Using NPK nanoparticles in multiplication and micro-tuber process of two potato variety (*Solanum tuberosum* L.) by using tissue culture techniques

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Abstract: Micro-tubers which are produced in tissue culture have benefits for disease free potato production. The effects of NPK nano-particles on *in vitro* micro-propagation of two potato variety (*Solanum tuberosum* L.) were evaluated. In the present study nodal explants of potato cultivars (Sponta and Seylon) were cultured on MS basal medium supplemented with different NPK (9: 0: 6 + 1 Silver) Nano particles (control, 10 ml, 20 ml, 30 ml, 40 ml, and 50 ml).. Analysis control (30 ml) exhibited highest number of nodes (4.0) followed by analysis control 10 ml, 20 ml and 40 ml (3.0) in Seylon Variety. While, Analysis control (30 ml) exhibited highest number of nodes (5.0) followed by analysis control 10 ml and 20 ml (4.0) in Sponta Variety. The highest number of micro-tuber was observed in treatment 20 ml (6) followed by treatment 10 ml, and 30 ml (4) in Seylon Variety. The highest number of micro-tuber were observed in treatment 30 ml (10) followed by treatment 40 ml, 20 ml, and 50 ml (8, 6, 6) in Sponta Variety.

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Key words: Potato cultivars, In Vitro propagation, multiplication.micro-tuberization, NPK nano-particle.

1. Introduction:

Potato (*Solanum Tuberosum L.*) is a very important crop in agricultural production in the republic of Egypt and around the world. It is grown in 180 countries worldwide. According to FAO data. *In Vitro* propagation of potato (*Solanum Tubersoum L.*) is commonly used in production of disease free seed tubers ***(Roca et al., 1979; Ranalli et al., 1994)***. *In vitro* approach facilitates production, mass multiplication and season independent production of diseases free planting material and conservation of potato in controlled and disease free conditions using space very effectively and efficiently ***(Manpreet et al., 2015)***. Availability of quality tuber is the prerequisite for successful potato production. Small size and weight microtubers have advantages in terms of disease free, storage, transportation and mechanization ***(Kefi et al., 2000b; Kanwal et al., 2006)***. Tuberisation process in potato is a very complex, but it can be induced under *in vitro* condition, and eliminate approximately 100% viruses in seed production programs and micro-tuber is one of the strategies in this perspective. Because of their small size and weight, micro-tubers have tremendous advantages in terms of storage, transportation, and production practices. They can be directly sown into the soil and can be produced in bulk in any season. They have similar morphological and biochemical characteristics compared to field produced tubers. Therefore, mass production of potato micro-tuber is likely to revolutionize the world potato production ***(Baciu et al. 2007; Shukla et al. 2007; Naresh et al. 2011).***

Studies on different crops have shown that growth of explants under laboratory condition is affected by many factors including genotype, media composition and growth conditions ***(Baciu et al. 2007; Shukla et al. 2007; Naresh et al. 2011)***.

A number of extensive physiological research has revealed that*in vitro* tuberisation is controlled by several factors, such as hormonal combination, ratio of photo period, nutrient compositions etc.

Fertilizers are chemical compounds applied to promote plant and fruit growth ***(Behera & Panda, 2009).*** Artificial fertilizers are inorganic fertilizers formulated in appropriate concentrations and the combinations supply three main nutrients: nitrogen, phosphorus and potassium (N, P and K) for various crops and growing conditions. N (nitrogen) promotes leaf growth and forms proteins and chlorophyll. P (phosphorus) contributes to root, flower and fruit development. K (potassium) contributes to stem and rootgrowth and the synthesis of proteins ***(Mandal et al. 2009 & Gu et al. 2009)***. This brings out the idea of developing encapsulated fertilizers, in which NPK fertilizers are entrapped within nanoparticles ***(Teodorescu et al., 2009).***

In the present study, an attempt has been made to compare the micro-propagation and micro-tuberization

potential of Two potato varieties by using NPK nanoparticles to found the best handling.

2. Materials and Method:

The experiment was conducted at The Central Laboratory of Date Palm, ARC, Giza, Egypt and Agricultural Systems Development Project during the period 2016- 2017.

The material for the present study comprised of micro-plants and micro-tubers of tow potato cultivars namely Sponta and Seylon were used as plant material for evaluation for their response to *in vitro* micro-tuberisation. Potato tubers were washed several times with detergent followed by several times rinsing with distilled water, dried and placed in dark room for one month till sprouting started.

Surface sterilization of explants

Single node cuttings about (1-3 cm) long were washed under running tap water for 20 min followed by surface sterilization dipped in 20% NaOCl solution for 15 - 20 min, given several washings with autoclaved distilled water,. Under the laminar airflow hood the single nodal segments were surface sterilized by treating with 0.1% mercuric chloride for 1min. These were thoroughly rinsed with sterile water 3-4 times after each step.

Multiplication of shoots (Experimental 1)

One apical explant was inoculated on medium in each test tube and 2 - 3 multinodal explants were inoculated in jars. Then explants were put on the MS basal medium supplemented with 1.5mg/l BAP and 0.5mg/l GA3 with 30gm/L sucrose, for shoot multiplication. Six concentrations of NPK (9: 0: 6 + 1 Silver) Nano particles (control,10 ml,20 ml,30 ml, 40 ml, and 50 ml), add in MS medium (***Murashige & Skoog, 1962)***. The medium was solidified with 0.7% agar and pH 5.8 was adjusted with 1N NaOH and 1N HCl prior to autoclaving. These cultures were incubated under 16 hours photoperiod from cool white fluorescent lights (approx. 50-60 µmolm-2s-1 light intensity) at 24° ±1°C. Observations were recorded; viz. microplants stem elongation, number of nodes, root length, number of rooting and number of leaf after 6 weeks of culturing.

Micro-tuber formation (Experimental 2)

Five double node cuttings were cultured for 21 days in a jar inoculated in jars. Then explants were put on the MS basal medium supplemented with 0.5mg/l BAP and 80gm/L sucrose, for micro-tuber formation. Six concentrations of NPK (9: 0: 6 + 1 Silver) Nano particles (control, 10 ml, 20 ml, 30 ml, 40 ml, and 50 ml) add in MS medium ***(Murashige & Skoog, 1962)***. The medium was solidified with 0.7% agar and pH 5.8 was adjusted with 1N NaOH and 1N HCl prior to autoclaving. These cultures were incubated under 16 hours photoperiod from cool white fluorescent lights (approx. 50-60 µmolm-2s-1 light intensity) at 25° ±1°C. Observations were recorded on the total number of micro-tubers and weights of micro-tubers and diameter of micro- tubers after 6 weeks of culturing.

Statistical Analysis:

The obtained data were subjected to analysis of variance. The mean values were compared using LSD test at the 5% level of probability. The data were tabulated and statistically factorial analyzed according to the randomized complete block design with three replicates (Snedecor & Cochran, 1980).

3. Result and Discussion:

Plant length

The maximum length was observed in analysis control 30 ml (5.0). Followed by analysis control 40 ml (4.1), then analysis control 20 ml and 50 ml (3.5). Minimum length was observed in analysis control 10(2.5) followed by analysis control 0(2.0). As shown in (Table 1).

Number of nodes

The number of nodes per plantlet is another important parameter in order to study the growth rate. A nodal cutting is one that has one node and one leaf and is usually 2-3 cm in length. Analysis control (30 ml) exhibited highest number of nodes (4.0) followed by analysis control 10 ml, 20 ml and 40 ml (3.0). These cultivars could be classified as high multiplying cultivars because of their higher number of nodes which indicated that at least 3-4 cuttings per plantlet could be obtained. Analysis control 0 and 50 ml on the other hand produce only (2.0). As shown in (Table 1 and chart 1).

Number of leaves

Green plant having large and numerous leaves have increased overall photosynthetic rate thereby increasing overall plant survival. Results have shown that analysis control 30 ml have highest number of leaves (4). Followed by analysis control 10 ml, 20 ml, and 40 ml (3.0). Analysis control 0 and 50 ml was recorded to have smallest number of leaves (2.0). As shown in (Table1).

Number of roots

Result has shown that analysis control 30 ml have the highest number of roots (6). Followed by analysis control 20 ml, and 40 ml (4). Analysis controls 0, 10 ml, and 50 ml have recorded to have smallest number of roots (3). As shown in (Table 1).

Length of roots

Maximum length was observed in analysis control 30 ml (3.5). Followed by analysis control 40 ml (2.5). Then analysis control 20 ml and 50 ml (2.0). Minimum length was observed in analysis control 10 ml (1.7). Followed by analysis control 0 (0.7). As shown in (Table 1).

Plant length

The maximum length was observed in analysis control 30 ml (5.5). Followed by analysis control 40 ml (4.2), then analysis controls 20 ml, 50 ml, and 10 ml (3.8, 3.5, and 3.1). Minimum length was observed in analysis control 0 (2.7). As shown in (Table 2).

Number of nodes

The number of nodes per plantlet is another important parameter in order to study the growth rate. A nodal cutting is one that has one node and one leaf and is usually 2-3 cm in length. Analysis control (30 ml) exhibited highest number of nodes (5.0) followed by analysis control 10 ml and 20 ml (4.0). These cultivars could be classified as high multiplying cultivars because of their higher number of nodes which indicated that at least 3-4 cuttings per plantlet could be obtained. Analysis control 0, 40 ml, and 50 ml on the other hand produce only (3.0). As shown in (table 2 and chart 2).

Number of leaves

Green plant having large and numerous leaves have increased overall photosynthetic rate thereby increasing overall plant survival. Results have shown that analysis control 30 ml have highest number of leaves (5). Followed by analysis control 10 ml and 20 ml (4.0). Analysis control 0, 40 ml, and 50 ml was recorded to have smallest number of leaves (3.0). As shown in (Table 2).

Number of roots

Results have shown that analysis control 30 ml have the highest number of roots (6). Followed by analysis control 20 ml (5). Then analysis controls 0, 10 ml, and 40 ml (3). Analysis control 50 ml has recorded to have smallest number of roots (2). As shown in (Table 2).

Length of roots

Maximum length was observed in analysis control 30 ml (2.5). Followed by analysis control 20 ml (2.0). Then analysis control 40 ml (1.8), and analysis control 50 ml (1.6). Minimum length was observed in analysis control 10 ml (1.3). Followed by analysis control 0 (1.2). As shown in (Table 2).

In vitro propagation methods using sprouts and nodal cutting are more reliable for maintaining genetic integrity of the multiplied clones ***(Liljana et al., 2012)***. Potato micro-propagation through double node cutting is an efficient way of mass multiplication of potato micro-plants under tissue culture conditions ***(Rai et al. 2012)***. The potato varieties showed varied response toward micro-propagation under laboratory condition ***(A. K. SRIVASTAVA et al. 2012)***. These reports are in agreement with results of the present investigation.

Number of micro-tuber

As shown in table 3 and chart 3, The highest number of micro-tuber was observed in statistical analysis 20 ml (6) followed by statistical analysis 10 ml,30 ml, and control ml (4, 4, 3). The lowest number of micro-tuber was observed in statistical analysis 50 ml (0), followed by statistical analysis 40 ml (2).

Average weight

Maximum average weight was found from statistical analysis 40 ml (0,95 g), followed by statistical analysis 30 ml (0,91 g), then statistical analysis 20 ml (0,73 g), then statistical analysis 10 ml (0,63 g). Minimum average weight was found from statistical analysis 50 ml (0), followed by statistical analysis control (0,45 g). (Table 3).

Average diameter

Maximum average diameter was found from statistical analysis 40 ml (2,9 mm), followed by statistical analysis 30 ml (2,7 mm), then statistical analysis 20 ml (2,4 mm). Minimum average diameter was observed in statistical analysis 50 ml (0), followed by statistical analysis control (2), then statistical analysis 10 ml (2.1 mm). (Table 3).

Average length

Maximum average length was observed in statistical analysis 30 ml (4,6 mm), followed by statistical analysis 20 ml (2,7 mm). Then statistical analysis 40 ml (2,4 mm). and statistical analysis 10 ml (2,1 mm). Minimum average length was observed in statistical analysis 50 ml (0), followed by statistical analysis 10 ml (2,0 mm). (Table 3).

Number of micro-tuber

As shown in table 4 and chart 4, The highest number of micro-tuber was observed in statistical analysis 30 ml (10) followed by statistical analysis 40 ml,20 ml, and 50 ml (8,6,6). The lowest number of micro-tuber was observed in statistical analysis 10 ml (1), followed by control (2).

Average weight

Maximum average weight was found from statistical analysis 30 ml (0,91 g), followed by statistical analysis 40 ml (0,58 g), then statistical analysis 50 ml (0,55 g), and statistical analysis 20 mlrecorded (0,53 g). Minimum average weight was found from statistical analysis 10 ml (0,37 g), followed by statistical analysis control (0,38 g). (Table 4)

Average diameter

Maximum average diameter was found from statistical analysis 30 ml (3,0 mm), followed by statistical analysis 20 ml (1,80 mm), then statistical analysis 40 ml and 50 ml (1,60 mm). Minimum average diameter was observed in statistical analysis control and 10 ml (1,40 mm). (Table 4).

Average length

Maximum average length was observed in statistical analysis 30 ml (4 mm,6 mm), followed by statistical analysis 20 ml (2,7 mm). Then statistical analysis 40 ml (2,4 mm). Minimum average length was observed in statistical analysis control and 10 ml (2,0 mm). (Table 4).

*In vitro*microtuber production is very beneficial to propagate and store valuable potato stock and may be adaptable for automated commercial propagation and largescale mechanized field planting ***(McCown & Joyce, 1991)***, in addition to economizing time, space and money ***(Venter & Steyn, 1997)***. ***(Dobranszki & Mendis, 1993)*** found that after culturing of shoots for 4 -weeks under long days, tuberization was induced with pouring of 8% sucrose in the culture medium. Although, many studies recommend solidified media for microtuberization of potato ***(Myeong et al., 1990; Kiji et al., 1997; Pelacho et al., 1999)***. These reports are in agreement with results of the present investigation.

Table 1: The effect of NPK nanoparticles *In vitro* shoots, roots, plantlets formation from sprouts Seylon explantsvariety in multiplication stage.

Treatment	Character				
	Plant length	Number of nodes	Number of leaves	Number of roots	Length of roots
Control	2	2.0	2.0	3.0	0.7
10 ml	2.5	3.0	3.0	3.0	1.7
20 ml	3.5	3.0	3.0	4.0	2.0
30 ml	5.0	4.0	4.0	6.0	3.5
40 ml	4.1	3.0	3.0	4.0	2.5
50 ml	3.5	2.0	2.0	3.0	2.0
$LSD_{0.05}$for	2.44	1.3	1.33	2.56	1.26

Chart 1: The effect of treatment NPK nanoparticles on the number of nodes in Seylon variety*in vitro*in multiplication stage.

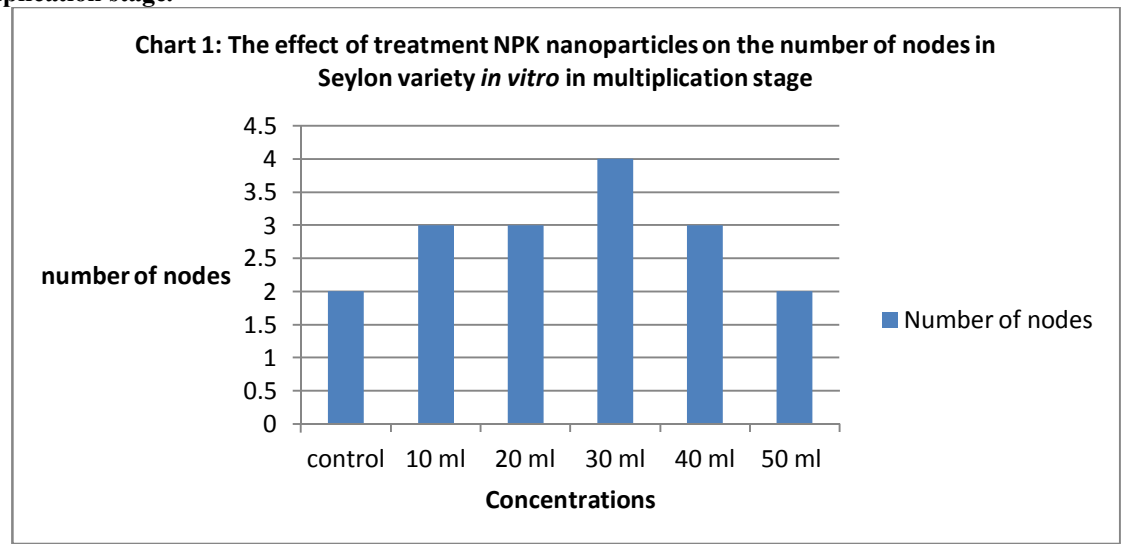


Table 2: The effect of NPK nanoparticles In *vitro* shoots, roots, plantlets formation from sprouts Sponta explants variety in multiplication stage.

Treatment	Character				
	Plant length	Number of nodes	Number of leaves	Number of roots	Length of roots
Control	2.7	3.0	3.0	3.0	1.2
10 ml	3.1	4.0	4.0	3.0	1.3
20 ml	3.8	4.0	4.0	5.0	2.0
30 ml	5.5	5.0	5.0	6.0	2.5
40 ml	4.2	3.0	3.0	3.0	1.8
50 ml	3.5	3.0	3.0	2.0	1.6
$LSD_{0.05}$for	2.71	1.34	1.34	2.68	1.81

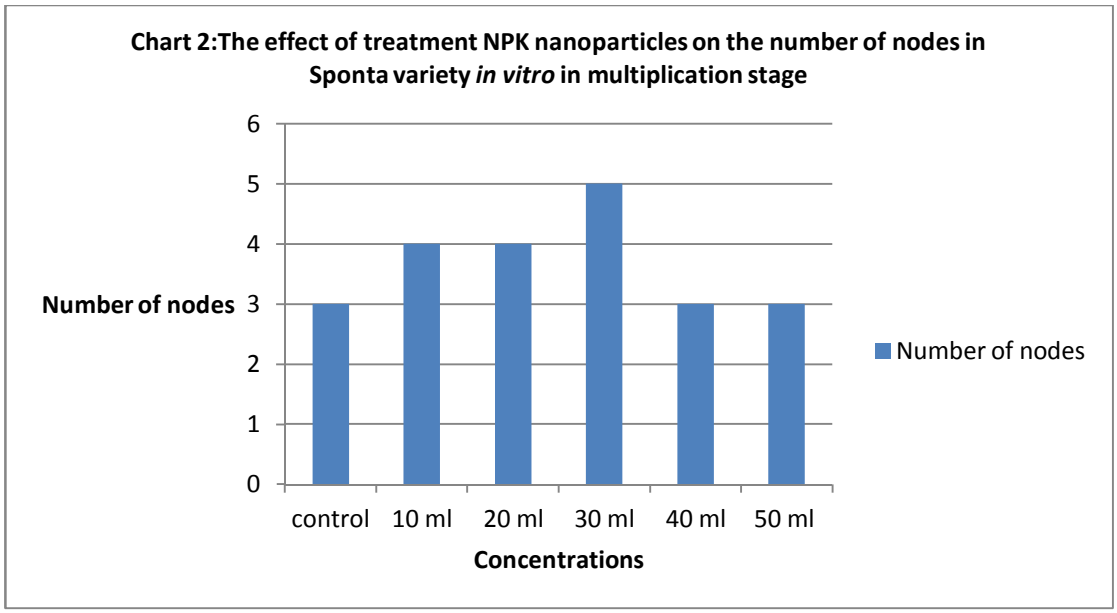


Table 3: The effect of treatment by NPK nanoparticles on the number of micro-tuber production of Seylon variety*in vitro*

Treatment	Character				Mean
	No. of micro-tuber / Plant	Average Weight/ micro-tuber (g)	Average Diameter/ micro-tuber (mm)	Average Length/ micro-tuber (mm)	
Control	3	0.45	1.2	2	2.11
10 ml	4	0.63	1.8	2.1	2.13
20 ml	6	0.73	2.4	2.7	2.95
30 ml	4	0.91	2.7	4.6	3.05
40 ml	2	0.95	2.9	2.4	2.06
50 ml	0	0	0	0	0
Mean	17.3	0.61	1.8	2.3	
$LSD_{0.05}$for	0.63	0.55	0.13	0.29	

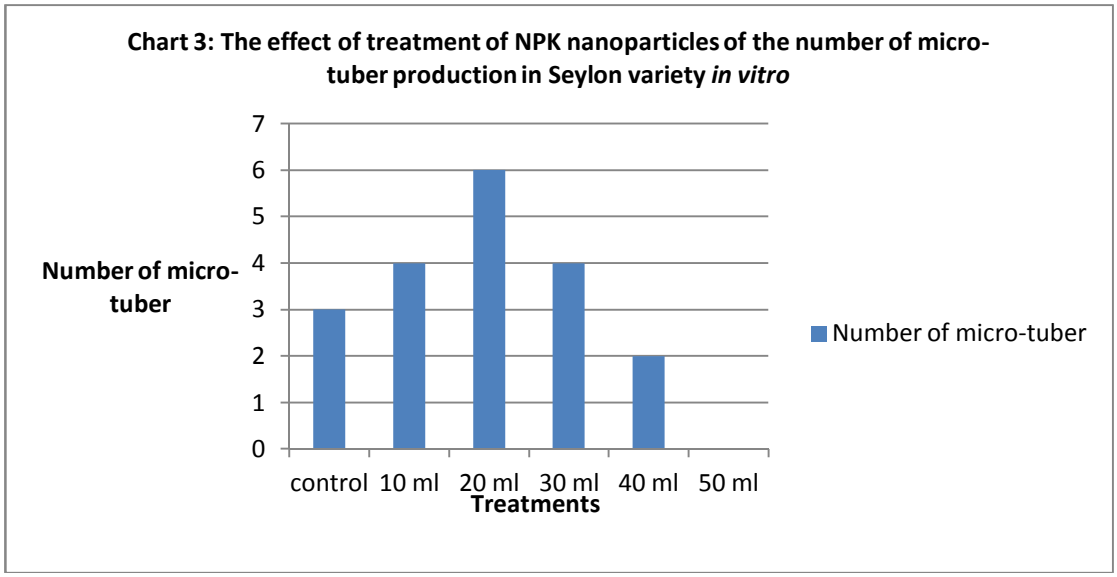
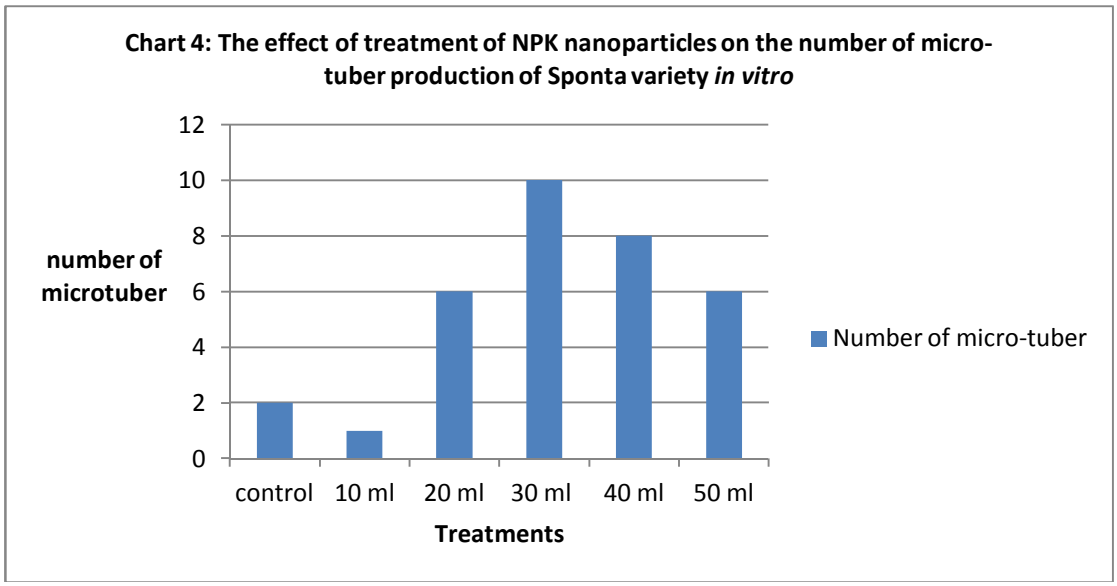


Table 4: The effect of treatment by NPK nanoparticles on the number of micro-tuber production of Sponta variety *in vitro*.

Treatment	Character				Mean
	No. of Micro-tuber/ Plant	Average Weight/ Micro-tuber (g)	Average Diameter/ Micro-tuber (mm)	Average Length/ Micro-tuber (mm)	
control	2	0.38	1.40	2	1.44
10 ml	1	0.37	1.40	2.1	1.21
20 ml	6	0.53	1.80	2.7	2.75
30 ml	10	0.91	3.00	4.6	4.62
40 ml	8	0.58	1.60	2.4	3.14
50 ml	6	0.55	1.60	2.2	2.58
Mean	5.5	0.55	1.8	2.66	
$LSD_{0.05}$for	1.34	0.05	0.43	0.44	



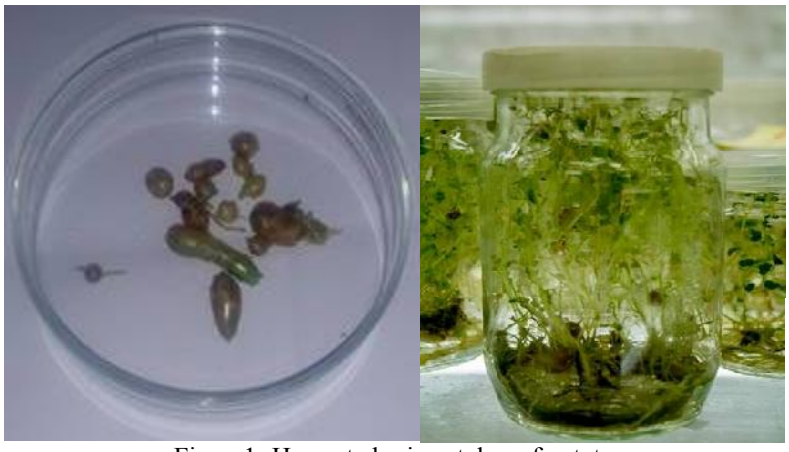


Figure1: Harvested micro-tuber of potato

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