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Biosynthesis of Silver Nanoparticles *via Haplophyllum tuberculatum* (Forssk.) A. Juss. (Rutaceae) and its Use as Bioherbicide

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THE PRESENT study was concerned with the synthesis of AgNPs *via* application of *Haplophyllum tuberculatum* crude aqueous extract (HTCAE) as a reducing agent. The resultant extract (HTAgAE) together with the crude one was compared in testing growth and some physiological and molecular parameters of the two recipient species; *Triticum aestivum* L. as a crop species and *Phalaris minor* Retz. as weed species. The effect of HTCAE on *P. minor* revealed significant reduction in growth germination percentage and both radicle and plumule lengths, nevertheless HTAgAE completely inhibited its germination. The HTAgAE at 5% and 10% stimulated the photosynthetic pigments in *T. aestivum* and reduced them in *P. minor* at 20% of HTCAE and HTAgAE. The total number of bands, polymorphism percentage and genomic template stability (GTS) % were generally arranged in a descending order by using HTCAE and HTAgAE. This order was reversed with HTAgAE in *P. minor* at 5% and in *T. aestivum* at 5% and 20% that reflected antagonistic effect of the nanosilver extract. At 5% HTCAE and 20% HTAgAE, *T. aestivum* accomplished more genetic stability than *P. minor* which may support their use as safe bioherbicide.

Keywords: Bioherbicides, Green chemistry, Molecular marker, Nanotechnology, Weed.

Introduction

The production of nanoparticles is one of the most recent fields of biological science. Many of the chemical and physical methods used to prepare them are very expensive and toxic to the environment (Kalaiarasi et al., 2013). Therefore, the use of green chemistry in many biological systems such as microorganisms and algae as well as higher plants that are considered low-cost and eco-friendly (Wang et al., 2007; Bansal et al., 2015; Shaik et al., 2017 and Rheder et al., 2018). These systems can convert inorganic metal ions into metal nanoparticles through the reduction capabilities of their proteins and metabolites (Kowshik et al., 2002; Rautaray et al., 2003; Scarano & Morelli, 2003; Lengke et al., 2007 and Govindaraju et al., 2008). The nanoparticles produced by plants are more stable and the synthesis rate is faster than microorganisms (Ahmad & Sharma, 2011 and Zahir et al., 2012). Silver nanoparticles (AgNPs) are one of the most commonly used nanomaterials due to their antioxidant and antimicrobial properties (Abou El-Nour et al., 2010 and Khatami et al., 2015).

Phalaris minor is a serious threat to productivity and sustainability of wheat cropping ecosystems that require large quantities of herbicides for control them (Om et al., 2002 and Chhokar & Malik, 2002). To overcome this problem, through an environmentally safe way, many plants can be used as bioherbicides because of their secondary metabolite contents (Dayan & Duke, 2014). Kumari et al. (2009) and Pérez-de-Luque & Rubiales (2009) noted out the efficacy of parasitic weed control through AgNPs, which is resulted in mitotic index decrease, inhibited the respiratory enzymes and bind to sulfur- and phosphorous-containing molecules involved in cell antioxidant defense.

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In Egypt, Rutaceae is monogeneric which represented by two species; *Haplophyllum tuberculatum* and *H. poorei* C.C. Towns. Previous investigations have confirmed intraspecific morphological variability and the presence of different chemotypes within *H. tuberculatum* (Raissi et al., 2016 and Marzouk et al., 2017). Its allelopathic potentiality has been recorded on seed germination and growth of several weeds especially *P. minor* due mainly to its phenolic contents (El-Darier et al., 2014 and Eissa et al., 2014).

The current study was focused on synthesizing AgNPs by applying *H. tuberculatum* crude aqueous extract (HTCAE) as a reducing agent. The resulting extract (HTAgAE) was compared with HTCAE in its effects on germination efficiency and some physiological and molecular parameters of two recipient species; *T. aestivum* (crop species) and *P. minor* (noxious weed) in pure and mixed cultures for use as bioherbicide.

Materials and Methods

Collection of plant materials

The vegetative part of *H. tuberculatum* collected from Matruh (260 km west of Alexandria city), dried in shade then grinded in a Wiley Mill to coarse uniform texture. The seeds of *T. aestivum* and *P. minor* were purchased from seed stores in Alexandria.

Biosynthesis and characterization of AgNPs

Seventy-five grams soaked in one liter of distilled water for 24hr at 25°C in the dark and the supernatant centrifuged at 3000r.p.m. for 15min. The crude extract was adjusted to pH 6.8 then series of dilutions (5, 10 and 20% as well as the control) was prepared and stored at 5°C until used (Singh et al., 2003 and El-Darier et al., 2014). Ten milliliters of crude extracts (5, 10 and 20%) were incubated with 100ml of 3mM aqueous silver nitrate and in shaker for 2hr, then at room temperature for 24hr in the dark until the brownish color was developed; indicating the formation of AgNPs (Parashar et al., 2009). These particles were also monitored by UV–Vis spectroscopy after dilution of the samples with deionized water at a scanning speed of up to 200–800nm (Leela & Vivekanandan, 2008 and Raut et al., 2009). The suspension containing AgNPs was prepared according to Elavazhagan & Arunachalam (2011) for transmission electron microscope (TEM). Elemental analysis was carried out using an energy dispersive X-ray fluorescence (EDX) spectrometer. TEM and EDX were performed at the special unit of Electron Microscope, Faculty of Science, Alexandria University.

Phytochemical screening of H. tuberculatum

The dried samples were qualitatively analyzed to determine the glycosides (Lewis & Smith, 1967), phenolic compounds (Evans et al., 1996), steroids, tannins, flavonoids and coumarins (Harborne, 1998), as well as alkaloids (Harborne, 1999). The essential oil was extracted from the fresh plant by a hydrodistillation method using a Clevenger apparatus (Awin, 2007). The tannins were expressed in terms of gallic acid mg/g of the extract (Sultana et al., 2012).

Germination bioassay

A germination bioassay experiment was carried out to investigate the biological activity of HTCAE and HTAgAE on germination percentage (GP), plumule (PL) and radicle (RL) lengths of *T. aestivum* and *P. minor*. Ten seeds of each species were immersed in 2% Chlorex for 2min, soaked in aerated distilled water for 24hr, then germinated under normal laboratory conditions; from 19-22°C with day and 12-14°C at night. Ten ml of the respective target species aqueous extracts (5, 10 and 20%) or distilled water as control were added daily to three replicates in a randomized complete block design.

Both inhibition and reduction percentages in plumule and radicle lengths were assessed according to Giaveno et al. (2007):

$$\% \text{ inhibition or reduction} = [(X - Y)/ X] \times 100$$

where, X= Maximum number of seeds germinated in control set and Y= Maximum number of seeds germinated in treated set.

Growth and photosynthetic bioassay

Ten seeds of the crop and weed species were soaked in different concentrations of both HTCAE and HTAgAE aqueous extracts and distilled water (control) for 24hr. The seeds planted in plastic pots (12X14cm) with about one Kg of sandy loam sterilized soil and the treatments were arranged in a completely randomized block design with three replicates. The plants were watered with normal tap water every two days under normal laboratory conditions (20±2°C temperature, 75±2% relative humidity, and 14/10hr light/dark photoperiod). After 21 days, the homogenous seedlings of *T.*

aestivum and *P. minor* in pure and mixed cultures experiments were collected, separated into shoots and roots and some growth parameters, photosynthetic pigments, chlorophyll fluorescence (Fv/Fm), chlorophyll stability indices (CSI %) and vegetative storage proteins (VSPs) were evaluated.

The photosynthetic pigments chlorophyll *a* (Chl. *a*), *b* (Chl. *b*) and carotenoids (Carot.) were extracted and determined (mg g fresh weight^{-1}) using the spectrophotometric method described by Metzner et al. (1965). Formula and extinction coefficients used for determination of photosynthetic pigments were:

Chl. *a*= 10.3 E_{665} - 0.918 E_{647},
Chl. *b*= 19.7 E_{647}-3.87 E_{665}
and Carot.= 4.2 E_{453}- (0.0264 Chl. *a* + 0.426 Chl. *b*).

The chlorophyll stability indices (CSI %) were measured according to Sivasubramaniawn (1992) as follows:

(Total Chl. content in stressed leaves/total Chl. content in control leaves) ×100.

Chlorophyll fluorescence was performed with OS-30P pulse modulated chlorophyll fluorimeter (Opti-sciences, Hudson, and USA) (Kooten & Snel, 1990).

Vegetative protein electrophoresis (VSPs) was performed through SDS-PAGE method of Laemmli (1970) and by using P-PER® Plant Protein Extraction Kit. The molecular weights of bands were determined by using UVP Doc-It®LS Image Analysis Software and the output dendrogram was performed by Unweighed Paired Group Method Average (UPGMA) based on Jaccard similarity coefficients. The percentage of polymorphism was determined according to Bisby (1995), while genomic template stability (GTS %) was calculated according to Cimino (2006) as follow:

% polymorphism= [(∑bands for each sample-∑common bands for all sample)/ ∑bands for all samples]

The genomic template stability (GTS%) was calculated as the following:

(GTS%) = (1- a/n) X 100

where a: average number of polymorphic bands detected in each treated sample (appearance of new bands and disappearance normal bands), n: total number of bands.

Statistical analysis

Results were reported as the average of three repetitions ±SE (standard error).The data subjected to standard one-way ANOVA and student's t-test using the COSTAT 2.00 statistical analysis software**.** Simple linear regression model was applied to account for possible differences in interdependence of different parameters and the concentration levels of the crude and AgNPs (Zar, 1984).

Results

Characterization of biosynthesized AgNPs

The color of the extract changes from pale yellow to brown color after addition of 3mM $AgNO_3$ which indicates the reduction of silver ions. The presence of nanoparticles was confirmed by obtaining a spectrum in the visible range of 400-430 nm using UV-Visible spectrophotometer (Fig. 1 A). The presence of elemental silver signal was confirmed by EDX as shown in Fig. 1B, the spectrum shows mainly Ag (40.1%) and (59.1%), respectively. The TEM image proved that the biosynthesized AgNPs are spherical and semispherical in shape with a smooth surface morphology and a diameter ranging from 20 to 30nm (Fig. 1 C).

Phytochemical screening of H. tuberculatum

Quantitatively, *H. tuberculatum* contained essential oils, flavonoids, glycosides, phenolic compounds, sterols, triterpenes, tannins and alkaloids. Quantitatively, the total flavonoids, total phenolics and essential oil content were 453.7mg/100g d.w., 1278.5mg/100g d.w. and 0.65%, respectively (Table 1).

Germination bioassay

The germination percentage (GP) of *T. aestivum* in pure (Tp) and mixed (Tm) cultures were unaffected (100%) at different concentrations of HTCAE. However, GP of *P. minor* showed significant reduction to nil values at 20% in pure (Pp) and mixed cultures (Pm). The effect of HTAgAE on the GP of Tp and Tm exhibited a different trend where a reduction of 40% was detected for both at 20%. On the other hand, all the applied concentrations of HTAgAE completely inhibited the germination of Pp and Pm (Fig. 2).

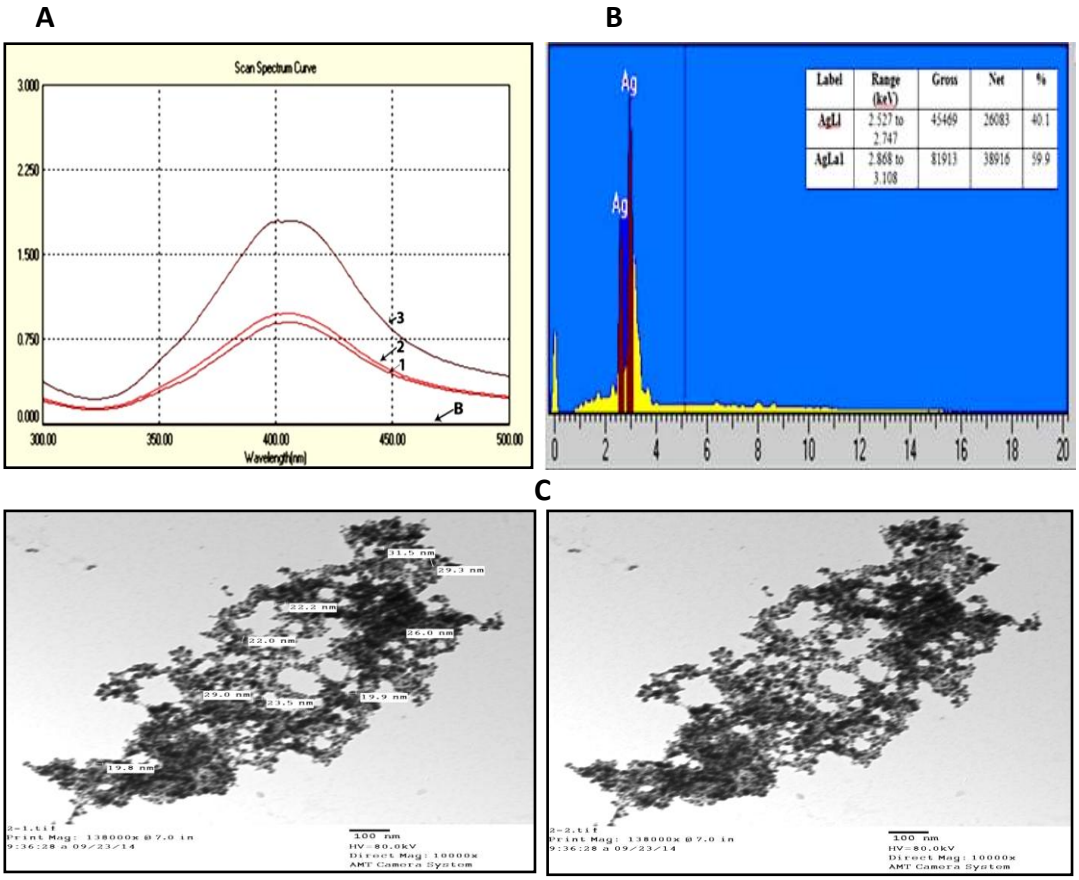

Fig. 1. UV–Vis absorption spectrum (A), EDX spectra (B) and TEM micrograph (C) of biosynthesized AgNPs by *Haplophyllum tuberculatum* aqueous extracts.

TABLE 1. Phytochemical screening of *Haplophyllum tuberculatum.*

Component	Qualitatively	Quantitatively
Alkaloids	++	-
Coumarins	++	-
Essential oil	++	0.65%
Flavonoids	++	453.7
Glycosides	+	-
Phenolic compounds	++	1278.5
Sterols and/or triterpenes	++	-
Tannins	+	-

Flavonoids and phenolic compounds were determined as mg rutin and mg chlorogenic acid /100g d.w. respectively.

The radicle length (RL) of *T. aestivum* in Tp and Tm cultures decreased significantly upon applying concentrations of HTCAE higher than 5%, while a gradual decrease in RL of *P. minor* (Fig. 3).

The HTCAE stimulated the plumule length (PL) of *T. aestivum* that the length increased from 10.3cm to 14.7 at 5% for Tp and from 11.5 to 12.8 for Tm, while the relative values were reached to 12.2 and 12.3 by using HTAgAE; Tp and Tm respectively. On the other hand, the HTCAE gradually reduced the PL of *P. minor* (Fig. 4).

Growth and photosynthetic bioassay

The HTAgAE and HTCAE inhibited lengths and weights (fresh and dry) for both shoot and root in *T. aestivum* and *P. minor* at all concentrations above 5%, where these reductions were more pronounced in *P. minor* (Fig. 5, 6 and 7).

The photosynthetic pigments, Chl. *a* and Chl. *b* and CSI were significantly increased in *T. aestivum*, Tp and Tm, at 5% and 10% HTAgAE. Whereas, the significant reduction in *P. minor*, Pp and Pm, was detected at 20% HTCAE. The Carot. content in *T. aestivum* and *P. minor* significantly improved at 10% and 20% of both HTCAE and HTAgAE (Table 2 and Fig. 8). Simple linear regression analysis confirmed that the variation in photosynthetic pigments was correlated to the extract concentrations.

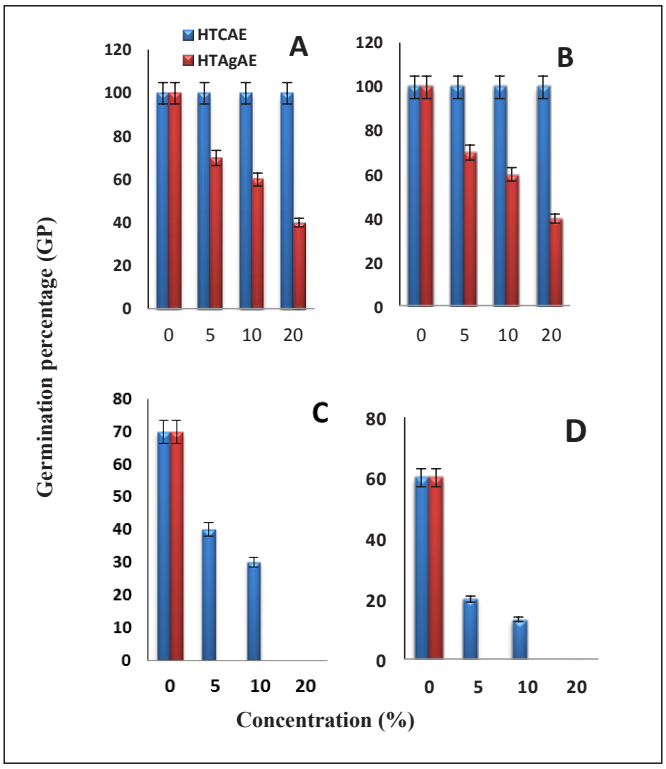

Fig. 2. Effect of *Haplophyllum tuberculatum* crude (HTCAE) and Ag- nanoparticles (HTAgAE) aqueous extracts on germination percentage (GP) of *Triticum aestivum* (A: Pure, B: Mixed) and *Phalaris minor* (C: Pure, D: Mixed) in culture experiments [Values are the means of three independent replicates± SE.tracts].

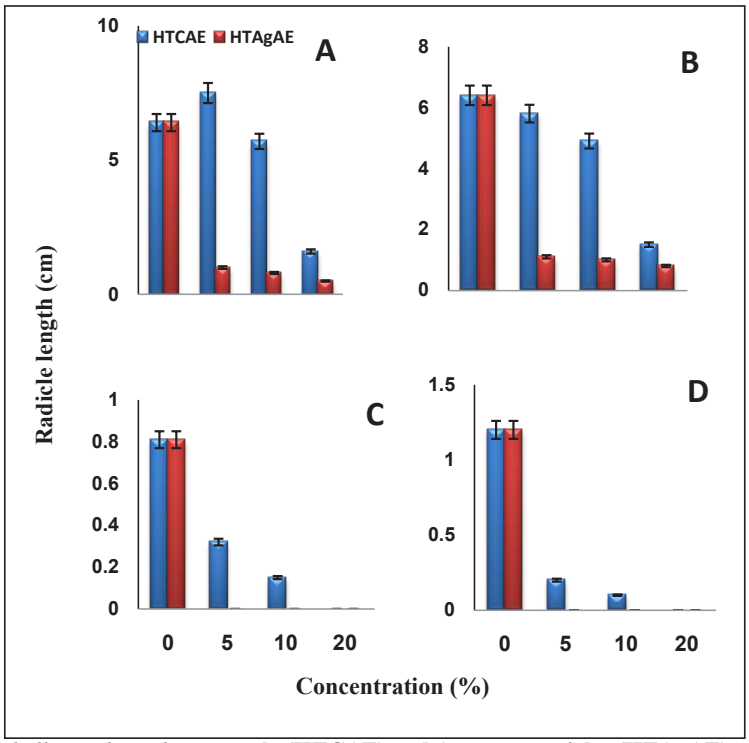

Fig. 3. Effect of *Haplophyllum tuberculatum* crude (HTCAE) and Ag-nanoparticles (HTAgAE) aqueous extracts on radicle length (RL) (cm) of *Triticum aestivum* (A: Pure, B: Mixed) and *Phalaris minor* (C: Pure, D: Mixed) in culture experiments [Values are the means of three independent replicates± SE.tracts].

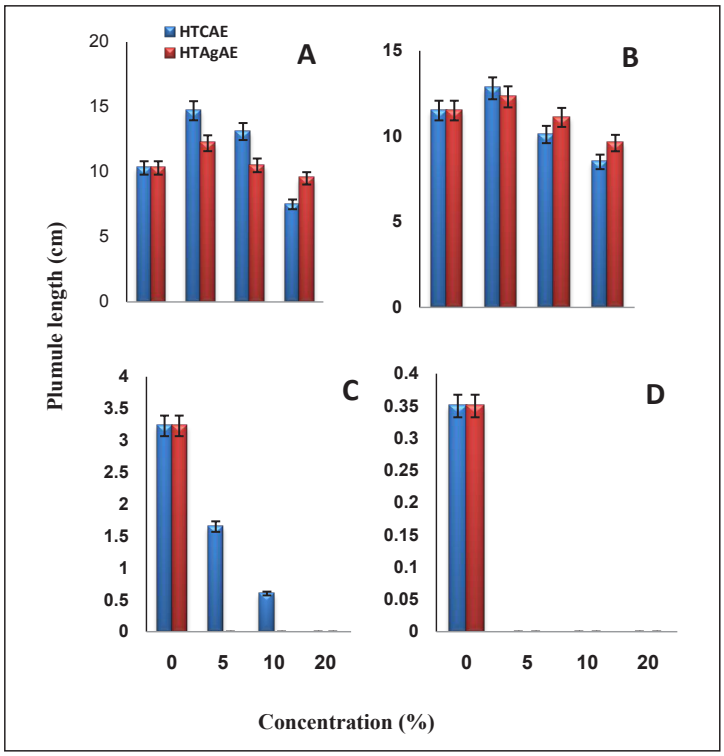

Fig. 4. Effect of *Haplophyllum tuberculatum* crude (HTCAE) andAg-nanoparticles (HTAgAE) aqueous extracts on plumule length (PL) (cm) of *Triticum aestivum* (A: Pure, B: Mmixed) and *Phalaris minor* (C: Pure, D: Mixed) in culture experiments [Values are the means of three independent replicates± SE.tracts].

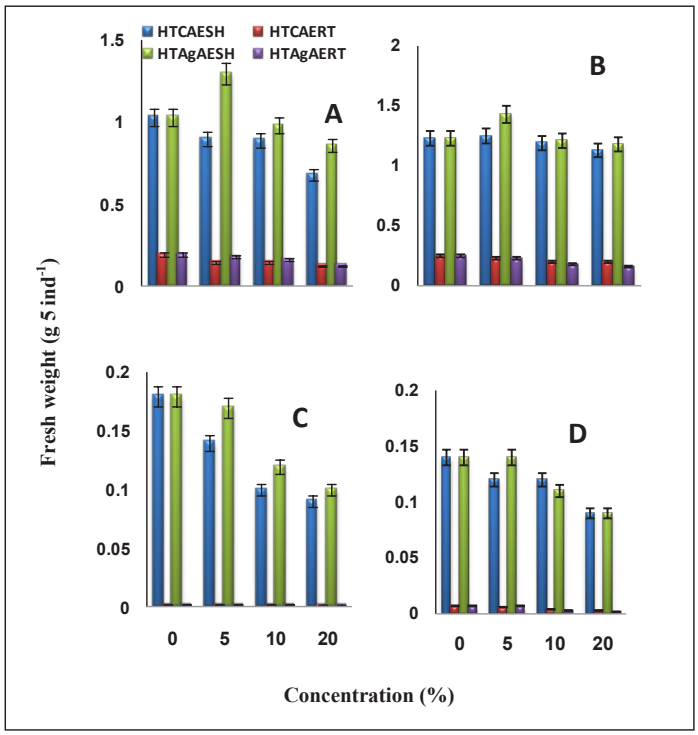

Fig. 5. Effect of priming with *Haplophyllum tuberculatum* (HTCAE) and Ag nanoparticles (HTAgAE) aqueous extracts on fresh weights (FW) (g 5 ind^{-1}) for both shoots (SH) and roots (RT) of *Triticum aestivum* (A: Pure, B: Mixed) and *Phalaris minor* (C: Pure, D: Mixed) in culture experiments [Values are the means of three independent replicates± SE.tracts].

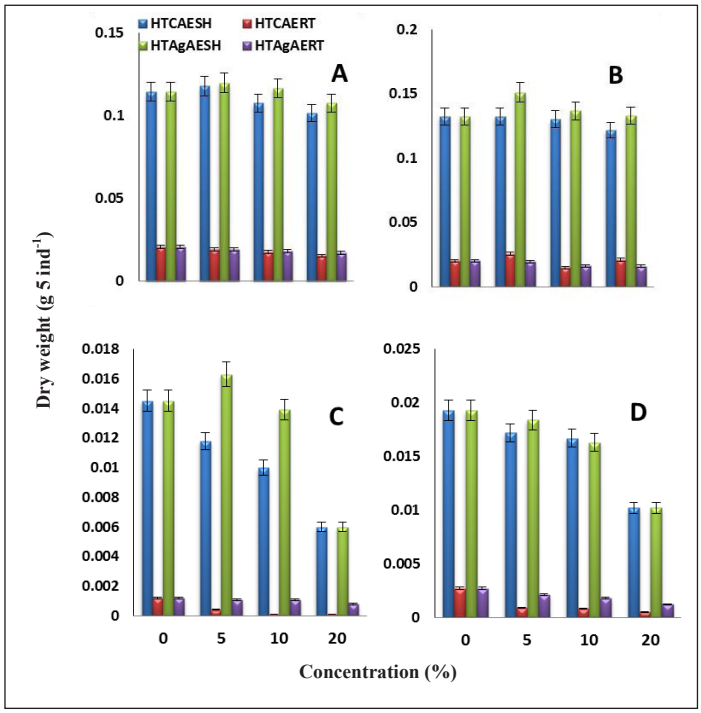

Fig. 6. Effect of priming with *Haplophyllum tuberculatum* crude (HTCAE) and Ag-nanoparticles (HTAgAE) aqueous extracts on dry weights (DW) (g 5 ind^{-1}) for both shoots (SH) and roots (RT) of *Triticum aestivum* (A: Purem B: Mixed) and *Phalaris minor* (C: Purem D: Mixed) in culture experiments (weights of five individuals) [Values are the means of three independent replicates± SE.tracts].

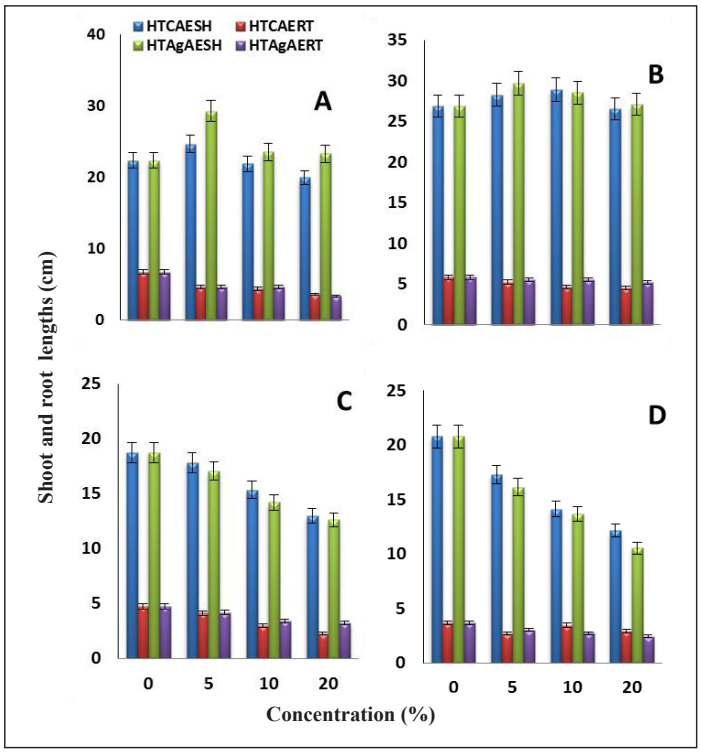

Fig. 7. Effect of priming with H*aplophyllum tuberculatum* crude (HTCAE) and Ag-nanoparticles (HTAgAE) aqueous extracts on shoot (SHL) and root (RTL) lengths (cm) of *Triticum aestivum* (A: Purem B: Mixed) and *Phalaris minor* (C: Pure, D: Mixed) in pure and mixed culture experiments [Values are the means of three independent replicates± SE.tracts].

TABLE 2. Effect of priming with *Haplophyllum tuberculatum* crude (HTCAE) and Ag-nanoparticles (HTAgAE) aqueous extracts on photosynthetic pigments (mg g^{-1}fresh weight) in leaves of *Triticum aestivum* and *Phalaris minor* in pure and mixed cultures in growth bioassay experiment . .

Treatment (%)	Currency	Chl. *a*	Chl. *b*	Chl. *a*+*b*	CSI	Chl. *a*/*b*	Carot.	Carot/*a*+*b*
Tp control	Distilled H_2O	5.247	1.660	6.907	100	3.160	0.365	0.052
5	HTCAE	5.240	1.660	6.900	100	3.156	0.465	0.067
	HTAgAE	5.785	1.960	7.745	112	2.951	0.486	0.062
10	HTCAE	4.989	1.575	6.564	95	3.167	0.626	0.095
	HTAgAE	5.687	1.716	7.403	107	3.314	0.520	0.070
20	HTCAE	4.760	1.469	6.229	90	3.240	0.690	0.110
	HTAgAE	4.830	1.190	6.020	87	4.058	0.570	0.094
t-test	P-value	0.435	0.642	0.202	0.040	0.561	0.112	0.141
Tm control	Distilled H_2O	5.210	1.680	6.890	100	3.101	0.290	0.042
5	HTCAE	4.987	1.660	6.647	96	3.004	0.406	0.061
	HTAgAE	5.343	1.879	7.222	105	2.843	0.490	0.067
10	HTCAE	4.695	1.602	6.297	91	2.930	0.507	0.080
	HTAgAE	5.196	1.875	7.071	102	2.771	0.540	0.070
20	HTCAE	4.531	1.530	6.061	88	2.961	0.585	0.096
	HTAgAE	4.330	1.535	5.865	85	2.820	0.580	0.098
t-test	P-value	0.224	0.367	0.121	0.030	0.512	0.627	0.431
Pp control	Distilled H_2O	3.997	1.480	5.477	100	2.701	0.235	0.043
5	HTCAE	3.760	1.370	5.130	94	2.744	0.363	0.071
	HTAgAE	4.000	1.420	5.420	99	2.469	0.425	0.078
10	HTCAE	3.607	1.343	5.013	92	2.733	0.510	0.102
	HTAgAE	3.620	1.420	5.040	92	2.549	0.593	0.117
20	HTCAE	2.580	1.170	3.750	68	2.205	0.532	0.142
	HTAgAE	3.420	1.320	4.740	86	2.591	0.555	0.117
t-test	P-value	0.042	0.566	0.612	0.02	0.741	0.444	0.222
Pm control	Distilled H_2O	3.660	1.510	5.170	94	2.424	0.316	0.061
5	HTCAE	2.813	1.343	4.156	76	2.094	0.332	0.079
	HTAgAE	3.608	1.493	5.101	93	2.416	0.376	0.074
10	HTCAE	2.420	1.305	3.725	68.	1.854	0.333	0.089
	HTAgAE	3.600	1.485	5.085	93	2.424	0.523	0.103
20	HTCAE	2.400	1.280	3.680	67	1.875	0.377	0.102
	HTAgAE	3.000	1.480	4.480	82	2.027	0.550	0.123
t-test	P-value	0.321	0.334	0.211	0.012	0.022	0.641	0.322

Data are means of three replicates. Tp: T. aestivum (pure) Tm: T. aestivum (mixed) Pp: P. minor (pure) Pm: P. minor (mixed). Values are the means of three independent replicates ± SE

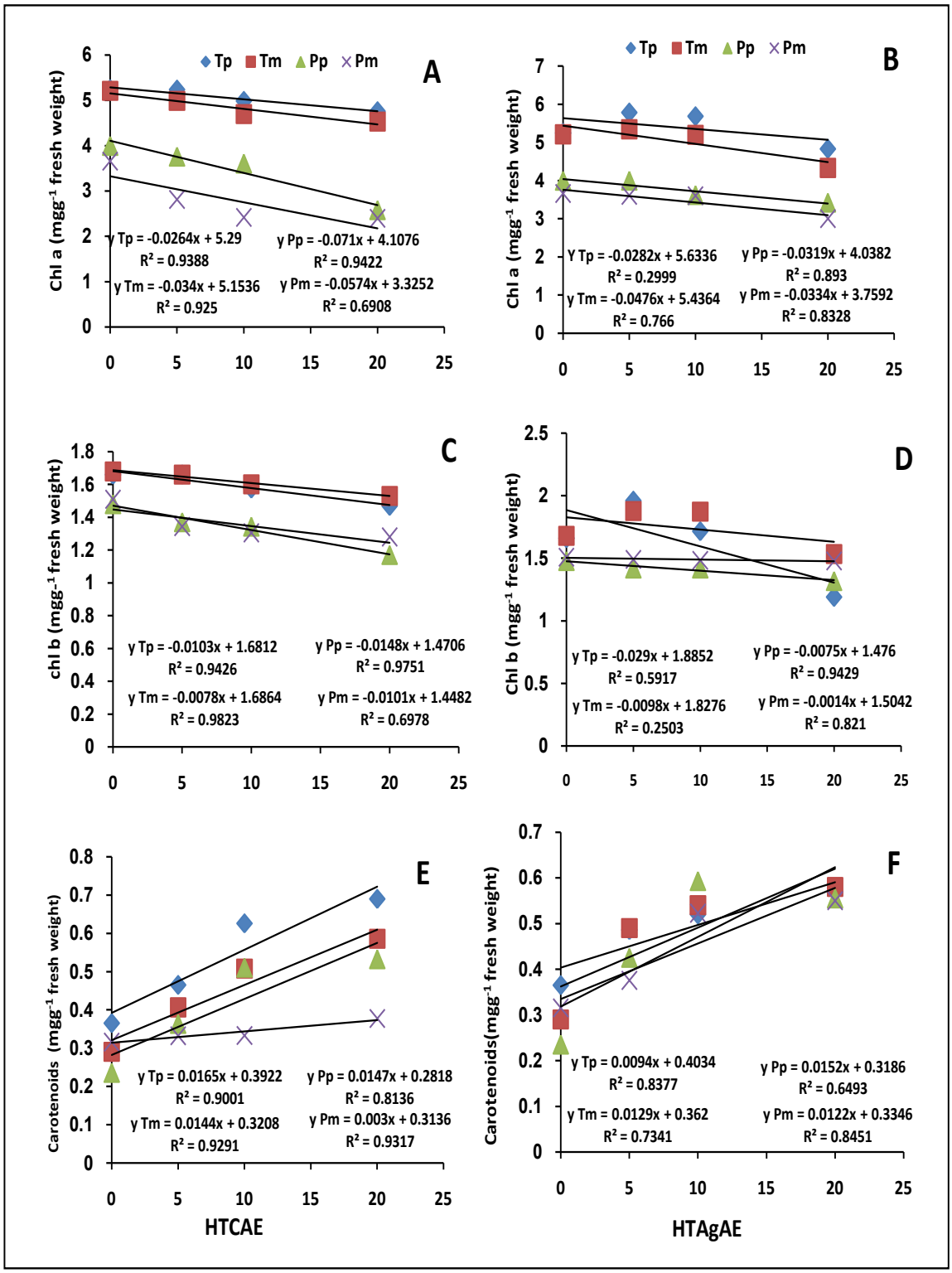

Fig. 8. Simple linear regression for the effect of priming with *Haplophyllum tuberculatum* crude (HTCAE) (A, C and E) and Ag-nanoparticles (HTAgAE) (B, D and F) aqueous extracts on photosynthetic pigments in leaves of *Triticum aestivum* and *Phalaris minor* in pure and mixed culture (A and B Chl. *a*), (C and D Chl. *b*) and (E and F Carotenoids) in growth bioassay experiment [Data are means of three replicates. Tp: *T. aestivum* (pure), Tm: *T. aestivum* (mixed), Pp: *P. minor* (pure), Pm: *P. minor* (mixed)].

As an indicator for the photosynthetic rate, the maximal photochemical efficiency of PSII (Fv/Fm), showed direct correlation with HTCAE and HTAgAE concentrations in both species, especially *P. minor* (Fig. 9).

Vegetative protein electrophoresis

The electrograms of *T. aestivum* and *P. minor* showed that 31 (16.5-113.2 KDa) and 44 bands (10.0-102.6kDa) were generated without common bands, respectively (Fig. 10). The highest values for the total and specific band numbers, the polymorphism percentage and the lowest GTS for both species were achieved at 5% HTAgAE (Table 3).

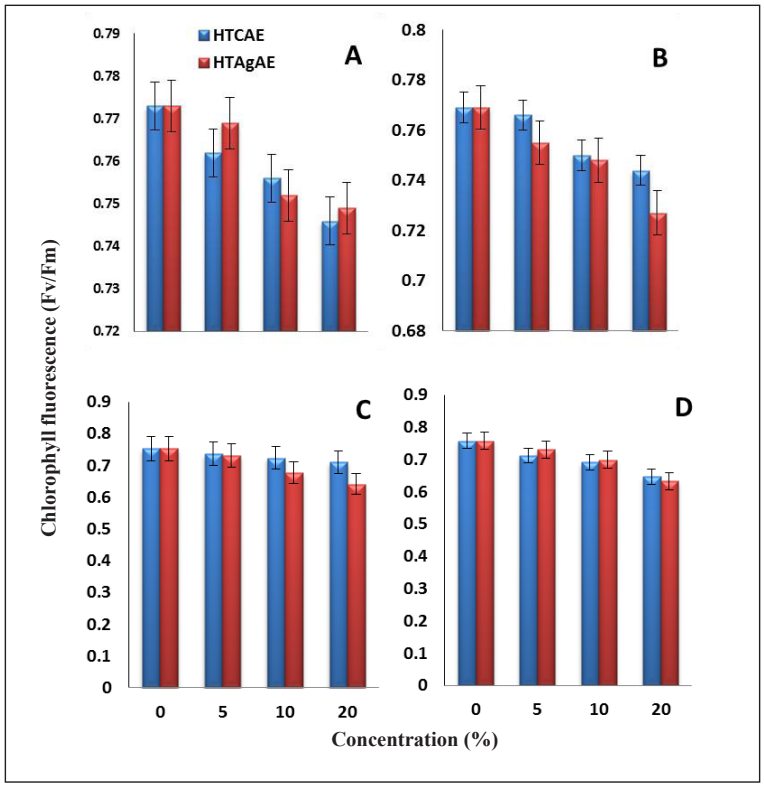

Fig. 9. Effect of priming with *Haplophyllum tuberculatum* crude (HTCAE) and Ag-nanoparticles (HTAgAE) aqueous extracts on chlorophyll fluorescence of *Triticum aestivum* (A: Pure, B: Mixed) and *Phalaris minor* (C: Pure, D: Mixed) in culture experiments [Values are the means of three independent replicates± SE].

TABLE 3. Total number of bands, percentage of polymorphism, new appeared bands, disappeared bands and genomic template stability (GTS%) of the vegetative proteins in *T. aestivum* and *P. minor* treated with 5 and 20% of *H. tuberculatum*, crude (HTCAE) and Ag-nanoparticles (HTAgAE) aqueous extracts in pure culture.

Treatment characters	*Triticum aestivum*					*P. minor*				
	C	HTCAE		HTAgAE		C	HTCAE		HTAgAE	
		5%	20%	5%	20%		5%	20%	5%	20%
Total number of Bands	10	6	6	9	7	10	10	10	13	9
Polymorphism %	32.2	19.4	19.4	29	22.6	22.7	22.7	22.7	29.5	20.5
Specific bands		2	5	8	4		6	6	9	6
New appeared bands		4	5	8	5		10	9	13	9
Disappeared bands		8	9	9	8		10	9	10	10
GTS%	100	61.3	54.8	45.2	58.1	100	54.5	59.1	47.7	56.8

The constructed dendrograms showed the relationship among different treatments on each species separately were congruent although different types of coefficient and sorting were used. For *T. aestivum*, the irrigated sample with 5% HTAgAE was segregated at the highest level of dissimilarity, followed with the control, then 20% HTCAE (Fig. 10 A). On the other hand, the *P. minor* samples irrigated with 5% and 20% HTAgAE were clustered together at 0.68 dissimilarity level (Fig. 10 B).

Discussion

The phytochemical screening of *H. tuberculatum* indicates the presence of alkaloids, essential oils, flavonoids, glycosides, sterols, triterpenes tannins and its relatively high phenolic content. Abou-Zeid et al. (2014) reported that *H. tuberculatum* contains many phenolics (23.6mg g^{-1} DM) and flavonoids (15.95mg g^{-1} DM); they detected chlorogenic, caffeic, gallic, 3,4-dicaffeoyl quinic acid, 4,5-dicaffeoyl quinic acid, benzoic acid, cinnamic acid, quercetin and catechin. Arasali & Kadimi (2009) indicated that silver ions were reduced to AgNPs because of the electron's ability to donate in phenolic compounds that constitutes a major group of compounds that act as primary antioxidants which are mainly responsible for the reducing property of the extract (Abou-Zeid & Ismail, 2018). Generally, plant extracts function as bioreducers besides having nanoparticle stabilizers in colloidal solutions of metals, such as silver and gold (Xue et al., 2016). In the present investigation, an attempt was made to use green biochemistry for obtaining AgNPs by using aqueous plant extract of *H. tuberculatum* as a simple, non-toxic and ecofriendly green material.

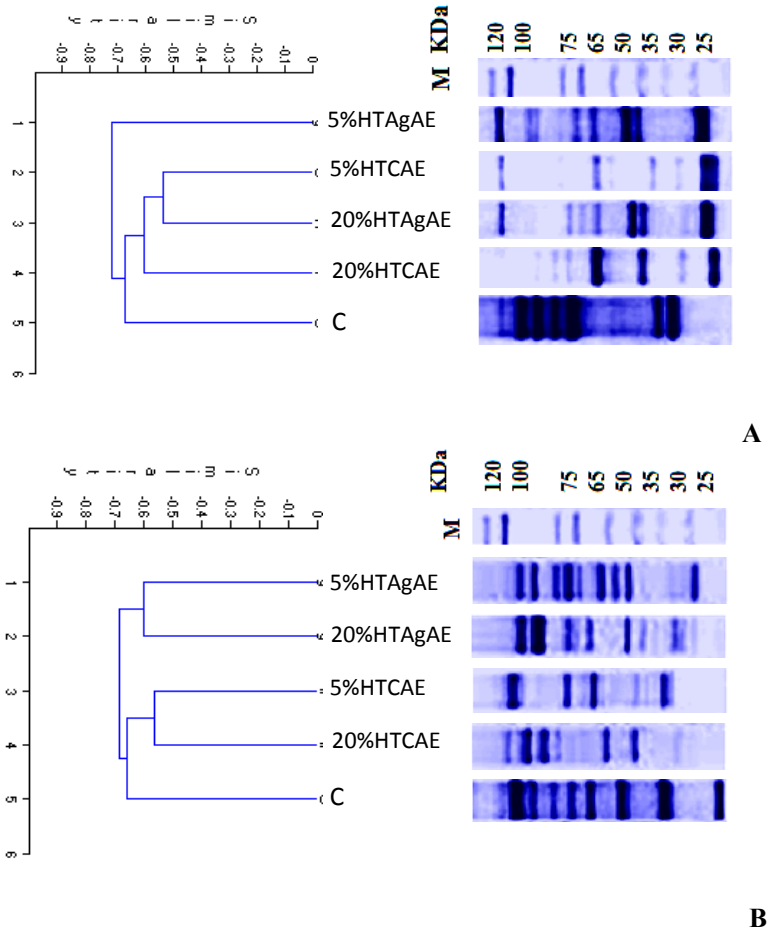

Fig. 10. The electrogram of the vegetative proteins in *Triticum aestivum* (A) and *Phalaris minor* (B) treated with 5 and 20% of *Haplophyllum tuberculatum*, crude (HTCAE) and Ag-nanoparticles (HTAgAE) aqueous extracts in pure culture and the dendrogram elucidated the relationships among them.

The effect of HTCAE on Pp and Pm shows a significant reduction in GP%, RL and PL, which reaches a maximum at 20%, whereas HTAgAE completely inhibits germination. The HTCAE achieves full germination in Tp and Tm, while reduction is detected to 40% at 20% HTAgAE. Farghaly & Nafady (2015) achieved the inhibitory effect of the AgNPs on GP% and the dry weight of wheat, while Yin et al. (2012) documented its enhancement on germination rate of *Eupatorium fistulosum*.

The fresh and dry weights, root lengths and shoot length in both *T. aestivum* and *P. minor* are negatively affected with HTCAE and HTAgAE, with the exception of 5% on the first species. Gruyer et al. (2013) showed that the positive and negative effect of nanoparticles on root elongation depended on plant species; the length was increased in barley and was decreased in lettuce. Ma et al. (2010) indicated that the inhibition of plant growth may not be direct from the phytotoxicity of nanoparticles, but may due to physical interactions between nanoparticles and plant cell transport pathways *via* apoplastic (blockage of the intercellular spaces in the cell wall or cell wall pores) or symplastic (blockage of the nano-sized plasmodesmata). Whereas, Asli & Neumann (2009) clarified that inhibition of leaf dimensions and transpiration rates as a result of the reduction in hydraulic conductivities.

Data of the present study showed that *P. minor* was more sensitive to AgNPs than *T. aestivum* this may be referred to smaller sized seeds of the first species. In accordance with our results, Wu et al. (2012) findings were that lettuce seeds are more sensitive to toxic NPs than radish seeds. They suggested that the adsorption of NPs on the seed surface could generate locally concentrated ions (released from NPs) and enhance NP phytotoxicity. Additionally, Farrag (2015) stated that AgNPs are toxic *to Lemna gibba* fronds and percent mortality was significantly increased in response to the increase of concentration and the prolonged time of exposure which caused significant changes in the growth parameters, some physiological changes and oxidative stress.

The HTAgAE at 5% and 10% stimulates the photosynthetic pigments in *T. aestivum* and reduces them in *P. minor* at 20% of HTCAE and HTAgAE. This is confirmed by reduction in the chlorophyll fluorescence (Fv/Fm), especially in *P. minor*, at 20% of HTCAE and HTAgAE, and is consistent with Racuciu & Creange (2007) and Pandey et al. (2014) reported that low concentrations of AgNPs enhanced the photosynthesis and the chlorophyll content. That is in congruent with Guerfel et al. (2009), Khaleghi et al. (2012) and Skrzypek et al. (2015) on the inhibitory effect of other plant extracts as olive and peppermint on the efficiency of photosystem II photochemistry (Fv/Fm). Abou-Zeid & El-Darier (2014), Hassanein et al. (2019) reported the reducing effect of *Moringa oleifera* leaf crude powder on the photosynthetic pigments and PSII - Fv/Fm that provided insights into a plant's ability to tolerate environmental stresses.

Results showed that *T. aestivum* seems to be more adapted to the biosynthesized AgNPs treatment, this may be explained by the enhancement of the different biochemical reactions and water absorption as indicated by a marked increase in GP, growth characteristics as well as photosynthetic pigments, CSI and photosynthetic efficiency. On the other hand a noticeable stress effect on *P*. minor plant was detected.

The vegetative proteins reveal remarkable differences between HTCAE and HTAgAE and the total number of bands, polymorphism percentage and GTS% are generally arranged in a descending manner. This manner is interrupted by using HTAgE at 5% in *P. minor* and both 5% and 20% in *T. aestivum*. The relatively low GTS%, especially at 5% in both species, reflects the antagonistic effect of *H. tuberculatum* in nanosilver form. The AgNPs caused an alteration of seedling proteins related to endoplasmic reticulum and vacuole (Vannini et al., 2013). The allelochemicals and especially phenolic compounds decline the incorporation of either phosphorous into DNA and RNA or certain amino acids into proteins (Padhy et al., 2000; Ni, 2004; Hegazy et al., 2007 and Baziramakenga et al., 2011). However, Rostami & Ehsanpour (2009) and Pozveh et al. (2014) noticed an increment in protein expression through the application of AgNPs. At 5% HTCAE 20% HTAgAE, *T. aestivum* achieves more genetic stability than *P. minor* which may support their use as safe bioherbicide.

Conclusion

The present work demonstrated a rapid green synthesis of AgNPs from *H. tuberculatum*.

The findings emphasis that both *H. tuberculatum* crude aqueous extracts and the biosynthesized AgNPs has a noticeable stress effect on *P. minor* as reduction in GP%, growth

characteristics, photosynthetic pigments and photosynthetic efficiency as well as alterations in protein profile. Metal nanoparticles may hold significant potential applications in agriculture, as they may selectively inhibit unwanted plants such as weeds.

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التخليق الحيوي للجسيمات النانونية الفضية عن طريق نبات المسيكا (الفصيلة السذبية) واستخدامه كمبيد حيوي للأعشاب

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أهتمت الدراسة الحالية بتخليق جزيئات الفضة النانونية عن طريق إستخدام المستخلص المائي الخام لنبات المسيكا (النوع المانح) كعامل مختزل. وقد تمت مقارنة المستخلص المائي المحتوي على جزيئات الفضة النانونية الناتج مع المستخلص المائي الخام وذلك بإختبارالنمو وبعض القياسات الفسيولوجية وأيضا الجزيئية لنوعين متلقيين وهما القمح كمحصول والفلارس كعشبة ضارة. وقد تم إثبات التخليق الحيوي لجزيئات الفضة النانونية في هذه الدراسة عن طريق التحول اللوني والتحليل الطيفي بالأشعة فوق البنفسجية والتحليل الطيفي بإستخدام الطاقة المتشتتة (EDS). وقد تم دراسة وفحص الشكل والحجم لجزيئات الفضة النانونية باستخدام الميكروسكوب الإلكتروني القاطع (TEM).

وقد أظهر المستخلص المائي المحتوي على جزيئات الفضة النانونية عند تركيز 5% تأثيرا محفزا كبيرا على معظم العوامل المقاسة في نبات القمح مقارنة بالمستخلص الخام. ومن ناحية أخرى، فقد أظهرت جزيئات الفضة النانونية المخلقة مع جميع التركيزات تأثيرا مثبطا على جميع العوامل المقاسة في نبات الفلارس.

وقد كشف قياس البروتين الخضري التخزيني في كل من نبات القمح والفلارس عند معاملتهم بالمستخلص المائي المحتوى على جزيئات الفضة النانونية عند تركيز 5% عن أعلى قيم في عدد الحزم ونسبة التحول والأستقرار الجيني بغض النظر عن العينة الضابطة. ويمكن أن نخلص إلى أن تخليق الفضة النانوية باستخدام المستخلص الخام لنبات المسيكا هو طريقة واعدة للسيطرة علي عشبة الفلارس الضارة. وقد أوصي الباحثون بإجراء المزيد من الدراسات لإنتاج أشكال أخرى من المعادن النانوية ليتم تطبيقها كمبيدات حيوية للأعشاب.