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Vegetative growth, chemical composition, and flavonoids content of *Azadirachta indica* plants as affected by application of yeast natural extract

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ABSTRACT

This experiment was carried out during 2013 and 2104 seasons on *Azadirachta indica* plants at National Research Centre greenhouse, Egypt. Experiment studied the effect of foliar spraying plants with dry yeast (*Saccharomyces cerevisiae*) extract at various concentrations (0, 5, 10, 15 and 20%) on growth, pigments, NPK (%), total soluble sugars, indoles, phenols and flavonoids leaves contents. Results showed that spraying neem plants with yeast extract at 15% significantly increased growth parameters (plants height, stem and root fresh and dry weights) and enhanced total chlorophylla, phosphorus, potassium and total soluble sugars content. Using the concentration 5% of dry yeast extract gave the highest values of chlorophyll b, total carotenoids and total chlorophyll content. Nitrogen content was produced at the highest value (3.55%) in plants treated with dry yeast extract at 10%.The foliar application of yeast extract at 10, 15 and 20% resulted the highest values of total soluble phenols (72.48, 72.27 and 73.46 mg/g D.W., respectively). The highest flavonoids leave content (3.23 and 3.14 mg CE/g D.W.) were obtained when the dry yeast extract was used at 15 and 20%, respectively. On the other hand, all treatments had no significant effect on stem diameter, number of leaves /plant and root length.

INTRODUCTION

Neem *(Azadirachta indica* A. Juss.), a member of the family Meliaceae, is an evergreen, tropical forest tree. It can grow on poor soils and wastelands and is famous for its drought resistance (Radwanski, 1977). This tree is a multipurpose timber tree and possess high value products that are extracted for use as insecticides, fertilizers and multipurpose medicines. Its chemical constituents have several biological activities, such as immune-stimulation, blood purification, anti- inflammation, anti-tumor, insect repulsion and bactericidal activity. All parts of these plants including fruit, seed, leaf, root and bark are used for their medicinal properties. It contains more than 100 bioactive compounds. The main active compounds are highly oxidized triterpenoids called limonoids. Azadirachtin is the most important important bioactive compound, it exists in all parts of the neem tree, but is concentrated in the seed kernel. Others are gedunin, nimbin and sodium nimbinate (Premananda, 2011). Biostimulants are an organic materials that have been shown to influence several metabolic processes such as respiration, photosynthesis, nucleic acid synthesis and ion uptake and when applied in small quantities, enhances plant growth and development. Active dry yeast is considered as biostimulant, natural source of cytokinins that stimulates cell division and enlargement as well as the synthesis of protein, nucleic acid and chlorophyll formation (El- Desouky *et al*., 1998; Wanas 2002 and Wanas, 2006). Moreover, it contains cryoprotective agent, i.e. sugars, proteins, amino acids and also several vitamins (Mahmoued, 2001). It may enhance water holding capacity, increase antioxidants, and enhance metabolism (Abbas, 2013). Bread yeast (*Saccharomyces cerevisiae*) is usually added to soil or as foliar application to crops (EL-Ghamriny *et al*., 1999)due to its content of many nutrient elements as well as its role in producing important substances like growth regulators such as gibberellins, auxins (Sarhan and Sharif, 1988) and its ability to

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produce a group of enzymes (Dinkha and Al-Khazragji, 1990). The goal of the present work was to evaluate the effects of these dry yeast natural extract as a biostimulant on plant growth, photosynthetic pigments and chemical composition of *Azadirachta indica* plants.

MATERIALS AND METHODS

The experiment was carried out during two successive seasons (2013 and 2014) at the greenhouse of the National Research Centre, Dokki, Egypt to study the effect of various dry yeast extract concentrations on growth, NPK (Total nitrogen, phosphorus and potassium), photosynthetic pigments, total soluble sugars, indoles, phenols and flavonoid leaves content of *Azadirachta indica* plants (two years old).

Neem plants 20-22cm with 10-14 leaves were obtained in May, 2014 in earthenware that were filled with media containing a mixture of sand and peat as 1:1 by volume. The plants were fertilized with 20 g/pot kristalon in four doses after 4, 8, 16 and 20 weeks from transplanting, after 50 days plants were transolocated to the pots (50 cm width) contained 8 Kg mixture of sand and peat as 1:1 by volume. The first spraying treatment of various dry yeast concentration in August, 2014 and the second after two months and were repeated in the two season.

Chemicals

The chemicals used in this study including the solvents were of analytical grade and used without testing and purification.

Extraction and determination

Yeast extract preparation

Saccharomyces cerevisiae newly produced active dry yeast was obtained (SIL-41, Zone Potuaire 59211 SANTES-France) and various concentrations(0, 5, 10, 15 and 20 g) of yeast were weighed and put with 25 cm^3 of water in a glass beakers with teaspoon full of sugar beaker. The beakers of each concentration were kept in a dark warm place for 30 minutes as described by Hanafy *et al*. (2012). Contents of the beakers were then filtered into a measuring flasks and water was added to 100 cm final volume for each one.

Data Recorded

Vegetative growth

The design of the experiment was complete randomized blocks in 3 replicates (each replicate contained 5 plants). Plants were randomly chosen at 80 days after treatment in both seasons to estimate plant height (cm), stem diameter (cm), number of leaves/ plant, stem fresh and dry weights (g)/ plant, root length (cm), root fresh and dry weights (g) /plant.

Photosynthetic pigments

Chlorophyll, a, b and carotenoids concentrations were determined as mg / 100g fresh weight, in leaves of *Azadirachta indica* using the method described by Saric *et al*. (1967).

NPK estimation

Samples of leaves were taken to determine total nitrogen (Horneck and Miller, 1998), phosphorus (Sandell, 1950) and potassium (Horneck and Hanson, 1998).

Total Soluble Sugars

It determined in the methanolic extract by using the phenol-sulfuric method according to Dubois *et al.* (1966).

Total indoles and phenols

The total indoles were determined by using "Erlic's reagent" according to Larsen *et al.* (1962). While, total soluble phenols were calorimetrically determined using Folin Ciocaltea reagent (A.O.A.C. 1985).

Total flavonoid

Total flavonoid was determined according tocolorimetric method by Adom *et al*. (2005), each volumetric flask were reacted with 5% sodium nitrite (200 µL). After 6 min, 10% $AlCl_3$ (0.3 ml) was added and at another 6 min. 1M NaOH (2 ml) was added, followed by adjusting the volume to 5 mL with deionized water and mixed well. Absorbance of the reaction mixture was read at 510 nm. Total flavonoid contents of each sample (three replicates per treatment) were expressed as mg catechin equivalents per gram of dry weight (mg CE g^{-1} DW) through the calibration curve with catechin.

Statistical analysis

The data obtained were statistically analyzed by using the least significant differences test (L.S.D) at0.05% according to Snedecor and Cochran (1980). The results were presented as mean values ±SD (standard deviations).

RESULTS AND DISCUSSION

Growth characteristics

Vegetative growth parameters of *Azadirachta indica* (plants height, stem and root fresh and dry weights)significantly influenced by all foliar application with yeast extract at 5,10, 15 and 20% as shown in Table (1).Maximum stimulatory effect on plant height (cm), stem fresh and dry weight (g) and root fresh and dry weights (g) was observed in plants treated with 15% yeast extract which produced (134.67 cm, 72.12, 21.78, 42.80 and 9.16 g, respectively) as compared with control plant which gave (97 cm, 38.76, 15.42, 17.7 and 5.96 g, respectively).However, all treatments had no significant effect on stem diameter, number of leaves /plant and root length. The enhancement effect of yeast extract might be attributed to its influence on metabolism, biological activity and photosynthetic pigments and enzyme activity which in turn encourage vegetative growth (Wanas, 2002 and El-Sherbeny *et al*., 2007). It is acting as a source of plant growth hormones, carbohydrates, amino acids and vitamins.

Table 1: Effect of dry yeast extract concentrations on vegetative growth characters of *Azadirachta indica* (as mean of the two seasons).

Character / Treatment	Plant height (cm)	Stem diameter (cm)	number of leaves /plant	Stem F.W. (g)	Stem D.W. (g)	Root length (cm)	Root F.W. (g)	Root D.W. (g)
Control (0%)	97	0.45	37.66	38.76	15.42	30	17.7	5.96
Yeast 5%	108	0.55	41.66	44.55	16.69	39	18.39	5.99
Yeast 10%	127.67	0.46	36.67	55.81	15.23	31.66	18.85	5.91
Yeast 15%	134.67	0.59	48	72.12	21.78	41.67	24.80	9.16
Yeast 20%	108.33	0.55	30.67	39.94	10.24	37.33	12.96	4.30
LSD at 5%	22.10	N.S.	N.S.	18.85	4.16	N.S.	4.82	1.65

Table 2: Effect of dry yeast extract concentrations on photosynthetic pigments (mg/100gF.W.) of *Azadirachta indica* leaves.

Treatment	Chl.a	Chl.b	Total carotenoides	Total chlorophyll	chl.*a*:chl.*b*	chl.*a*+chl.*b*: carotenoids
Control (0%)	264.45	92.6	186.09	357.05	2.86	1.92
Yeast 5%	450.54	226.82	366.38	677.36	1.99	1.85
Yeast10%	367.07	115.43	226.48	482.5	3.18	2.13
Yeast15%	469.73	188.88	290.81	658.61	2.49	2.26
Yeast 20%	397.29	133.91	240.05	531.19	2.97	2.21
LSD at 5%	0.005	3.32	3.04	3.32	-	-

Table 3: Effect of dry yeast extract concentrations on NPK (%) of *Azadirachta indica* plants.

Treatment	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Control (0%)	1.51±0.14	0.021±0.002	1.03±0.025
Yeast 5%	3.43±0.15	0.111±0.004	1.04±0.03
Yeast 10%	3.55±0.11	0.261±0.020	1.29±0.05
Yeast 15%	1.66±0.06	0.323±0.012	1.49±0.05
Yeast 20%	0.48±0.03	0.143±0.003	0.78±0.03
LSD at 5%	0.18	0.014	0.069

Table 4: Effect of dry yeast extract concentrations on total soluble sugars, indoles, phenols and flavonoids content of *Azadirachta indica* plants.

Treatment	Total soluble sugars (Mg/100gF.W.)	Total indoles (Mg/100gF.W.)	Total phenols (Mg/g D.W.)	Total flavonoides (Mg CE /g D.W.)
Control (0%)	28.35±1.36	41.45±0.60	55.06±1.96	2.03±0.22
Yeast 5%	30.62±1.53	51.46±0.87	56.39±1.84	2.42±0.21
Yeast 10%	32.31±1.17	51.89±1.56	72.48±1.97	2.45±0.04
Yeast 15%	42.95±1.28	60.18±1.03	72.27±1.98	3.23±0.24
Yeast 20%	41.09±1.25	41.17±0.57	73.46±1.39	3.14±0.20
LSD at 5%	2.49	1.90	3.72	0.14

Photosynthetic pigments

Data presented in Table (2) indicated that addition of different concentrations of dry yeast extract significantly increased pigment concentrations in leaves of *Azadirachta indica.* Applying yeast extract at 15% to plants increased contents of chlorophyll a to the highest value (469.73mg/100g F.W.). Whereas, using the concentration 5% of yeast extract gave the highest values of chlorophyll b, total carotenoids and total chlorophyll (226.82, 366.38 and 677.36 mg/100g F.W., respectively) comparing with control. Similar results were found when yeast was applied to field bean plants and increased contents of chlorophyll a, b, and total chlorophyll (Homme *et al*., 1992).

Enhancing the leaf chlorophyll might be attributed to the important role of dry yeast extract as biostimulant action on increasing the availability of water and minerals (Mady, 2009). Moreover, the improvement of photosynthetic pigments in response to the foliar application of active dry yeast may be attributed to bioregulators which affect the balance between photosynthesis and photorespiration in plants (Olaiya, 2010; Abou El-Yazied and Mady, 2011)

Mineral content

Data in Table (3) illustrated that yeast extract increased NPK (%)content of Neem plants. Nitrogen content was at the highest value (3.55%) in plants treated with foliar application at 10% of yeast extract, while the concentration15% was favored treatment for increasing phosphorus and Potassium plant content (0.323 and 1.49%, respectively) as compared with control plants. Increasing the content of leaves nutrient elements may be due to the positive effective of yeast extract on increasing vegetative growth as a result of dry yeast content in many nutrient minerals and its compounds like growth regulators (Sarhan and Sharif, 1988). Homme *et al*. (1992) mentioned that yeast also facilitate the growth of plants by improving the uptake of nutrients and production of some phytohormones and convert insoluble form of phosphorous into soluble one, enhancing phosphorous availability to plants.

Total soluble sugars, indoles, phenols and flavonoids content

As shown in Table (4), most of dry yeast extract treatments caused significant increase in total soluble sugars,

indoles, phenols and flavonoids leaves contents as compared with control plants. The best results were obtained from total soluble sugars (42.95 and 41.09 mg/100gF.W.) and total flavonoids (3.23 and 3.14 mgCE/g D.W.) with yeast extract foliar application at 15 and 20%, respectively. The highest total indoles leaves content (60.18 mg/100gF.W.) was obtained with foliar application (15%) of dry yeast extract. It can also noticed that foliar application of yeast extract more than 5% (10, 15 and 20%) resulted in highest values of total soluble phenols (72.48, 72.27 and 73.46 mg/g D.W., respectively).

Concerning the concentration of total soluble sugars, the best values of total sugar content and beneficial effect on carbohydrate accumulation in leaves of field bean were observed with yeast extract (Mady, 2009). For indoles, similar results were found when the maximum values in auxins were obtained with foliar application of yeast extract at 5 ml/L combined with boron at 50 ppm (Abou EL-Yazied and Mady, 2012).

As for phenolic compounds content, Yeast extract augments the enzyme activities of phenylpropanoid metabolism which leads to phenolic compounds (Ramachandra and Ravishankar, 2002).

The important roles of plant phenolics materialize in protection against biotic and abiotic stressors. They exhibit a wide range of biological activities including antibacterial, anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic and vasodilatory (Soobrattee *et al*., 2005). Because of the pharmaceutical and economical values of phenolics, it is essential to recognize under which conditions they are synthesized. Ozgur and Sule (2009) observed that, cultures of *Astragalus chrysochlorus* treated with yeast extract inhibited the growth and coupled with coloring, the cells turned to claret-red. It was thought that the substances which are responsible for claret-red color, may correspond phenolic compounds that account for pink, red, orange, scarlet, purple colors. Gang *et al.* (2012) studied the effects of Yeast polysaccharide (YPS) on the growth and flavonoids accumulation of *Fagopyrum tataricum* sprout cultures, which were dependent on both YPS concentration and its treatment period. The results provide further evidence for the elicitor activity the yeast polysaccharide (YPS) in stimulating the stress responses and secondary metabolism of *Fagopyrum tataricum* sprout cultures.

CONCLUSION

Applying of dry yeast extract positively affected most of studied growth characters, i.e. plants height, stem and root fresh and dry weights as well as pigments content and NPK (%) of Neem plants. Moreover, total soluble sugars, indoles, phenols and flavonoids contents increased by application of dry yeast extract.

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