompanied by a significant increase also in fresh weight of callus. This can be seen in the results of the present study, as illustrated in table (6). Fresh weight of callus increased from 3.28 g on control medium to 5.3 g in calli treated with 50 and 150 mM mannitol. Mitoi et al. (2009) revealed that long term exposure to mannitol at high concentrations, may activate the adaptive mechanisms by which the expression of antioxidant enzymes are correlated. Hadi et al. (2014) explained that there is a big difference between gradual and shock exposure of callus to mannitol treatment. Working with fenugreek calli, Hussein and Aqlan (2011) found out that osmotic stress created by low mannitol concentration enhanced callus growth. Ziegler (1990) stated that while shock exposure results in a significant decrease in callus fresh weight, the gradual exposure results in no adverse effects on callus fresh weight and in some cases may even improve callus growth. The same author reported that gradual exposure of *Ruta graveolens* calli to mannitol exhibited no adverse effects on callus fresh weight. Even more, the same author added that they observed a significant increase in callus growth in response to 240 g/l mannitol after twelve weeks of exposure and concluded that it seems that callus growth increased slowly with increasing the time of exposure to mannitol. The gradual exposure of callus to mannitol induces high expression for a number of genes like osmo-proteins (shock proteins), but these proteins do not forme when calli expose to shock treatment with mannitol. According to Peleg et al. (2011), the results observed may be due to the over expression of specific genes that has activated the expression of many downstream genes thus plants express improved stress tolerance.

**Table (6).** Effect of biotic and abiotic elicitation on fresh weight of callus of *S. nigrum*.

| Mannitol (mM) | Fungal filtrate (%) | | | |
|---|---|---|---|---|
| | **0.0** | **0.5** | **1.0** | **1.5** |
| **0** | 3.28 b | 1.69 def | 3.15 bc | 1.50 def |
| **50** | 5.30 a | 1.89 cdef | 2.77 bcd | 1.05 ef |
| **100** | 4.57 a | 1.99 cde | 0.82 ef | 1.89 cdef |
| **150** | 5.30 a | 0.40 f | 0.79 ef | 1.80 cdef |

Each value is a mean of three determinations. Different letters means significant value, LSD 0.05 = 0.921

With respect to fungal filtrate, the results showed that the lower and higher concentrations resulted in a decrease in fresh weight of callus, while the medium concentration of 1% showed insignificant increase in comparison to the corresponding control. It is worthy to mention that the treatment which resulted in the minimum callus size had resulted also in the minimum callus fresh weight (0.4 g). In general, the maximum fresh weight, which also represents a significant increase over all the other treatments, was obtained in control callus (3.28 g). The results obtained may agree and confirm the results which were obtained by El-Nabarawya et al. (2015), who reported that fungal biotic elicitation showed negative effect on both callus fresh and dry weights compared to control.

With respect to the dry biomass, the results illustrated in table (7) show that calli, which exhibited the maximum fresh weight (control calli) had shown the maximum dry weight (0.31 g). It is clear from the same table that all the fungal treatments resulted in an insignificant decrease in callus dry biomass. The minimum dry biomass (0.04 g) resulted from the treatment with 0.5% fungal filtrate and 150 mM mannitol.

With respect to alkaloids, the results illustrated (Table 8) show that the amount of alkaloids in calli ranged between 10 and 200 mg/g dry callus powder. Treatment with autoclaved fungal filtrate resulted in an increase in alkaloid content from 30 mg/g in control calli to 130 and 140 mg/g dry callus powder in response to 0.5 and 1.5%, respectively. The amount of alkaloids of 170 mg/g dry callus powder was recorded in calli treated with 50 mM mannitol, which can be considered proper for alkaloid biosynthesis, while the higher concentrations suppressed alkaloid biosynthesis or accumulation. The results of interaction of the two elicitation factors ranged from enhancement of alkaloid biosynthesis to suppression. For example treatment with 1% autoclaved fungal filtrate plus 100 mM mannitol resulted in the maximum accumulation of alkaloids (200 mg/g dry powder), a value which means that the treatment increased the amount of alkaloids more than six folds of the amount of alkaloids detected in control calli. Probably, the combination of the two eliciting factors in these concentrations was proper

and that some sort of synergism have resulted and triggered the biosynthesis of alkaloids. On the other hand, treatment with 0.5% fungal filtrate in combination with 150 mM mannitol was severe enough to decrease the amount of alkaloids to 10 mg/g dry callus powder. In general, it may be mentioned that working out the proper combination and concentration of elicitors may exhibit positive effects on the biosynthesis of alkaloids by calli of *S. nigrum in vitro*.

**Table (7).** Effect of biotic and abiotic elicitation on dry weight of callus of *S. nigrum*.

| Mannitol (mM) | Fungal filtrate (%) | | | |
|---|---|---|---|---|
| | **0.0** | **0.5** | **1.0** | **1.5** |
| **0** | 0.31 a | 0.20 abcde | 0.21 abcd | 0.20 abcde |
| **50** | 0.29 ab | 0.14 bcdef | 0.23 abcd | 0.11cdef |
| **100** | 0.27 abc | 0.17 abcdef | 0.08 def | 0.17 abcdef |
| **150** | 0.31 a | 0.04 f | 0.05 ef | 0.17 abcdef |

Each value is a mean of 3 determinations. Different letters means significant value, LSD 0.05 = 0.094

**Table (8).** Effect of biotic and abiotic elicitation on total alkaloids (mg/g dry plant material) of calli of *S. nigrum* induced from stem nodal segments.

| Mannitol (mM) | Fungal filtrate (%) | | | |
|---|---|---|---|---|
| | **0.0** | **0.5** | **1.0** | **1.5** |
| **0** | 30 | 130 | 15 | 140 |
| **50** | 170 | 70 | 100 | 100 |
| **100** | 30 | 60 | 200 | 50 |
| **150** | 20 | 10 | 30 | 20 |

Although the treatments applied exhibited in some cases positive effects and in other cases negative effects on alkaloids. The situation differed with respect to flavonoids. All the applied treatments (as illustrated in table 9) triggered flavonoid biosynthesis, a result which may show for some extent that flavonoids play a role in osmotic adjustment, defense mechanisms and anti-oxidative effects against biotic or abiotic stress or elicitation factors. The amount of flavonoids in control calli was 20 mg/g dry callus powder, while in treated calli it ranged between 60 mg (i.e three folds the concentration of flavonoids in control calli) and 430 mg (i.e. more than 21 folds the concentration of flavonoids detected in control calli).

**Table (9).** Effect of biotic and abiotic elicitation on total flavonoids (mg/g dry plant material) of calli of *S. nigrum* induced from stem nodal segments.

| Mannitol (mM) | Fungal filtrate (%) | | | |
|---|---|---|---|---|
| | **0.0** | **0.5** | **1.0** | **1.5** |
| **0** | 20 | 360 | 430 | 350 |
| **50** | 280 | 200 | 230 | 210 |
| **100** | 390 | 210 | 60 | 410 |
| **150** | 120 | 60 | 160 | 170 |

The amounts of tannins (Table 10) increased from 10 mg/g in control calli to a maximum value of 310 mg/g, in calli treated with 150 mM mannitol. It is worthy to mention that except for treatment with 1% fungal filtrate and 150 mM mannitol, all the other treatments markedly resulted in amounts of tannins higher than the corresponding control. So, It can be freely mentioned that both biotic and abiotic treatments individually or combined exhibited positive effects on secondary metabolism and accumulation of alkaloids, flavonoids and tannins. The results obtained in this study are in agreement with other studies, which found out that *in vitro* cultured plant cells could synthesize extra amounts of secondary metabolites like phenolics (Grace and Logan, 2000), anthraquinone (Nazif et al., 2000), flavonoids and indole alkaloids (Chutipaijit et al., 2009) and anthocyanin (Chan et al., 2010) in response to various abiotic stress factors. It is to be mentioned that osmotic stress tends to accumulate sugars and compatible solutes in the cytoplasm (Chen and Murata, 2002) and other osmotically active low molecular weight compounds (Roychoudry et al., 2008). The results of the present study may agree with Dixon and Priva (1995), who mentioned that soluble phenolics, which represent the most widely distributed secondary metabolites in the plant kingdom, could be enhanced as a powerful antioxidant in plant tissues suffering from different stress factors. The results obtained in this study that biotic elicitation may enhance the biosynthesis of secondary metabolites in callus cultures may agree with previous observations, which were made on other plants by Baldi et al. (2009), Karwasara et al. (2011), Mendhulkar and Vakil (2013), Swaroopa et al. (2013), Tahsili et al. (2013), Ahmed and Baig (2014), Ebrahimi and Zarinpanjish (2015) and Gadzovska Simic et al. (2015). Elicitation is done to enhance the production of secondary metabolites in plant cultures by using biotic/abiotic molecules. They are considered as signaling molecules as their incorporation in the cultures generates signal transduction cascade and leads to activation and expression of the related genes with the biosynthesis of metabolites (Zhao et al., 2005) and also stimulates plant's antioxidant defense system (De Gara et al., 2003).

**Table (10).** Effect of biotic and abiotic elicitation on total tannins (mg/g dry plant material) of calli of *S. nigrum* induced from stem nodal segments.

| Mannitol (mM) | Fungal filtrate (%) | | | |
|---|---|---|---|---|
| | **0.0** | **0.5** | **1.0** | **1.5** |
| **0** | 10 | 110 | 200 | 210 |
| **50** | 130 | 200 | 200 | 120 |
| **100** | 110 | 30 | 130 | 140 |
| **150** | 310 | 110 | 10 | 80 |

The results illustrated in table (11) and fig. (2) show the antibacterial activity of methanolic extracts of calli of *S. nigrum* against four pathogenic bacterial strains (*Staphyllococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Escherichia coli*). The inhibition zones of bacterial growth were measured in mm. The results in general indicate that the extracts of elicited calli were much more powerful in inhibiting the growth of the tested pathogenic bacterial strains. The diameter of inhibition zone of *S. aureus* reached up to a maximum of 18 mm in calli treated with 1 or 1.5 fungal filtrate plus 50 mM mannitol, while the diameter of the inhibition zone that resulted from extracts of untreated calli was only 6 mm. The treatment with 0.5% fungal filtrate increased the inhibition zone of *Bacillus cereus* up to 14 mm in comparison to 6 mm in the extracts of control calli. With respect to *E. coli*, the maximum inhibition zone caused by treated calli reached up to 10 mm (1% fungal filtrate with or without 0.5 mM mannitol; 100 mM and 0.5% fungal filtrate plus 150 mM mannitol), while the inhibition zone caused by control extracts was 6 mm. In contrast to the previously mentioned cases, the methanolic extract of untreated calli was more powerful in inhibiting the growth of *Pseudomonas aeruginosa*. The inhibition zone reached up to 15 mm, while in the best cases (with extracts of treated calli, the inhibition zones reached 14 mm). It has been also observed that under some treatments, the extracts of calli could not inhibit the bacterial growth, probably due to the severity or improperly applied treatments or due to the biosynthesis of metabolites with less antibacterial effects. However, the results obtained in inhibiting the bacterial growth may be due to the elevated biosynthetic potential of the treated calli of secondary metabolites, which already known to inhibit bacterial growth like alkaloids, flavonoids and tannins, which were determined in this study.

**Table (11).** Effect of biotic and abiotic elicitation on antibacterial activity of methanolic extracts of calli of *S. nigrum* obtained from nodal explants as measured in mm inhibition zones by the disc-plate method.

| Mannitol (mM) | Fungal filtrate (%) | Pathogenic bacteria | | | |
|---|---|---|---|---|---|
| | | ***Staphyllococcus aureus*** | ***Bacillus cereus*** | ***Eschershia coli*** | ***Pseudomonas aeroginosa*** |
| **0** | **0.0** | 6 | 6 | 6 | 15 |
| | **0.5** | 9 | 14 | 8 | 9 |
| | **1.0** | 14 | 12 | 10 | 9 |
| | **1.5** | 16 | 10 | 9 | 12 |
| **50** | **0.0** | 14 | 9 | 9 | 8 |
| | **0.5** | 12 | 10 | 9 | 11 |
| | **1.0** | 18 | 12 | 10 | 14 |
| | **1.5** | 18 | 13 | 8 | 9 |
| **100** | **0.0** | 14 | 13 | 10 | 12 |
| | **0.5** | 16 | -ve | -ve | -ve |
| | **1.0** | 14 | 12 | 10 | 12 |
| | **1.5** | -ve | 10 | 10 | 10 |
| **150** | **0.0** | 14 | 12 | 8 | 11 |
| | **0.5** | 10 | 9 | 10 | -ve |
| | **1.0** | 12 | 9 | -ve | -ve |
| | **1.5** | 12 | -ve | 8 | -ve |

Hussein (2009) found out that callus cultures from leaf and stem explants of *Cordia africana* exceeded the mother plant parts in antibacterial activity. The results obtained by Chaudhry et al. (2014) indicated that the extracts of elicited calli obtained from explants of *Nigella sativa* (biotic and abiotic) showed better antibacterial activity against pathogenic Gram +ve and Gram –ve bacterial strains even more than some commercially available antibiotics. The same authors explained the high antibacterial activity on the basis of elevated biosynthesis of secondary metabolites like alkaloids and flavonoids, which were also determined in their study. The authors concluded that the results of their study have revealed clear potentiality of *N. sativa* callus extracts as an alternative source for antimicrobial drugs. The results of the present study are in agreement with the previous findings that methanolic plant extracts give higher antimicrobial activities as stated by Soniya et al. (2013). The higher antimicrobial activity of the callus extract could be either related to the production of a compound/group of compounds produced only in undifferentiated callus cells or may be produced in higher amounts in these cells when compared to differentiated cells as reported in the previous studies (Nagananda et al., 2012), suggesting that *in vitro* cell

cultures contain potential active antimicrobial components. Elicitation also played a remarkable role in elevating the antibacterial activity of callus extracts in many cases.

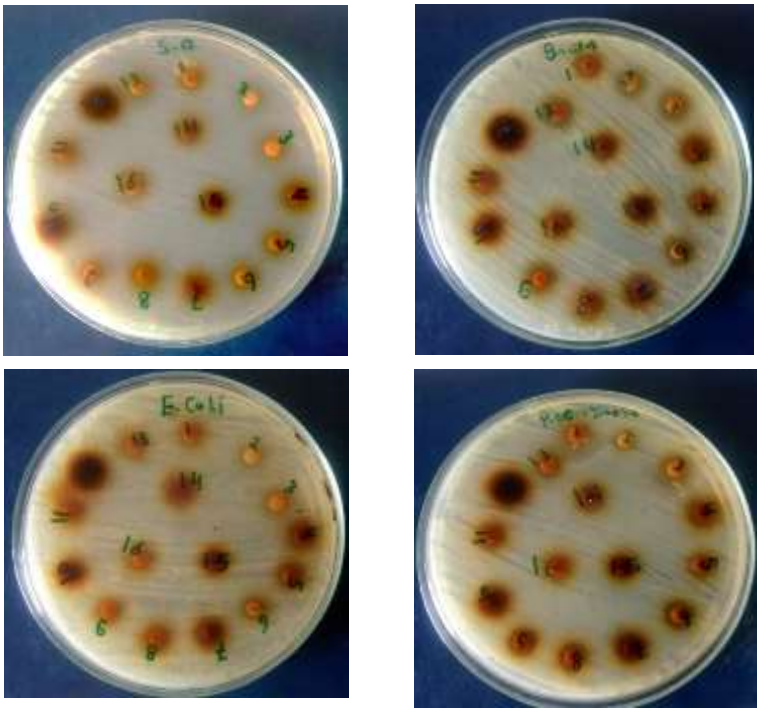

**Fig. (2).** Antibacterial activity of alcoholic extracts of calli of *S. nigrum* produced under biotic and abiotic elicitation.

## REFERENCES

Ahmed, S.A. and M.M.V. Baig (2014). Biotic elicitor enhanced production of psoralen in suspension cultures of *Psoralea corylifolia* L. Saudi J. Biol. Sci., 21: 499–504.

Ali, A.A., S.A. Ross, M.K. Mesbah and S.A. El-Moghazy (1991). Phytochemical study of *Limonium axillare* (Forssk.). Bull. Fac. Pharm. Cairo Univ., 29: 59-62.

Baldi, A., A.K. Srivastava and V.S. Bisaria (2009). In "Fungal Elicitors for Enhanced Production of Secondary Metabolites in Plant Cell Suspension Cultures". Symbiotic Fungi, Soil Biology (Varma, A. and A.C. Kharkwal Eds.). Springer-Verlag, Berlin/Heidelberg, Germany, 18, p. 373–380.

Bohm, B.A. and R. Kocipai-Abyazan (1974). Flavonoids and condensed tannins from leaves of Hawailan *Vaccinium vaticulatum* and *V. calycinium*. Pacific Sci., 48: 458-463.

Chan, L.K., S.S. Koay, P.L. Boey and A. Bhatt (2010). Effects of abiotic stress on biomass and anthocyanin production in cell cultures of *Melastoma malabathricum*. Biological Res., 43: 127–135.

Chaudhry, H., F. Nida and I. Ahmad (2014). Evaluation of *Nigella sativa* L. callus extracts under elicitation for phytochemical and antibacterial activity. Int. J. Pharm. Bio. Sci., 5 (4): (B) 903 – 916.

Chen, T.H.H. and N. Murata (2002). Enhancement of tolerance of abiotic stress by metabolic engineering of betains and other compatible solutes. Curr. Opin. Plant Biol., 5: 250-257.

Chutipaijit, S., S. Cha-Um and K. Sompornpailin (2009). Differential accumulations of proline and flavonoids in indica rice varieties against salinity. Pakistan J. Bot., 45: 2497-2506.

De Gara, M. De Pinto and F. Tommai (2003). The antioxidant system vis-à-vis reactive oxygen species during plant pathogen interaction. Plant Physiol. Biochem., 41: 863-870.

Dixon, R. and N. Priva (1995). Stress induced phenyl propanoid metabolism. Plant Cell, 7: 1085-1097.

Ebrahimi, M.A. and N. Zarinpanjeh (2015). Bio-elicitation of β-carboline alkaloids in cell suspension culture of *Peganum harmala* L. J. Med. Plants, 14 (55): 43-57.

El-Nabarawya, M.A., S.H. El-Kafafia and M.A. Hamzab (2015). The effect of some factors on stimulating the growth and production of active substances in *Zingiber officinale* callus cultures. Annals Agricult. Sci., 60 (1): 1-9.

Gadzovska, S.S., O. Tusevski, S. Maury, C. Hano, A. Delaunay, B.M. Chabbert, F. Lamblin, E. Laine, C. Joseph and D. Hagege (2015). Fungal elicitor-mediated enhancement in phenylpropanoid and naphtodianthrone contents of *Hypericum perforatum* L. cell cultures. Plant Cell Tiss. Org. Cult., 122: 213–226.

Grace, S.C. and B.A. Logan (2000), Energy dissipation and radical scavenging by the plant phenyl propanoid pathway. Phil. Trans. Royal Soc. B., 355: 1499-1510.

Hadi, S.M., K.M. Ibrahim and S.I. Yousif (2014). Effect of shock and elevated levels of mannitol on callus growth, regeneration and proline accumulation in *Ruta graveolens* cultures. International Journal of Microbiology and Applied Sci., 3 (11): 479-488.

Harborne, J.B. (1984). In "Phytochemical Methods". A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London.

Hussein, E.A. (2009). Callus from leaf and stem explants of *Cordia africana* Lam., exceeded the mother plant parts in antibacterial activity. Bull. Fac. Appl. Sci., Taiz Univ., 1: 41-49.

Hussein, E.A. and E.M. Aqlan (2011). Regeneration of *Solanum villosum* Mill., *via* direct organogenesis *in vitro*: A novel study. Intern. J. Bot., 7 (2): 177-182.

Karwasara, V., P. Tomar and V. Dixit (2011). Influence of fungal elicitation on glycyrrhizin production in transformed cell cultures of *Abrus precatorius* Linn. Pharmacogn. Mag., 7 (28): 307–313.

Malik, S., S. Bhushana, M. Sharma and P. Ahuja (2016). Biotechnological approaches to the production of shikonins: a critical review with recent updates. Critical Reviews in Biotechnology, 36 (2): 327-340.

Mendhulkar, V.D. and M.M.A. Vakil (2013). Chitosan and *Aspergillus niger* mediated elicitation of total flavonoids in suspension culture of *Andrographis paniculata* (Burm. f.). Nees. Int. J. Pharma. Bio. Sci., 4: 731–740.

Mitoi, E. M., I. Holobiuc and R. Blindu (2009). The effect of mannitol on antioxidantive enzymes *in vitro* term cultures of *Dianthus tenuifolius* and *Dianthus spiculifolius*. Rom. J. Biol. Plant Biol., 54 (1): 25-33.

Murashige, T. and F. Skoog, (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15: 473-497.

Nagananda, G.S., A. Krishnamoorthy, A. Das and S. Bhattacharya (2012). Phytochemical screening and evaluation of antimicrobial activities of *in vitro* and *invivo* grown plant extracts of *Lobelia inflata* L. Int J Phar Bio Sc., 3 (3): 433–442.

Namdeo, A.G. (2007). Plant cell elicitation for production of secondary metabolites: A review. Pharmacognosy Reviews, 1(1): 69-79.

Nazif, N.M., M.R. Rady and M.M. Seif El-Nasr (2000). Stimulation of anthraquinone production in suspension cultures of *Cassia acutifolia* by salt stress. Fitoterapia, 71: 34-40.

Peleg, Z., M.P. Apse and E. Blumwald (2011). Engineering salinity and water-stress tolerance in crop plants. Getting closer to the field. Adv. Bot. Res., 57: 407 - 432.

Radman, R., T. Saez, C. Bucke and T. Keshavarz (2003). Elicitation of plant and microbial systems. Biotechnol. Appl. Biochem., 37: 91-102.

Rates, S.M.K. (2001). Plants as sources of drugs. Toxicon, 39: 603-613.

Roychoudry, A., S. Basu, S.N. Sarka and D.N. Sengupta (2008). Comparative physiological and molecular responses of common aromatic indica rice cultivars to high salinity with non-aromatic indica rice cultivars. Plant Cell. Rep., 27: 1395-1410.

Salie, F., P.F.K. Eagles and H.M.J. Leng (1996). Preliminary antimicrobial screening of four South African Asteraceae species. J. Ethonopharmacol., 52: 27-33.

Soniya, M., T. Kuberan, S. Anitha and P. Pankareswari (2013). *In vitro* antibacterial activity of plant extracts against gram positive and gram negative pathogenic bacteria. Intern. J. of Microbiol. Immunol. Res., 2 (1): 1-5.

Swaroopa, G., M. Anuradha and T. Pullaiah (2013). Elicitation of forskolin in suspension cultures of *Coleus forskohlii* (wild.) Briq. using elicitors of fungal origin. Curr. Trends Biotechnol. Pharm., 7: 755–762.

Tahsili, J., M. Sharifi, N. Safaie, S. Esmaeilzadeh-Bahabadi, and M. Behmanesh (2013). Induction of lignans and phenolic compounds in cell culture of *Linum album* by culture filtrate of *Fusarium graminearum*. J. Plant Interact., 9: 412–417.

Verpoorte, R. and J. Memelink (2002). Engineering secondary metabolite production in plants. Curr. Opin. Biotechnol., 13: 181-187.

Zhao, J., L. Davis and R. Verpoorte (2005). Elicitor signal transduction leading to production of secondary metabolites. Biotechnol. Adv., 23: 283-333.

Ziegler, H. (1990). Role of plant physiology in assessing productivity potential under stress environment. Proceedings of the International Congress of Plant Physiol., New Delhi, India, 88: 10 -17.

# تأثير التظهير الحيوي وغير الحيوي على الشكل الظاهري والنمو والمركبات الفعالة والنشاط الضد بكتيري لكالس نبات السولانم نيجروم المتكون في المعمل

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يعتبر نبات السولانم نيجروم نبات عشبي طبي تابع للعائلة الباذنجانية يستخدم كمطهر، مضاد للبكتيريا ومدر للبول كما يستخدم كمضاد للإلتهابات. لقد تم في هذه الدراسة إستخدام القطع الساقيه العقدية كمنفصلات نباتية للحصول على الكالس، حيث تم زراعة تلك المنفصلات النباتية على وسط موراشيج وسكوج المزود بالبنزيل أدينين مع ثنائي كلوروفينوكسي حمض الخليك (2,4-D) لإستحثاث تكون الكالس تحت تأثير التظهير الحيوي (براشح الوسط الغذائي المعقم لفطر فيوزاريوم أوكسي سبورام) والتظهير غير الحيوي (بإستخدام سكر المانيتول لإحداث حالة من الإجهاد المائي على الخلايا المنزرعة). لقد أثبتت نتائج هذه الدراسة أن التظهير قد أثر بشكل متفاوت على نسبة النمو وطبيعة السطح ولون وقوام الكالس ووزنيه الطازج والجاف، وكذلك حجم الكالس ومحتواه من المواد الفعالة. تدل الملاحظات والتحاليل التي أجريت على أن التظهير بشكل عام قد عزز التكوين الحيوي وتراكم المواد الفعالة في أنسجة الكالس. وأظهرت النتائج أن القلويدات الناتجة من الكالس المعامل بـواحد بالمائة من مستخلص الراشح الفطري مع ١٠٠ مليمول مانيتول زادت من ٣٠ إلى ٢٠٠ مليجرام لكل جرام ماده نباتية جافة. أما بالنسبة للفلافونويدات المقدرة فى الكالس المعامل بـواحد بالمائة من مستخلص الراشح الفطري منفردًا، فقد زادت من ٢٠ إلى ٤١٠ مليجرام لكل جرام مادة نباتية جافة في حين أن كمية التانينات المقدرة في الكالس المعامل بـنصف بالمائة من مستخلص الراشح الفطري مع 50 مليمول أو واحد بالمائة من مستخلص الراشح الفطري منفردًا زادت من ١٠ مليجرام لكل جرام جاف من أنسجة الكالس غير المعامل (كنترول) إلى ٣١٠ مليجرام لكل جرام مادة نباتية جافة للكالس المعامل. أشارت نتائج تقدير النشاط الضد بكتيري التي أجريت في هذه الدراسة إلى أن الكالس الناتج من التظهير الحيوي وغير الحيوي قد أظهر تأثير ضد بكتيري متفاوت مقابل السلالات البكتيرية الممرضة التي تم إختبارها في هذه الدراسة، وهي ستافيلوكوكس أوريوس وباسيلس سيريوس وسيدوموناس إيروجينوزا وإيشريشيا كولاي. تشير النتائج التي تم الحصول عليها في هذه الدراسة إلى أن التظهير الحيوي وغير الحيوي قد يعززان من إنتاج المواد الأيضية الثانوية والنشاط الضد بكتيري لخلايا كالس نبات سولانم نيجروم.