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Growth Quality and Bioactive Compounds of Poplar Seedlings as Affected by Different Fertilization Treatments

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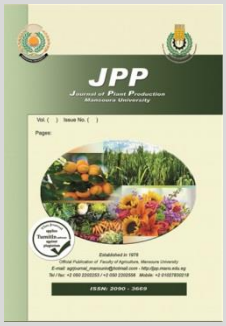

ABSTRACT

Pot experiments were conducted during two successive seasons of 2019 and 2020 at the Fac. of Agric., Minia Univ. to investigate effects of four application rates of compost (0, 1, 2 and 3%) (w/w) and six treatments of bio-fertilizers and/or mineral NPK fertilization (control, *Azotobacter chroococcum* + *Bacillus circulans*, *Azotobacter chroococcum* + *Bacillus megaterium*, mixture of bio., mixture of bio. + 75% NPK and 100% NPK) on vegetative criteria, accumulation of bioactive compounds (phenolic compounds and flavonoids) through improving plant's secondary metabolism and antimicrobial activity of methanolic and ethanolic extracts of *Populus alba*. All compost treatments significantly improved all transplant vegetative growth parameters such as plant height, diameter, aerial part fresh and dry weights, main root length and roots fresh and dry weights, as well as, total chlorophylls and NPK% as compared to control. Biofertilizers and/or mineral NPK fertilization treatments significantly increased all the previous traits, the mixture of three species of bacteria plus 75% NPK followed by mineral full dose of NPK treatments were the most effective than other used treatments. The highest values were obtained when *Populus alba* transplants were grown in sandy soil contained 3% compost plus inoculation with three species of bacteria plus 75% NPK or compost at 3% plus 100 % NPK and these two treatments give the highest values of phenolic compounds in the methanolic and ethanolic extracts. This research confirmed that poplar is a promising wooden tree to be grown on sandy soils for timber production in Egypt, woody products and bioactive compounds.

Keywords: Poplar, Bioactive Compounds, Antimicrobial Activity, Biofertilizers.

INTRODUCTION

Poplar trees (*Populus alba*) are among the best fast-growing trees worldwide and it belongs to Salicaceae family (Liu *et al.*, 2009). Poplar trees are a promising woody tree that can be grown successfully on the Egyptian vast areas of infertile sandy soils for timber production and various wood products including paper, pulp, packing crates, reconstructed boards, pallets, plywood, sawn timber, veneer, boxes, roundwood and furniture (Hewidy *et al.*, 2020). In addition, poplar trees are used as protective belts for sand dunes, windbreaks to control soil erosion by wind (Scaysbrook *et al.*, 1992). Poplar plays significant role in phytoremediation (i.e., uptake of heavy metal to purify polluted soils) of contaminated sites, rehabilitation of friable ecosystem including fighting desertification and restoration of forest landscape (Ball *et al.*, 2005).

Populus alba (White poplar) is a native to the Mediterranean region. The genus *Populus* is widely distributed all over the world especially in the Northern hemisphere. They are dioecious, medium-sized woody tree with simple, glabrous and scale-covered buds (Hewidy *et al.*, 2020). White poplar leaves can be used as bio-monitors of soil pollution (Soliman *et al.*, 2017). The genus *Populus* is the main model scheme for poplar genomic and physiological research. The poplar represents a significant model for woody perennial biotechnology due to its *in vitro* culture amenability and genetic engineering via *Agrobacterium*- mediated conversion (Soliman *et al*., 2017).

By tradition, the Salicaceae family comprises *Salix* (willow) and *Populus* (poplar), common in northern moderate regions (Kwon and Bae, 2009). Several secondary metabolites including flavonoids and phenolic compounds, salicin derivatives, anthocyanin and polysaccharides can be isolated from *P. alba* (Alcalde-Eon *et al.*, 2016). *P. alba* show many microbiological activities involving *in vitro* antioxidant activity, cytotoxic, antiviral, antifungal and weak antibacterial activities (Boudkhili *et al.*, 2012). The bioactive compounds extracted from genus *Populus* are acknowledged by traditional medicine for various microbiological activities including antifungal, antioxidant, antitumoral, antiseptic and antiviral (Christov *et al.*, 2006).

The chloroform extract derived from *Populus alba* flowers displays an antiproliferative activity against carcinomatous cell lines and in the treatment of herpes and dental cavities (Wamidh and Mahasneh, 2010). By tradition, *Populus alba* can be used as a skin disinfectant since its properties involve bioactive substrates (Adam *et al.*, 2009). Methanolic and ethanolic extracts of *Populus alba* leaves have antibacterial and antifungal properties versus a significant number of microorganisms and these characteristics have also been obviously demonstrated by Al-Hussaini and Mahasneh, (2011). In accordance with the previous researches, this research was directed to investigate the antimycobacterial effect of this plant and to confirm

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previous explored results. This research consists of a chromatographic and phytochemical analysis designed to separate and identify the poplar chemical contents responsible for antimycobacterial activity.

Organic manures can serve as alternative to mineral fertilizers for improving soil structure (Dauda *et al.,* 2008) and microbial biomass (Suresh *et al*., 2004). Compost is one used to improve soil physical, chemical and biological properties (water retention capacity, drainage, pH, better availability of soil micro-organisms and reducing the negative impact of chemical-based pesticides and fertilizers in the ecosystems (Ali *et al.,* 2002; Zheljazkov and Warman, 2003; Ahmed *et al.* 2006; Kevin *et al.,* 2011; Abdel-Mola, 2014; Abo El-Wafa, 2014; and Abdou and Ibrahim, 2015) on *Populus spp*., mentioned that organic manures treatments led to increase vegetative and root growth traits and pigments, as well as, N, P and K% in the leaves.

Bio-fertilizers (microbial inoculants) applied to seeds, soil and/or seedlings in order to increase soil fertility with increasing the number of microorganisms and accelerate certain microbial processes in rhizophere zone. Such microbiological processes can increase biological nitrogen fixation (*Azotobacter chroococcum*) can convert unavailable forms of nutrients to available, P and K dissolving bacteria (*Bacillus megaterium* and *Bacillus circulans*, respectively). Cole, (1982); Hauwaka, (2000); Ahmed *et al.,* (2005), Abdou and Ibrahim (2015) on poplar plant, Mossad (2016) and Soliman (2019) on moringa plants found that biofertilizers treatments significantly increased all vegetative and root growth parameters, as well as, some chemical constituents, pigments and NPK % as compared to control. Mineral NPK fertilizers increased the vegetative growth characters of *Populus* spp. (Zabek, 1995, Kohan *et al.*, 2000, Ali *et al*., 2002; and Abdel-Mola, 2014).

Therefore, the scientific aim of this research was mainly to explore agronomic responses of *Populus alba* transplants in terms of growth parameters and accumulation of the bioactive compounds grown on sandy soil to different fertilization regimes of compost, biofertilizers and/or mineral NPK. In addition, this research was conducted to investigate the antimycobacterial effect of poplar using a chromatographic and phytochemical analysis allowing the separation and identification of chemicals responsible for antimycobacterial activity.

MATERIALS AND METHODS

This work was carried out during two growing seasons of 2019 and 2020 at the Nursery of Ornamental Plant, Faculty of Agriculture, Minia University, Egypt. The scientific aim of this study was to evaluate effects of organic fertilizer (compost), bio-fertilizers (Nutrient Stabilizing Bacteria) and/or mineral NPK fertilization on poplar (*Populus alba*) grown in sandy soil and in turn antimycobacterial effect of these poplars' extracts. The *Populus alba* plant traits examined were vegetative criteria, accumulation of the bioactive compounds and improving the plant's secondary metabolism, the accumulation of secondary metabolites and its vital role as antimicrobial activity.

Plant material:

Nodal cuttings of plant were obtained from Malawi Agricultural Research Station, Agriculture Research Center, Egypt. Cuttings (20 cm length) were planted on first day of March for both seasons in plastic pots (45 diameters) as each was filled with 30 kg of sandy soil. Each pot contained two cuttings and transplants were thinned to one transplant/pot after one month from planting date. Plant leaves were collected, air dried in the shade at the agricultural biochemistry laboratory of Beni-Suef University. Leaves were then ground to a powder for the preparation of various extracts.

Physical and chemical properties of the soil used was analyzed with the standard methods of Jackson, (1975) and Page *et al*., (1982) are listed in Table (1).

Table 1. Some physiochemical properties of the soil used.

Soil Property			Value	
Particle-Size Distribution%	Coarse sand 56.30%	Fine sand 33.45%	Silt 7.60%	Clay 2.65%
Texture grade			Sand	
F.C%			17.63	
PWP %			4.97	
WHC %			19.66	
A. V. (F.C. – PWP) %			12.66	
A. V. (WHC-PWP) %			14.69	
Bulk density (BD) g/cm^3			1.64	
Particle density (PD) g/cm^3			2.51	
pH (1:2.5 water)			7.76	
$CaCO_3$ (g kg^{-1})			105	
CEC (cmol$_c$ kg^{-1})			3.2	
EC (dS m^{-1} at 25 ^{0}C)			0.70	
OM (g kg $^{-1}$)			6.6	
Total N (g kg $^{-1}$)			0.58	
Total P (g kg $^{-1}$)			0.19	
Total K (g kg $^{-1}$)			3.2	

Randomized complete block design (split plot design) with three replicates each replicate contain three transplants was followed in this trial. Four application rates of compost (zero, 1, 2 and 3%) (w/w) were allocated as main plot treatments (A) and the six bio fertilizers and/or mineral NPK fertilization treatments assigned as the sub plot treatments (B), consequently the interaction treatments (A×B) were 24 treatments. The examined compost (compost El-Neel) was attained from the Egyptian Co. for Solid Waste Recycling, New Minia City. Compost was incorporated into sandy soils inside pots (40 cm depth), physicochemical properties of the compost investigated are shown in Table (2).

The sub plot treatments were as follows:

1- Without any fertilizers (control)
2- *Azotobacter chroococcum* + *Bacillus circulans*
3- *Azotobacter chroococcum* + *Bacillus megaterium* var. *phosphaticum*
4- *Azotobacter chroococcum* +*Bacillus circulans* + *Bacillus megaterium* var. *phosphaticum*
5- Three strains of bacteria + 75% NPK
6- 100% NPK as recommended dose. (El-Kayal, 1996).

Active three strains of bacteria (*Azotobacter chroococcum* as N-fixing bacteria, *Bacillus circulans* as solubilizing potassium silicate and *Bacillus megaterium* var. phosphaticum as solubilizing phosphate) were obtained from Agricultural Research Center, El-Giza, Egypt. Biofertilizers were applied at four times at a month interval starting 45 days after transplanting (April 14st for both seasons). A mixture of bacteria was mixed with soils around transplants and then pots were irrigated immediately.

Recommended chemical NPK fertilizers were 4 g/pot of ammonium sulphate (20.6% N) + 4 g/pot of calcium superphosphate (15.5% P_2O_5) + 3 g/pot of potassium sulphate (48% K_2O), while 75% NPK were 3+3+2.25 (g), respectively (El-Kayal, 1996). The amounts of chemical NK fertilizers were divided into four equal groups and added at one-month interval, starting April 21th in both seasons. Whereas, all amounts of P were added with the first dose of NK. All other local agronomic activities were performed as usual in both seasons.

Table 2. Some physiochemical properties of the compost used.

Compost property	Value	Compost property	Value
Dry weight of 1 m^3	450 kg	C/N ratio	26.50
Fresh weight of 1 m^3	650-700 kg	N/P ratio	2.00
Moisture weight (%)	36.60 %	Total P (g kg^{-1}) (D.M.)	5.0
pH (1:2.5)	7.90	Total K (g kg^{-1}) (D.M.)	9.0
EC (ds m^{-1} at 25 C^0)	2.20	Total Ca (g kg^{-1}) (D.M.)	26.3
CEC ($cmol_c$ kg^{-1})	45.66	Total Mg (g kg^{-1}) (D.M.)	6.6
Dry solids %	63.40	NaCl (%)	0.72-0.75
Ash%	9.90	Fe (mg kg^{-1})	150-200
Total N (g kg^{-1}) (D.M.)	10.0	Mn (mg kg^{-1})	25-56
Total Organic Matter (%)	32-34 %	Cu (mg kg^{-1})	75-150
Total Organic carbon (%)	18.5-19.7 %	Zn (mg kg^{-1})	150-225

Extract preparation:

The polyphenols of poplar leaves (control and the best interaction treatments) were extracted by maceration as described by Benhammou *et al.,* (2008) with roughly modified changes. Shriveled plant leaves (5 g) were macerated in 100 mL of different organic solvents with different polarity (ethanol and methanol). After 24 hours, samples were filtered (Whatman No.1), then, filtrates were collected and evaporated to dryness using a rotavapor (Buchi R-200) at 40°C.

Determination of phenolic compounds by HPLC:

Phenolic substrates identification was carried out via HPLC. The system of HPLC comprises a gas vacuum device, auto sampling device, and German dual pump with maximum pressure of 400 bar (Agilent 1260, Agilent Technologies). A calibration curve was obtained for phenolic quantitative analysis, by dotting peaks against different concentrations for each recognized substrate at 280 nm. The attained curves for all recognized substrates displayed a positive linearity average of $r^2 = 0.99$.

Microbial strains:

Antimicrobial activity of the extracts obtained from *P. alba* were verified against 4 microorganisms including two Gram-positive bacteria, including (*Staphylococcus aureus*, *Bacillus subtilis*) and two Gram-negative bacteria, including (*Escherichia coli*, *Pseudomonas aeruginosa*). Bacteria strains were obtained from the Department of Microbiology, Faculty of Agriculture, Minia University.

Growth quality and biochemical compounds measurements:

At the first week of November (in both seasons) the following data were recorded:

Vegetative growth parameters: plant height (cm), stem diameter (mm), aerial parts fresh and dry weights (g).

Root growth parameters: main root length (cm), fresh and dry weights of roots (g).

determination of some chemical compositions:

Total chlorophylls (mg/g. f.w.) was determined as described by Moran (1982).

Concentrations of N%, P% and K% in the dry matter (leaves) were assessed according to Page *et al*. (1982).

Fractionation of phenolic compounds.

The antimicrobial activity of methanolic and ethanolic extracts for *P. alba*.

Statistical analysis:

Experimental data were analyzed using MSTAT-C (1986). Significant differences among treatments were compared by analysis of variance with the L.S.D. test at P <5%.

RESULTS AND DISCUSSION

Vegetative growth characters:

Data obtained in Tables (3 and 4) indicated that seedling height, stem diameter and aerial part fresh and dry weights/transplant were significantly increased in the first and second seasons due to the use of 1, 2 and 3% compost. The treatment of 3% compost was more effective than other used treatments. Such superior treatment increased transplant height, stem diameter, aerial part fresh and dry weights over control by 39.59 and 47.59% for transplant height, 76.70 and 76.39% for stem diameter, 39.45 and 47.80% for aerial part fresh weight and 67.28 and 47.97% for aerial part dry weight in both seasons, respectively.

The role of compost treatments on increasing vegetative growth traits may be due to compost improved essential nutrients availability, such stimulation of nutrients uptake drives to improve the organic biosynthesis, cell division, cell elongation, carbohydrates buildup and dry matter accretion (Nijjar, 1985). Comparable results attained by Ali *et al.* (2002), Ahmed *et al*. (2006), Abo El-Wafa (2014) and Abdou and Ibrahim (2015) on *Populus* spp., Mosaad (2016), Soliman (2019) on *Moringa spp*., Abdou *et al*. (2020) on *Delonix regia* and Ali *et al.* (2020) on *Taxodium distichum*.

Regarding the effect of bio. and/or mineral NPK treatments, all used five treatments significantly increased transplant height, stem diameter, aerial part fresh and dry weights/transplant compared to control. The treatment of *Azotobacter chroococcum* plus two species of *Bacillus* was more (triple inoculation) effective than used *Azotobacter chroococcum* plus one species of *Bacillus* (dual inoculation). Moreover, the treatment of a mixture of three strains of bacteria (*Azotobacter chroococcum* + *Bacillus circulans* + *Bacillus megaterium*) plus 75% NPK (reduced dose of recommended mineral NPK) and mineral NPK (full dose) recorded the best values without insignificant differences between them in all cases. Biofertilizers are important source for supplementing plant nutrients *Azotobacter chroococcum* have a symbiotic nitrogen

fixation, as well as, *Bacillus* spp. Bacteria which lower the pH in soil rhizosphere and produce chelating substance, which lead to either solubilization phosphates (by *Bacillus megaterium* var. phosphaticum) or potassium (by *Bacillus circulans*) and consequently enhanced nutritional states and growth of plant (Subha-Rao, 1981 and Hauwaka, 2000).

These findings go parallel with those of Abdel-Mola (2014), Abo El-Wafa (2014) and Abdou and Ibrahim (2015) on *Populus* spp. and Soliman (2019) on *Moringa peregrina*. The interaction between compost and bio and/or mineral NPK fertilization treatments was significant in both seasons for the four studied traits. The highest values were obtained due to supplying *Populus alba* transplants with compost at 3% in combination with three bacteria strains + 75% NPK dose or mineral NPK at full dose.

Table 3. Influence of the experimental treatments on transplant height and stem diameter of *Populus alba* plants.

Treatments (B)	Compost% (Treatments A)									
	1st season (2019)					2nd season (2020)				
	0 %	1%	2%	3%	Mean (B)	0 %	1%	2%	3%	Mean (B)
	Transplant height (cm)									
Control	75.60	83.92	94.00	106.22	89.94	81.65	90.63	101.51	114.80	97.15
Azoto. + *B. circulans*	90.72	99.79	111.76	125.18	106.86	97.98	107.78	129.33	144.85	119.99
Azoto. + *B. megaterium*	95.83	105.82	118.85	133.55	11٣.51	103.81	114.81	137.55	153.91	127.52
Azoto. + B. circ.+ B. mega.	102.11	112.32	125.81	141.89	120.53	108.6٠	121.71	145.81	162.11	134.56
Bio. + 75 % NPK	108.21	119.51	133.92	151.00	128.16	115.11	127.90	152.90	170.00	141.48
100 % NPK	110.01	122.45	137.81	155.22	131.37	116.33	129.80	155.81	174.51	144.12
Mean (A)	97.08	107.30	120.36	135.51		103.91	115.45	137.15	153.36	
L.S.D. at 5 %	A: 2.11		B: 2.61		AB: 5.22	A: 2.12		B: 2.76		AB: 5.52
	Stem diameter (mm)									
Control	8.21	9.03	10.10	11.13	9.62	9.11	10.02	11.12	12.36	10.65
Azoto. + *B. circulans*	9.85	12.02	14.68	17.95	13.63	10.81	12.97	15.83	19.34	14.74
Azoto. + *B. megaterium*	12.81	15.37	18.75	22.92	17.46	13.05	15.66	19.11	23.35	17.79
Azoto. + B. circ.+ B. mega.	15.42	18.51	22.76	27.84	21.13	15.89	19.07	23.45	28.85	21.82
Bio. + 75 % NPK	18.88	22.66	27.87	34.37	25.95	19.51	23.41	28.80	35.52	26.81
100 % NPK	18.50	22.21	27.31	33.68	25.43	19.30	23.17	28.51	35.17	26.54
Mean (A)	13.95	16.63	20.25	24.65		14.61	17.38	21.14	25.77	
L.S.D. at 5 %	A: 0.89		B: 2.19		AB: 4.38	A: 1.01		B: 1.76		AB: 3.52

Azoto.: ***Azotobacter chroococcum***
B. circ.*: *Bacillus circulans
***B. mega.*: *Bacillus megaterium* var. phosphaticum**
Bio.: ***Azotobacter chroococcum* + *Bacillus circulans* + *Bacillus megaterium* var. phosphaticum**

Table 4. Influence of experimental treatments on aerial part fresh and dry weights (g) of *Populus alba* plants.

Treatments (B)	Compost% (Treatments A)									
	1st season (2019)					2nd season (2020)				
	0 %	1%	2%	3%	Mean (B)	0 %	1%	2%	3%	Mean (B)
	Aerial part fresh weight of transplant (g)									
Control	83.16	92.31	103.40	116.84	98.93	89.90	99.78	111.76	126.40	106.96
Azoto. + *B. circulans*	99.80	109.77	122.94	137.70	117.55	107.81	118.65	142.30	159.34	132.03
Azoto. + *B. megaterium megaterium megaterium megaterium*	105.41	117.40	130.75	146.91	125.12	114.21	126.29	151.31	169.30	140.28
Azoto. + B. circ.+ B. mega.	112.35	123.55	138.39	156.22	132.63	119.56	133.88	160.39	179.32	148.29
Bio. + 75 % NPK	119.04	131.46	147.35	166.15	141.00	126.62	140.69	168.19	187.50	155.75
100 % NPK	122.01	134.85	152.59	171.11	145.14	127.96	142.95	171.55	192.13	158.65
Mean (A)	106.96	118.22	132.57	149.16		114.34	127.04	150.92	169.00	
L.S.D. at 5 %	A: 2.51		B: 2.57		AB: 5.14	A: 2.88		B: 3.07		AB: 6.14
Control	30.85	34.29	38.78	43.37	36.82	32.95	36.95	41.38	46.77	39.51
Azoto. + *B. circulans*	37.12	40.62	45.49	50.95	43.55	39.89	43.95	52.65	58.96	48.86
Azoto. + *B. megaterium megaterium*	39.11	43.45	48.39	54.38	46.33	42.26	46.73	56.00	62.64	51.91
Azoto. + B. circ.+ B. mega.	41.٧0	45.72	51.21	57.81	49.11	44.25	49.54	59.34	66.35	53.62
Bio. + 75 % NPK	44.10	48.64	54.55	61.48	52.19	46.85	52.10	62.23	69.38	57.64
100 % NPK	45.15	49.89	56.46	63.33	53.71	47.35	52.89	63.47	71.10	58.70
Mean (A)	33.01	43.70	49.15	55.22		42.26	46.19	55.85	62.53	
L.S.D. at 5 %	A: 0.99		B: 1.93		AB: 3.86	A: 1.03		B: 1.55		AB: 3.10

Azoto.: ***Azotobacter chroococcum***
B. circ.*: *Bacillus circulans
***B. mega.*: *Bacillus megaterium* var. phosphaticum**
Bio.: ***Azotobacter chroococcum* + *Bacillus circulans* + *Bacillus megaterium* var. phosphaticum**

Root growth parameters:

Data obtained in Tables (5 and 6) showed that the three used percentages of compost significantly increased main root length and fresh and dry weights of roots/transplant as compared with control in both seasons. The treatment of 3% compost resulted in highest values as given by 16.67 and 18.51% increase for main root length, 22.87 and 25.53% for roots fresh weight and 30.46 and

31.57% for roots dry weight in both seasons, respectively over the control treatment. The increase in root growth features may be because of compost improving the physicochemical properties of the investigated sandy soil (Abo El-Fadle *et al.*, 1968). Similar outcomes were obtained by Abdel-Mola (2014), Abo El-Wafa (2014) and Abdou and Ibrahim (2015) on *Populus* spp., Soliman (2019) on *Moringa peregrina* and Abdou *et al*. (2020) on *Delonix regia*.

Concerning the effect of bio. and/or mineral NPK fertilization, figures presented in Tables 5 and 6 indicated that investigated fertilization treatments significantly enlarged main root length and fresh and dry weights of roots/transplant compared to control treatment. The treatments of mineral NPK (100%) or followed by three bacteria strains + 75% NPK recorded the highest values for the three parameters of root system, without significant differences between such two superior treatments. These findings could be attributed to that different fertilization systems increased perennial tissue partitioning as coarse roots. In addition, different fertilization systems increased plant biosynthesis and metabolic processes which subsequently augmented carbohydrates buildup in the roots (Soliman *et al*., 2017).

The interaction effects of different fertilization treatments (compost and bio. and/or mineral NPK) were significant for main root length and root fresh and dry weights in both growing seasons. The tallest main root length was obtained by the treatment of compost 3% plus triple inoculation plus 75% NPK or 100% mineral NPK. Moreover, the heaviest root weight were obtained with the interaction treatments of fertilization *Populus alba* with 3% compost in combination of mineral NPK (full dose) followed by triple inoculation + 75% NPK then the three mixture of bacteria such without significant differences between three interaction treatments.

Total Chlorophyll pigments (mg/g f.w.):

Data obtained in Table (6) indicated that total chlorophylls content in the fresh leaves of *Populus alba* were significantly increased due to three percentages of compost with high content 3.226 in the first season and 3.234 mg/g f.w. in the second one, while, control recorded the least contents as gave 3.182 and 3.189 mg/g. f.w. in both seasons respectively. This finding is supported by Ali *et al.* (2002), Abdel-Mola (2014) on *Populus spp*. and Mossad (2016) on *Moringa oleifera*. Data presented in Table (6) showed that all five used treatments significantly augmented total chlorophylls as compared to control. The treatment of mineral NPK (full dose) was more operative than other treatments in this regard. Similar trend was obtained by Ali *et al.* (2002) on *Populus nigra*, Abdou *et al.* (2006) on *Khaya senegalensis*, Ferrini and Baietto (2006) on different tree species, Osman and Abo Hassan (2010) on mangrove, Abdou *et al*. (2020) on *Delonix regia* and Ali *et al*. (2020) on *Taxodium distichum*.

These results are in agreement with Abo-El-Khair (1993) on *Casaurania gluca*, Badran *et al*. (2003) on *Acacia saligna*, Ouahmane *et al.* (2009) on *Pinus halepensis* and Mossad (2016) on *Moringa oleifera*. However, the role of chemical NPK was obtained by Moustafa (2004) and Revathi *et al.* (2013) on *Dalbergia sissoo*, Abdou *et al.* (2006) on *Khaya senegalensis*, El-Morshedy (2007) on *Grevillea robusta*, Amin (2013) on *Pinus radiate* and Abdou and Ibrahim (2015) on *Populus alba*. The interaction between main and sub (A×B) was significant for total chlorophylls in both seasons. The highest contents of total chlorophylls due to compost at 3% in combination with three mixture bacteria + 75% NPK or 100% NPK.

Table 5. Influence of experimental treatments on main root length (cm) and fresh weight of roots/transplant (g) of *Populus alba* plants.

Treatments (B)	Compost% (Treatments A)									
	1st season (2019)					2nd season (2020)				
	0 %	1%	2%	3%	Mean (B)	0 %	1%	2%	3%	Mean (B)
	Main root length (cm)									
Control	11.51	11.96	12.51	13.16	12.29	11.88	12.43	12.98	13.63	12.73
Azoto. + *B. circulans*	12.31	12.81	13.42	14.13	13.17	12.69	13.29	13.98	14.76	13.68
Azoto. + *B. megaterium*	13.22	13.83	14.54	15.35	14.24	13.59	14.29	14.88	15.76	14.63
Azoto. + B. circ.+ B. mega.	13.81	14.32	14.93	15.54	14.65	14.19	15.79	15.48	16.26	15.18
Bio. + 75 % NPK	14.92	15.63	15.44	17.06	16.01	14.91	15.79	16.69	17.65	16.26
100 % NPK	14.51	15.12	16.83	18.45	15.73	14.78	15.48	16.28	19.16	16.43
Mean (A)	13.38	13.94	14.28	15.61		13.67	14.35	15.05	16.20	
L.S.D. at 5 %	A: 0.30		B: 0.80		AB: 1.60	A: 0.36		B: 0.83		AB: 1.66
	Fresh weight of roots/transplant (g)									
Control	25.32	26.43	27.70	29.10	27.15	26.15	27.50	28.70	30.14	28.12
Azoto. + *B. circulans*	27.10	28.31	29.66	31.25	29.08	29.19	30.70	33.50	35.41	32.20
Azoto. + *B. megaterium*	31.73	33.20	34.90	36.84	34.17	33.98	35.73	37.20	39.40	36.58
Azoto. + B. circ.+ B. mega.	35.91	38.66	40.31	48.51	40.85	36.90	39.95	43.35	51.16	42.84
Bio. + 75 % NPK	40.28	43.77	44.81	50.51	44.84	41.75	45.79	49.30	54.00	47.71
100 % NPK	41.35	45.81	46.85	51.67	46.42	42.81	46.00	49.55	54.50	48.22
Mean (A)	33.62	36.03	37.37	41.31		35.13	37.61	40.27	44.10	
L.S.D. at 5 %	A: 0.91		B: 1.58		AB: 3.16	A: 0.98		B: 1.68		AB:3.36

Azoto.: ***Azotobacter chroococcum***
B. circ.*: *Bacillus circulans
***B. mega.*: *Bacillus megaterium* var. phosphaticum**
Bio.: ***Azotobacter chroococcum* + *Bacillus circulans* + *Bacillus megaterium* var. phosphaticum**

Table 6. Influence of experimental fertilization treatments on dry weight of roots/transplant (g) and total chlorophylls content (mg/g. f.w.) of *Populus alba* plants.

Treatments (B)	Compost% (Treatments A)									
	1st season (2019)					2nd season (2020)				
	0 %	1%	2%	3%	Mean (B)	0 %	1%	2%	3%	Mean (B)
	Dry weight of roots/transplant (g)									
Control	12.91	13.75	14.68	15.71	11.76	13.60	14.30	14.95	15.68	14.63
Azoto. + *B. circulans*	14.09	15.00	16.02	17.34	15.61	15.18	16.27	17.76	19.12	17.08
Azoto. + *B. megaterium*	16.82	17.93	19.20	20.63	18.65	18.01	19.30	20.46	22.06	19.96
Azoto. + B. circ.+ B. mega.	19.39	21.26	22.57	28.15	22.84	19.93	21.58	23.85	28.65	23.50
Bio. + 75 % NPK	22.15	24.51	25.55	29.30	25.38	22.96	25.64	28.10	31.86	27.14
100 % NPK	23.16	26.11	27.17	30.49	26.73	23.97	26.22	28.33	32.16	27.67
Mean (A)	18.09	19.76	20.87	23.60		18..94	20.55	22.24	24.92	
L.S.D. at 5 %	A: 0.85		B: 1.35		AB: 2.70	A: 0.96		B: 1.76		AB: 3.52
	Total chlorophylls content (mg/g. f.w.)									
Control	2.975	3.016	3.056	3.095	3.036	2.981	3.021	3.071	3.110	3.046
Azoto. + *B. circulans*	3.125	3.131	3.138	3.147	3.135	3.132	3.138	3.145	3.154	3.142
Azoto. + *B. megaterium*	3.176	3.183	3.191	3.201	3.188	3.183	3.189	3.196	3.204	3.193
Azoto. + B. circ.+ B. mega.	3.227	3.234	3.242	3.251	3.239	3.234	3.241	3.249	3.260	3.246
Bio. + 75 % NPK	3.279	3.289	3.301	3.315	3.296	3.285	3.296	3.309	3.324	3.304
100 % NPK	2.309	3.320	3.333	3.348	3.328	3.316	3.329	3.341	3.352	3.335
Mean (A)	3.182	3.196	3.210	3.226		3.189	3.202	3.219	3.234	
L.S.D. at 5 %	A: 0.011		B: 0.017		AB: 0.034	A: 0.015		B: 0.015		AB:0.030

Azoto.: ***Azotobacter chroococcum***
B. circ.*: *Bacillus circulans
***B. mega.*: *Bacillus megaterium* var. phosphaticum**
Bio.: ***Azotobacter chroococcum* + *Bacillus circulans* + *Bacillus megaterium* var. phosphaticum**

Nitrogen, phosphorus and potassium poplar contents.

Data presented in Table (7) reported that N, P and K concentrations (%) in the dry leaves was significantly increased due to fertilizing *Populus alba* transplants at 1, 2 and 3% compost application rates comparing with control in both seasons. Moreover, these increases were gradually increased in accordance with increases in compost application rates. These results can be devoted to the augmentation of N, P and K% in the rhizosphere by means of adding compost thus enhanced uptake of plant nutrients (Awad *et al*., 2003). The same findings were attained by Ali *et al.* (2002) and Ahmed *et al.* (2006) on *Populus* spp., Wroblewska *et al.* (2009) on *Salix purpurea*, Ahmadloo *et al*. (2012) on *Cypress* sp., Abo El-Wafa (2014) on *Populus nigra*, Abdou *et al.* (2020) on *Delonix regia* and Ali *et al*. (2020) on *Taxodium distichum.*

Table 7. Influence of experimental treatments on nitrogen and phosphorus percentages of *Populus alba* plants.

Treatments (B)	Compost% (Treatments A)									
	1st season (2019)					2nd season (2020)				
	0 %	1%	2%	3%	Mean (B)	0 %	1%	2%	3%	Mean (B)
	N%									
Control	1.931	2.133	2.321	2.511	2.224	1.989	2.197	2.391	2.586	2.291
Azoto. + *B. circulans*	1.963	2.161	2.360	2.544	2.257	2.022	2.226	2.431	2.620	2.325
Azoto. + *B. megaterium*	1.968	2.171	2.369	2.549	2.264	2.027	2.236	2.440	2.625	2.332
Azoto. + B. circ.+ B. mega.	2.010	2.221	2.414	2.603	2.312	2.070	2.287	2.486	2.681	2.381
Bio. + 75 % NPK	2.116	2.236	2.405	2.615	2.343	2.179	2.303	2.477	2.693	2.413
100 % NPK	2.101	2.230	2.401	2.603	2.334	2.164	2.297	2.473	2.681	2.404
Mean (A)	2.015	2.192	2.378	2.571		2.075	2.258	2.450	2.648	
L.S.D. at 5 %	A: 0.089		B: 0.032		AB: 0.064	A: 0.096		B: 0.033		AB: 0.066
	P%									
Control	0.215	0.234	0.255	0.276	0.245	0.219	0.239	0.260	0.282	0.250
Azoto. + *B. circulans*	0.219	0.251	0.270	0.293	0.258	0.223	0.256	0.275	0.299	0.263
Azoto. + *B. megaterium*	0.244	0.265	0.286	0.306	0.275	0.249	0.270	0.292	0.312	0.281
Azoto. + B. circ.+ B. mega.	0.226	0.258	0.268	0.281	0.258	0.231	0.263	0.273	0.287	0.263
Bio. + 75 % NPK	0.246	0.269	0.292	0.311	0.280	0.251	0.274	0.298	0.317	0.285
100 % NPK	0.245	0.266	0.287	0.310	0.277	0.250	0.271	0.293	0.316	0.283
Mean (A)	0.233	0.257	0.276	0.296		0.237	0.262	0.282	0.302	
L.S.D. at 5 %	A: 0.018		B: 0.012		AB: 0.024	A : 0.019		B: 0.010		AB: 0.020
	K%									
Control	1.64	1.75	1.86	1.95	1.80	1.66	1.77	1.88	1.97	1.82
Azoto. + *B. circulans*	1.92	2.02	2.13	2.19	2.07	1.94	2.04	2.15	2.21	2.09
Azoto. + *B. megaterium*	1.81	1.91	2.04	2.10	1.97	1.83	1.93	2.06	2.12	1.98
Azoto. + B. circ.+ B. mega.	1.86	1.96	2.08	2.19	2.02	1.88	1.98	2.10	2.21	2.04
Bio. + 75 % NPK	2.06	2.16	2.27	2.38	2.22	2.08	2.18	2.29	2.40	2.24
100 % NPK	1.99	2.21	2.31	2.31	2.21	2.01	2.23	2.33	2.33	2.23
Mean (A)	1.88	2.00	2.12	2.19		1.90	2.02	2.14	2.21	
L.S.D. at 5 %	A: 0.06		B: 0.15		AB: 0.30	A: 0.07		B: 0.17		AB: 0.34

Azoto.: ***Azotobacter chroococcum***
B. circ.*: *Bacillus circulans
***B. mega.*: *Bacillus megaterium* var. phosphaticum**
Bio.: ***Azotobacter chroococcum* + *Bacillus circulans* + *Bacillus megaterium* var. phosphaticum**

From Table (7) it was clear that all used fertilization treatments caused significant increase in N, P and K% in both seasons, in comparison with that of control. Regarding N% the treatments of triple inoculation + 75% NPK followed by mineral NPK% then triple inoculation biofertilizers caused significant increases in N% in both seasons over other used treatments without significant differences between them. Concerning P% it was mentioned that these element percentage was significantly increased by fertilizing *Populus alba* with three mixtures of bacteria + 75% NPK followed by mineral NPK (full dose) then a combined of *Azotobacter chroococcum* + *Bacillus megaterium* var. phosphaticum during both seasons without significant differences between them. The same trend was observed regarding potassium in the dry leaves as significantly increased due to fertilizing *Populus alba* transplants at 1, 2 and 3% compost application rates compared with control in both seasons. Moreover, the K increases were gradually increased by increasing compost application rates. Likewise, each of the three treatments of mixture of biofertilizers + 75% NPK followed by mineral NPK (100%) then *Azotobacter chroococcum* + *Bacillus circulans* gave the best results with significantly differences among them. Similar results were obtained by Ahmed *et al.* (2006) and Abdel-Mola (2014) on *Populus nigra*, Yolina and Minda (2003), Aditya *et al.* (2009), Joseph *et al.* (2010) and Mohan and Radhakrishnan (2012) on *Tectona spp*.

The interaction between the main and sub plot (A×B) treatment was significant for N, P and K% in both seasons. The best interaction treatments were obtained by compost at 3% plus bio. +75% followed 100% NPK then (*Azotobacter chroococcum* + *Bacillus circulans* + *Bacillus megaterium*) for N%. While for P% the treatments of 3% in combination with bio.+75% or 100% NPK or *Azotobacter chroococcum* + *Bacillus megaterium* or 2% ×bio. +75% or mineral NPK 100%.

Poplar contents of phenolic compounds.

HPLC separation of the phenolic compounds from the methanolic extract of control treatment and organic, biological treatment of *P. alba* are shown in Fig. (1). The separation detected the presence of p-coumaric acid (0.65, 1.03 mg/g), ellagic acid (1.96, 2.85 mg/g), kaempferol (0.74, 1.08 mg/g) in the extracts tested. The HPLC fingerprinting of *P. alba* extract revealed significant differences in the phenolic composition between all interaction treatments and control. Falcao *et al.*, (2010) similarly displayed the occurrence of p-coumaric acid and apigenin in poplar-type propolis and some chromatograms undetermined due to unavailability of certain chemical ingredients.

Gifston *et al.* (2010) mentioned that phenolic substrates also execute a critical part in antioxidant plant system. Moreover, Gad El-Hak *et al.* (2012) mentioned that phenolic compound stimulated vegetative growth, protein content, total carbohydrate, nitrogen, phosphorus, potassium and yield of different plants. Flavonoids are common in plants, with many jobs since phenolic and flavonoids compounds are chemical envoys, cell cycle inhibitors and physiological regulators. Flavonoids and antimicrobial compounds extract of *Populus alba* secreted by poplar roots assist symbiotic relationships with Rhizobia in the infective phase. Besides, antimicrobial compounds and some flavonoids have inhibitory activity against soil pathogenic microorganisms, e.g., *Fusarium oxysporum* Galeotti, *et al.* (2008). The flavonoids, one a type of polyphenolic substrates, have a wide-range of feats in soils and plants. They defend against pathogen attack, act as attractants for pollinators and plant-microbe symbiotic associations signal initiation (Zhang *et al.*, 2013 and Hewidy, *et al.*, 2020).

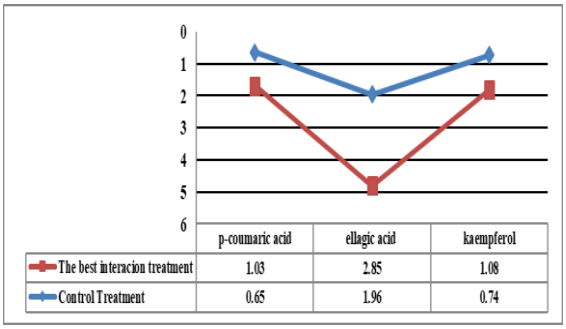

Figure 1. HPLC analysis of methanolic extract of *P. alba*

The antimicrobial activity of methanolic and ethanolic extracts of *P. alba*

In the present study, antimicrobial activity of different poplar organic extracts against 4 bacteria strains was tested qualitatively *in vitro* by presence or absence of inhibition zone (IZ) diameter. The rudimentary extracts of *Populus alba* displayed equinoctial activity against Gram-positive bacteria, including *S. aureus* (M. E. 17.66, E. E. 14.33) and *B. subtillis* (M. E. 13.33, E. E. 6.66). Least activity of polar organic extracts was documented contra Gram negative bacteria, *E. coli* (M. E. 10.66, E. E. 9.66), *P. aeruginosa* (M. E. 11.33, E. E. 10.16) as shown in Fig. (2).

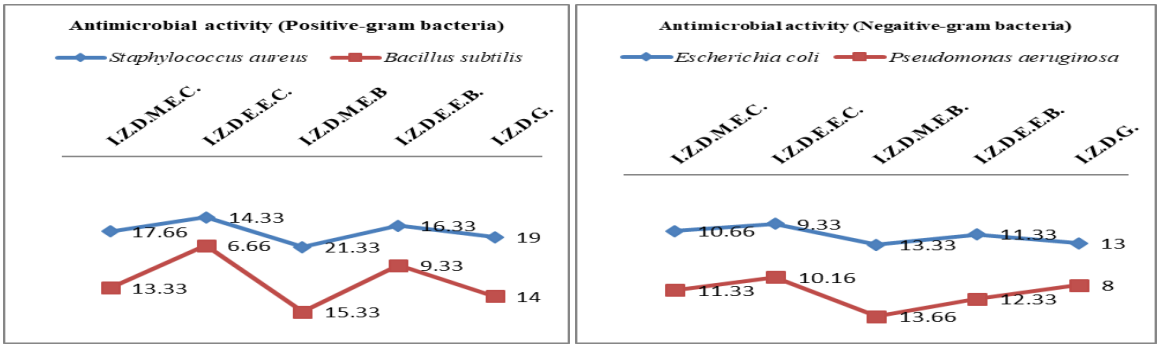

Figure 2. Antimicrobial activity of positive bacteria and negative bacteria of methanolic and ethanolic extracts of *P. alba*

***I.Z.D.G.: Inhibition Zone Diameter Gentamicin (15µg/disk), I.Z.D.E.E.B.: Inhibition Zone Diameter of Ethanolic extract for the Best interaction treatment, I.Z.D.M.E.B.: Inhibition Zone Diameter of Methanolic extract for the Best interaction treatment, I.Z.D.E.E.C.: Inhibition Zone Diameter of Ethanolic extract for Control treatment, I.Z.D.M.E.C.: Inhibition Zone Diameter of Methanolic extract for Control treatment.**

The interaction effects between treatments (A×B) were significant for producing more effective organic extracts against Gram-positive and Gram-negative in both seasons compared to control. The best interaction treatments were obtained by compost at 3% plus bio. +75% followed by (*Azotobacter chroococcum* + *Bacillus circulans* + *Bacillus megaterium*) for Gram-negative bacteria. Experimental analysis exposed more effective organic extracts against Gram-positive than Gram-negative bacteria by different fertilization treatments (compost, biofertilizers and/or mineral NPK) applied to sandy soil. Higher resistance of Gram-negative bacteria is probably correlated to the impervious nature for bacteria outer membranes, which are impermeable to lipophilic compounds (Djenane *et al.,* 2012). Gram-positive bacteria have only an outer layer of peptidoglycans making them more sensitive and less protected against polyphenolic agents and preventing only molecules diffusion of molar mass greater than 50 000 D (Abirami *et al.,* 2012). Smith-Palmer *et al.,* (1998), Marino *et al.,* (1999) and Inouye *et al.,* (2001) attained comparable results to current study, confirming research findings of that Gram-positive bacteria are more sensitive to poplar organic extracts than Gram-negative bacteria.

Different fertilization treatments (compost, biofertilizers and/or mineral NPK) applied to sandy soil significantly increased all vegetative criteria, accumulation of bioactive compounds (phenolic compounds and flavonoids) by improving the plant's secondary metabolism, the antimicrobial activity of methanolic and ethanolic extracts of *Populus alba*. In addition, pigments showed higher values chlorophyll a and b and phenolic compounds due to investigated treatments compared to control. However, in this study, application of different compost rates and biofertilizers and NPK percentages resulted in different values in growth, morphological, bioactive compounds and physiological properties of *P. alba*. The highest agronomic improvements values were attained when *Populus alba* transplants were grown in sandy soil treated with 3% compost plus inoculation with three species of bacteria plus 75% NPK or compost at 3% plus 100 % NPK.

In Egypt, soil and water quality must receive urgent attention because arable soils and water resources in Egypt are very limited and of variable certainty at present. It could be concluded that pathogenic and heavy metal pollution constitute the main effects on soil and water quality under conducted intensive agricultural systems. In addition, water pollution originates mainly from point sources such as agricultural activities with analogous impacts from contaminated irrigation water with municipal effluents. Under intensive agricultural systems in Egypt, urgent environmental ecosystem recovery should be implemented as soil and water resources became sick with salts, pathogens and heavy metals. Therefore, it is of supreme importance for monitoring and attempting to remedy and manage the degradation of soil and water resources in Egypt (Abd El-Azeim *et al*., 2020). It has been clearly evidenced by different researchers that ethanolic and methanolic extracts of *Populus alba* leaves have antibacterial and antifungal properties against many microorganisms (Al-Hussaini and Mahasneh, 2011). Pathogenic antimicrobial extracts and flavonoids are common in poplar trees and poplar rhizosphere, with many functions.

Secreted flavonoids and the antimicrobial compounds by *Populus alba* roots have inhibitor activities against soil pathogenic bacteria, e.g., *E. Coli; Fusarium oxysporum* (Galeotti, *et al*. 2008; Zhang *et al*., 2013 and Hewidy, *et al*. 2020). In alternative medicine, bioactive materials created by poplars are well-known for different anti-microbiological activities counting antibacterial, antiviral, antioxidant, antifungal, antiseptic and antitumoral (Christov *et al*., 2006). Chloroform derived extracts from poplar flowers displays an activity of antiproliferative against carcinogenic cell lines and for herpes treatment and dental infections (Wamidh and Mahasneh, 2010). By tradition, *Populus alba* is applied for skin disinfectant (Adam *et al*., 2009). In addition, poplar trees can be used as a biomonitoring of soil heavy metal pollution (Soliman *et al* 2017).

CONCLUSION

In this study, the use of different rates of compost and combinations of biofertilizers and NPK resulted in different significant improvements in vegetative growth quality parameters, extracts of phenolic and antibiotic compounds, morphological and physiological responses of *P. alba*. The highest agronomic improvements values were attained when *Populus alba* transplants were grown in sandy soil treated with 3% compost plus inoculation with three species of bacteria plus 75% NPK or compost at 3% plus 100 % NPK. Therefore, results of this research stated that poplar as a promising woody tree can be grown on the Egyptian desert soils under arid conditions for wood production with good quality parameters is confirmed.

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جودة النمو والمركبات النشطة بيولوجيا لشتلات الحور ومدى تأثرها بمعاملات التسميد المختلفة

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أجريت تجربة أصص خلال موسمي ٢٠١٩ \ ٢٠٢٠ بكلية الزراعة – جامعة المنيا بهدف دراسة تأثير أربع نسب من الكمبوست (صفر، ١، ٢ ، ٣ %) وستة معاملات من التسميد الحيوي و/أو التسميد المعدني (NPK) (كنترول - بكتريا *Azotobacter chroococcum* - بكتريا *Azotobacter chroococcum* + *Bacillus circulans* بكتريا *Azotobacter chroococcum* + *Bacillus megaterium* var. phosphaticum – الثلاثة انواع من البكتريا - الثلاثة انواع من البكتريا + ٧٥ % (NPK) - ١٠٠ % (NPK)) على صفات النمو الخضري والجذري وبعض المكونات الكيماوية وعلى زيادة المركبات الكيماوية النشطة بيولوجيا (الفينول – الفلافينويد) لنبات الحور. أدت كل المعاملات المستخدمة من الكمبوست الى زيادة معنوية فى الصفات الخضرية (الارتفاع – قطر – وزن الجزء الهوائي الطازج والجاف وطول الجذر الرئيسي وكذلك الوزن الطازج والجاف للجذور بالإضافة الى الصبغات الكلية والنسبة المئوية لعناصر (NPK) مقارنة بالكنترول. وقد تأثرت كل الصفات سالفة الذكر معنويا بالمعاملات السمادية بالاسمدة الحيوية مع /أو الاسمدة الكيماوية (NPK). وقد سجلت المعاملة بالثلاثة أنواع من البكتريا + ٧٥% (NPK) أعلى عند زراعة شتلات الحور في الأراضي الرملية المعاملة ٣% كمبوست والملقحة بالثلاثة أنواع من البكتريا. وهذه المعاملة أعطت كذلك أعلى القيم للمركبات الفينولية ومضادات البكتريا لنباتات الحور. ومن خلال هذه الدراسة يمكننا أستنتاج أن شجرة الحور شجرة واعده كمصدر لإنتاج الاخشاب والصناعات الخشبية ويمكنها النجاح بصورة جيدة تحت ظروف الأراضي الرملية بمصر