Int.J.Curr.Microbiol.App.Sci (2017) *6*(1): 834-843

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 1 (2017) pp. 834-843**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2017.601.098

Influence of Silver and Copper Nanoparticles on Physiological Characteristics of *Phaseolus vulgaris* L. *in vitro* and *in vivo*

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Keywords

Phaseolus vulgaris L., silver nanoparticles, copper nanoparticles.

Article Info

Accepted:
28 December 2016
Available Online:
10 January 2017

A B S T R A C T

Phaseolus vulgaris seeds were surface sterilized using ethanol 90%, soaked in a sterile distilled water for 60min, then soaked in copper and silver nanoparticles at different concentrations (25, 50 and 100mg/ml) for 15 and 30min. The effect of nanoparticles on the plant physiological characteristics *in vivo* was detected. Callus was initiated from treated and non–treated seed embryos, then embryos were maintained and transferred to callus induction medium Murashige and Skoog (MS) medium supplemented with combinations of plant growth regulators including Dichlorophynoxyacetic acid (2,4-D) (2.5mg/l), Benzyl adenine (BA) (0.5mg/l). The effects of nanoparticles on %callus induction, callus fresh weight and callus dry weight were observed. Results indicated that the effective concentration of nanoparticles that affect the physiological parameters of treated plant, % callus induction, callus fresh and dry weight was 50mg/ml sliver nanoparticles at 30min.

Introduction

Medicinal plants are used for screening and development of natural products for the quality evaluation of crude therapeutic compounds found in the plants; so to understanding their mechanism of action (Corpa and Pendrya, 2013). The studying of drugs and food supplements obtained from natural sources like plant extracts have been increased (Frankic *et al*., 2009). Among many plant species, common bean (*Phaseolus vulgaris*) is a warm season annual legume crop, grown primarily for it is protein and energy-rich dry seeds. Common bean grains are a good source of iron, zinc and other minerals (Buruchara *et al*., 2011). *P. vulgaris* has an increasing attention, because to it is rich with a variety of active compounds for health benefits such as proteins, amino acids, carbohydrates, oligosaccharides, phenols, saponins, flavonoids, alkaloids and tannins (Mishra *et al*., 2010). Ability of secondary metabolites production in different plant species *in vitro* minimize environmental variations due to the using of defined nutrient media, controlled conditions and homogeneity of stress applications. In addition, the simplicity of such manipulations enables the studying of a large plant population and stress treatments in a limited space and a short

period of time (Sakthivelu *et al*., 2008). According to the importance of nanoparticles, scientists have been deal with the idea of nanotechnology is a novel area of science that combines biology, chemistry, agriculture and physics (Rezaei-Zarchi *et al*., 2012; Demir *et al*., 2014). Silver and copper nanoparticles based compounds have been used widely in medicine. Also have a strong toxicity against many microbes. Thus act as anti-microbial agent (Mirzajani *et al*., 2011). Recently, many researches lead to using the NPs in plants and determine their efficiency. However, nanoparticles are able to interact with plants causing many morphological and physiological changes, depending on the properties of NPs. Efficacy and potential activity of it is determined by their chemical composition, size, surface and most importantly the dose of it (Khodakovskaya *et al*., 2012). Researchers explain that by finding suggests on both positive and negative effects on plant growth and development, and the impact of engineered nanoparticles (ENPs) on plants depends on the concentration, size, physical and chemical properties of ENPs as well as plant species and varies from plants to plants. However, the NPs roles in seeds germination, roots, plant growth and photosynthesis for growth and development (Ma *et al*., 2010).

Materials and Methods

Collection of plant materials

Seeds of *Phaseolus vulgaris* were collected from the nearby plantation area, Baghdad, Iraq.

Treatment with nanoparticles

Seeds of *Phaseolus vulgaris* were sterilized by washing with 90% ethanol for 5min followed by thorough washing with sterile distilled water using magnetic stirrer. Then soaked in the sterile distilled water for 60min, after that seeds were soaked in a different concentrations of copper (800mg/l) and silver (4000mg/l) (Oraibi, *et al*., 2015) nanoparticles (25, 50 and 100mg/ml) for 15 and 30min (Razzaq *et al*., 2016).

Germination of *Phaseolus vulgaris* seeds

Treated and non-treated seeds were germinated in Petri dishes inside an incubator maintained at 25°C, for 14 days. Seedlings were transferred to test tubes (15x2.5cm) containing half strength MS medium. The test tubes were placed in a growth chamber at 16/8hrs (light/dark) photoperiod at a light intensity of 1000lux, and at an ambient temperature of 25 ± 2^0C; the medium was changed weekly. Means of shoot, root length and plant fresh weight were recorded after six weeks. Dry weights were recorded after drying plant tissues for 48hrs at 72°C. Completely randomized design with ten replications was employed for germination test with 30 seeds in each petri dish. Germination percentage was measured after 7 days by the following formula:

Germination Percentage = (Number of germinated seeds / Total number of seeds) x 100 (Razzaq *et al*., 2016).

Callus induction from *Phaseolus vulgaris* seeds embryos

Medium preparation

Murashige and Skoog, 1962 (MS) medium components were prepared and supplemented with sucrose, myo-inositol and growth regulators. The pH of the medium was adjusted to 5.8 using 0.1N NaOH or 0.1N HCl, then 8g/l agar was added to the medium. The medium was dispensed into 15x2.5cm tubes (10ml/tube). The medium was sterilized by autoclaving at 121°C for 15min.

Callus induction

Treated and non-treated white bean seeds (*Phaseolus vulgaris* L.) were surface sterilized using 70% (v/v) ethanol, then rinsing with stirring in 2.5% sodium hypochlorite. . Seeds were taken to the laminar air flow and washed three times with sterile distilled water. Callus cultures were initiated on hypocotyls of the newly germinated embryos using MS medium containing 0.5mg/l BA and 2.5mg/l 2,4-D. All the cultures were incubated in a growth room under a 16hrs photoperiod (cool, white fluorescent light) and the temperature was maintained at 25 ± 2°C with 2-7% relative humidity (Mahamune *et al*., 2011). Callus induction frequency (%) was calculated using the following formula (Yousif, 2002).

Callus induction frequency (%) = (No. seeds produced callus/total seeds cultured) x100.

Callus fresh weight was measured after 8 weeks of sub culturing into a callus growth medium (Ahmad *et al.*, 2011).

Measurement of callus fresh and dry weights

Callus fresh weight was measured after eight weeks of sub culturing onto a callus growth medium. Callus tissues were then dried at 40°C for 48hrs using an oven, and then dry weights were recorded (Ahmad *et al.*, 2011).

Results and Discussion

Effect of nanoparticles on % seed germination, shoot length, root length, fresh weight, dry weight, number of axillary buds, number of adventitious buds and number of leaves.

Results exhibited in table 1 shown that there was a significant increase in the mean % seed germinations at 50mg/ml sliver nanoparticle for 30min treatment type recording 93%. While there were no significant differences obtained in the treatments 25, 50, 100mg/ml for 15min of sliver nanoparticles, while mean %seed germinations decreased significantly in other treatments in comparison to the -control. Data also revealed that mean shoot length significantly increased at the treatments 25 and 100mg/ml for 15min; 50mg/ml at 30min sliver nanoparticles which recorded 24cm, 28cm and 27cm respectively, but no significant differences were recorded at other treatments in the mean shoot length.

Also data in table 1 showing that there was a significant increase in the mean root length at 25mg/ml sliver nanoparticles for 30min, 100mg/ml copper nanoparticles at 15min and 100mg/ml copper nanoparticles for 30min time of exposure recording 13cm, 15cm and 12cm respectively in comparison to the – control (8cm), while there was no significant differences reported in the mean root length in other treatments in comparison to the – control.

For plant fresh weights, results concluded that a significant increase in the mean fresh weight in treatment of 50mg/ml sliver nanoparticles for 30min occurred only which recorded 139mg in comparison to the -control (104mg), while the mean plant fresh weight significantly decreased at the treatments 25mg/ml and 100mg/ml with copper nanoparticles for 15min; 25, 50, 100mg/ml copper nanoparticles for 30min time of exposure recording 72mg, 79mg, 62mg, 59mg and 51mg respectively and there was no significant difference in all others treatments in comparison with the -control.

Data in table 1 revealed that there was a significant decrease in the mean dry weight at all treatments except 100mg/ml sliver nanoparticles for 15min, 25 and 50mg/ml for 30min time of exposure with no significant differences in comparison with the -control.

Results also indicated that there was no significant difference recorded in all treatments in mean numbers of axillary buds except the treatment of 50mg/ml copper nanoparticles for 15min; 25, 50 and 100mg/ml copper nanoparticles for 30min (1, 1, 1 and 0 respectively). The mean number of axillary buds significantly decreased at these treatments in comparison to the -control (3).

Additionally, results illustrated in table 1 showed no significant differences recorded in the mean number of adventitious buds at all types of treatment except 100mg/ml copper nanoparticles for 15min; 25, 50 and 100mg/ml copper nanoparticles in 30min time of exposure which recorded 1, 0, 0 and 0 respectively which considered a significant reduction in the mean number of adventitious buds compared with –control that recording 6.

Finally, data in table 1 exhibited that there was a significant increase occurred in the mean number of leaves at the treatment of 50mg/ml sliver nanoparticles for 30min recorded 22, while a significant decrease in mean number of leaves obtained in all other treatments except 25, 100mg/ml sliver nanoparticles for 15min and 25mg/ml sliver nanoparticles for 30min treatment which statistically not significant compared with –control that recorded 17.

Results presented in table 1 are in agreement with those of Salama (2012) who studied the effects of silver nanoparticles on some crop plants such as common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.) and reported that increasing the concentration of silver nanoparticles up to 60mg/ml led to an increase in shoot and root lengths, leaf area and chlorophyll content in the two tested crop plants compared with control plants, but the enhancing level of silver nanoparticles resulted in a reduction of these compounds.

Figure 1 showed the effect of silver and copper nanoparticles on physiological characteristics of *Phaseolus vulgaris* after six weeks of culture under growth chamber conditions.

Results are in line with Hojjat and Hojjat (2015) who studied the effect of nano-silver on seed germinations and yield. They concluded that an accumulation and uptake of nanoparticle was dependent on the time of exposure and concentration.

Adhikari *et al.,* (2012) demonstrated the effect of Cu-nanoparticles on germination and growth of soybean and chickpea seeds, they cocluded that Cu is an essential element for plants at low concentration, but it was phytotoxic for morphological, physiological, biochemical and molecular levels of plants materials at high levels.

Results are in agreement with Dimkpa et al. (2015) who studied the interference of Cu-NPs on nutrition of plants and showed that inhibition of growth by CuO-NPs was more apparent in roots (10–66%) than shoots (9–25%).

Effect of nanoparticles on % callus induction, callus fresh weight and callus dry weight

Results indicated that a significant increase occurred in the mean %callus inductions at 50mg/ml sliver nanoparticles for 30min recorded 97% compared with the –control (86%), while a significant decrease in the mean %callus induction obtained in all other treatment types except 25, 50mg/ml sliver nanoparticles for 15min; 25mg/ml sliver nanoparticles for 30min with no significant differences in the mean callus induction in comparison to the -control (table 2).

Table.1 Effect of nanoparticles on mean %seed germination, shoot length (cm), root length (cm), fresh weight (mg), dry weight (mg), number of axillary buds, number of adventitious buds and number of leaves, after six weeks of culture under a growth chamber conditions. n=30.

Type of treatment			Mean							
Type of Nano.	**Conc. (mg/ml)**	**Time of exposure (min)**	**seed germination (%)**	**shoot length (cm)**	**root length (cm)**	**fresh weight (mg)**	**dry weight (mg)**	**number of axillary buds**	**number of adventitious buds**	**number of leaves**
- cont.	**- cont.**	**- cont.**	84	16	8	104	39	3	6	17
Silver	**25**	**15**	81	24	8	91	26	2	3	14
Silver	**50**	**15**	78	21	11	95	24	2	2	13
Silver	**100**	**15**	87	28	9	122	37	4	4	19
Silver	**25**	**30**	76	22	13	119	35	3	5	17
Silver	**50**	**30**	93	27	10	139	41	4	7	22
Silver	**100**	**30**	69	20	6	107	28	2	2	13
copper	**25**	**15**	62	18	11	72	21	3	4	9
copper	**50**	**15**	68	21	10	86	26	1	3	12
copper	**100**	**15**	74	14	15	79	19	2	1	5
copper	**25**	**30**	69	16	7	62	22	1	0	2
copper	**50**	**30**	51	10	9	59	15	1	0	1
copper	**100**	**30**	51	13	12	51	17	0	0	1
Mean			73	19.2	9.9	91.2	27.0	2.1	2.8	11.1
L.S.D 0.05	**%seed germination= 6.1; shoot length= 6.1; root length= 3.7; fresh weight= 24.6; dry weight= 9.1; number of axillary buds; 1.9; number of adventitious buds=4.1 and number of leaves=3.6**									

Table.2 Effect of nanoparticles on mean %callus induction, callus fresh weight (mg) and callus dry weight (mg), after inoculating explants for callus induction onto solid MS medium for four weeks or inoculating callus pieces onto solid MS medium for six weeks at initial weight 100mg for callus growth. n=30.

Type of treatment			Mean		
Type of Nanoparticles	**Conc. (mg/ml)**	**Time of exposure (min)**	**Callus induction (%)**	**Callus fresh weight (mg)**	**Callus dry weight (mg)**
- cont.	**- cont.**	**- cont.**	86	636	48
Silver	**25**	**15**	92	529	35
Silver	**50**	**15**	84	692	60
Silver	**100**	**15**	73	468	65
Silver	**25**	**30**	91	710	53
Silver	**50**	**30**	97	721	69
Silver	**100**	**30**	82	694	57
Copper	**25**	**15**	73	538	63
Copper	**50**	**15**	71	598	43
Copper	**100**	**15**	63	694	59
Copper	**25**	**30**	41	478	38
Copper	**50**	**30**	29	248	41
Copper	**100**	**30**	11	119	27
Mean			68.6	548.0	50.6
L.S.D 0.05		**% Callus induction= 9.31; Callus fresh weight= 82.70; Callus dry weight = 20.17**			

Fig.1 *Phaseolus vulgaris* seedlings originated from seeds treated and non-treated with silver and copper nanoparticles after six weeks of culture under growth chamber conditions. n=30.

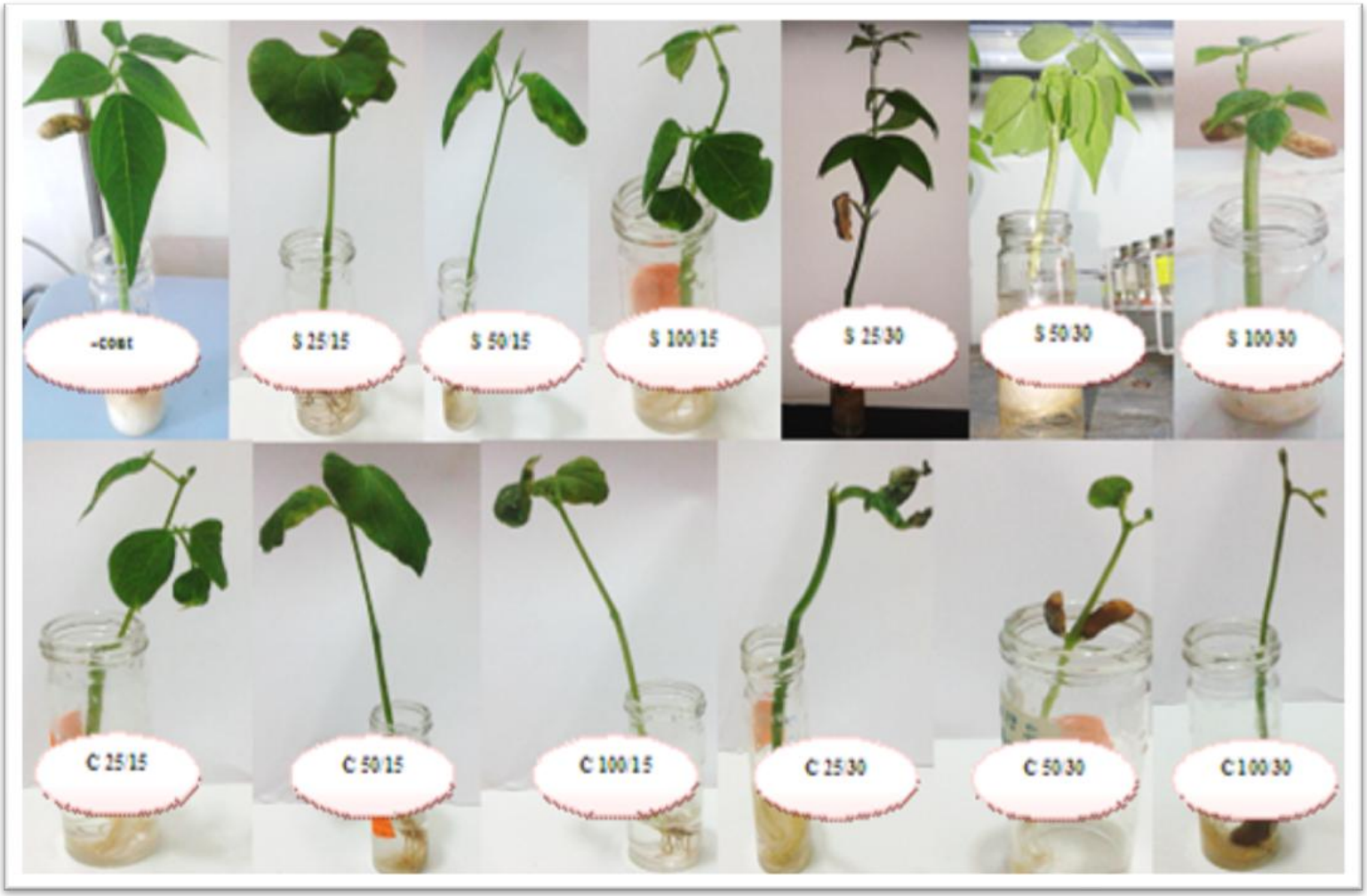
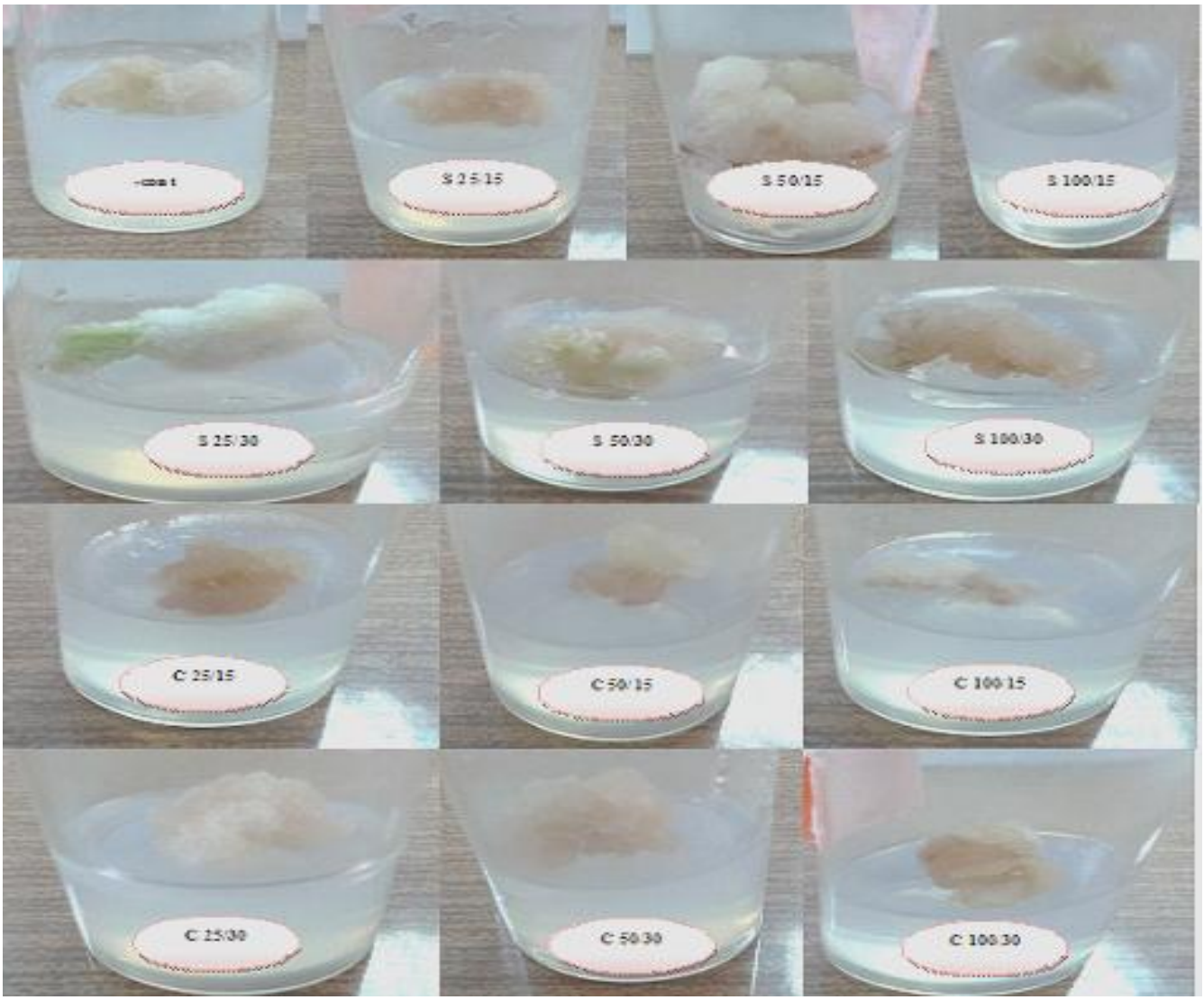


Fig.2 *Phaseolus vulgaris* callus cultures originated on embryos raised from treated and non-treated seeds after exposure to different concentrations of nanoparticles showing the changes in the callus mass grown on MS medium for eight weeks. n=30.



The represented data also reported that mean callus fresh weight increased significantly at 50mg/ml sliver nanoparticles treatment for 30min recording 721mg, but mean callus fresh weight significantly decreased at the treatments 25, 100mg/ml AgNPs for 15min; 25mg/ml CuNPs for 15min, 25, 50 and 100mg/ml CuNPs for 30min which recorded 529, 468, 538, 478, 248 and 119mg respectively compared with –control (636mg), while no significant differences were found in the mean callus fresh weight in other treatment types in comparison to the -control.

Finally results in table 2 declared that a significant increase in the mean callus dry weight obtained at the treatment 50mg/ml AgNPs for 30min recording 69mg. While other treatments recorded no significant differences in the mean callus dry weight,

except the treatment of 100mg/ml copper nanoparticles for 30min which showed shown a significant reduction in the mean callus dry weight (27mg) compared to the –control (48mg). Figure 2 demonstrated the effects of different concentrations of nanoparticles on *Phaseolus vulgaris* callus cultures that originated from non-treated and treated seeds showing the changes in the callus mass grown on MS medium for eight weeks.

Results demonstrated in table 2 were in agreement with those obtained by Emad et al. (2015) who studied the effect of AgNPs on growth, anatomy, protein and DNA of *Solanum nigrum* callus in vitro culture, the changes in *S. nigrum* callus morphology, anatomy, especially biomass (weight) and deforming cell shape and color with a variation observed in genetic instability in callus exposure to AgNPs. They stated that exposure to AgNPs increased callus fresh weight. While Hendawey et al. (2015) concluded the presence of biochemical role of nanoparticles in *Stevia rebaudiana* L. callus at different concentrations on dry weights. Decreases occurred especially at high concentrations of CuNPs causing inhibitory effect on production of *Stevia* callus.

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How to cite this article:

Harir S. Mustafa, Asma G. Oraibi, Kadhim M. Ibrahim and Nabeel K. Ibrahim. 2017. Influence of Silver and Copper Nanoparticles on Physiological Characteristics of *Phaseolus vulgaris* L. *in vitro* and *in vivo. Int.J.Curr.Microbiol.App.Sci.* 6(1): 834-843.
doi: http://dx.doi.org/10.20546/ijcmas.2017.601.098