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Effects of Nanosilver and Nitroxin Biofertilizer on Yield and Yield Components of Potato Minitubers

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ABSTRACT

Effects of nanosilver and Nitroxin® biofertilizer containing nitrogen stabilizing bacteria on the efficiency of potato seed tuber production from minitubers of Sante cultivar obtained from virus-free tissue cultured plantlets were evaluated in the field. The experiment was a completely randomized block design consisting 7 treatments each in 3 replications including: Normal N similar to the region (T1, control), Normal N+25 mg/L Nanosilver (T2), Normal N+50 mg/L Nanosilver (T3), Normal N+75 mg/L Nanosilver (T4), No N+Nitroxin (T5), 1/2 N+Nitroxin (T6), 1/2 N+Nitroxin+50 mg/L Nanosilver (T7). The results showed that nanosilver had its best effects in a concentration of 50 mg/L (T3) compared to the control. The highest records regarding tuber diameter, number of tubers per each plant, average weight of each tuber and tuber yield were obtained in T7. This combination of Nanosilver and Nitroxin (T7) increased tuber yield 85% and 117% compared to T5 and T1, respectively. According to the results, application of Nitroxin biofertilizer on potato minitubers can reduce the amount of mineral nitrogen fertilizer to half, while producing higher yield. © 2011 Friends Science Publishers

Key Words: Potato; Minituber; Nano silver; Nitrogen fertilizer; Biofertilizer

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important crops in the world and also in Iran with annual plantations of over 190000 ha. Since potato is propagated vegetatively, it is exposed to bacterial, fungal, viral and micoplasma diseases more often resulting severe yield reduction in the infected plantations. Therefore, obtaining higher yield efficiency has been always considered important and can be achieved by healthy seed tubers production from minitubers grown in the field. There are great potentials and benefits for growing potato micro and minitubers in the field (Kawakami *et al.*, 2003).

Fertilizer management is one of the most important factors in successful cultivation of crops affecting yield quality and quantity. Applications of mineral fertilizers have unfavorable impacts on our environment and health. Thus, any operation and method that try to reduce the environmental pollutions will have a positive role in our life. Today, environmental protection is more important for the agrarian, considering the sustainable agriculture (Pepo *et al.*, 2005). Bio-fertilizers are more environmental friendly and in many cases, they have given the same or even better crop yields compared to mineral fertilizers (Saghir Khan *et al.*, 2007; Vessey, 2003).

Bio-fertilizers include mainly the nitrogen fixing, phosphate solubilizing and plant growth- promoting microorganisms (Goel *et al.*, 1999) providing a more balanced nutrition for plants (Belimov *et al.*, 1995). Presently, Azotobacter, Azospirillium, Cyanobacteria, Rhizobium, Endophytic diazotrophs, Blue green algae, Azolla, Mycorrhizae and Sinorhizobium are being used as bio-fertilizers, benefiting the crop production (Hegde *et al.*, 1999). Chemical fertilizers are made up of only few minerals and impede the uptake of other minerals that results the imbalance the whole minerals pattern of the plants body. Biofertilizers can restore or increase the amount of deficient nutrients and enrich the soil nutrients. Free-living nitrogen-fixing bacteria *eg Azotobacter chroococcum* and *Azospirillum lipoferum,* not only have the ability to fix nitrogen but also the ability to release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients and photosynthesis (Fayez *et al.,* 1985).

Application of biofertilizers can increase the production efficiency of potato seed tubers from minitubers and reduce the production costs. Production of high quality and standard healthy potato seed materials would be best possible when the production efficiency of minitubers is high enough. Nitroxin® biofertilizer consists the most

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effective species of nitrogen stabilizing bacteria including the genus Azotobacter, Azospirillum and phosphate solubilizing bacteria including the genus Pseudomonas has been recommended for potato as well as other crops by producing company. However, its effect has not been studied on production efficiency of potato minitubers grown in the field.

Sharaf (1995) showed that inoculation with a mixture of *Azotobacter* and *Azospirillum* with full doses of rock phosphate and inorganic N-fertilizer, in combination with inoculation with vascular arbuscular mycorrhiza (VAM), improved growth of both datura (*Datura stramonium*) and ammi (*Ammi visnaga*: Fam. Umbelliferae) plants. Badran and Safwat (2004) and El-Ghadban *et al.* (2006) found that fennel responded to biofertilizer by increasing growth and oil yield and changing the chemical composition.

Nanosilver is a new classes of material with remarkably different physiochemical and biological characteristics such antimicrobial activity (Choi *et al.*, 2009) has shown to have antibacterial, antifungal and antiviral effects (Nomiya *et al.,* 2004; Sondi & Salopek-Sondi, 2004) and can reduce the damages and the lost caused by these diseases (Russell & Hugo, 1994). The nanosilver products make broad claims about the power of their nanosilver ingredients, such as: “eliminates 99% of bacteria” renders material “permanently antimicrobial and antifungal”, “kills approximately 650 kinds of harmful germs and viruses” and “kills bacteria in a short time as 30 min” and it was 2-5 times faster than other forms of silver (Emtiazi *et al.*, 2009). Nanosilver is a solution containing nanosilver and used for its antimicrobial effects. Potato minitubers can benefit from antimicrobial effect of nanosilver before culture in the field. No published research was found regarding the effect of nanosilver on production efficiency of potato mimitubers grown in the field.

The aim of the present research was to evaluate the effects of nanosilver and Nitroxin on yield and yield components of the potato minitubers grown in the field.

MATERIALS AND METHODS

The experiment was conducted in the farmer's conditions in Ghorveh region, Sanadaj, Kurdistan province, Iran in 2009. Potato minitubers, cv. Sante obtained from virus-free tissue cultured plantlets were kindly supplied by Hamadan Agricultural and Natural Resources Research Center. The field preparation was done first by a 25 cm depth plough in May followed by disc, rotivator, leveler and hen manure dispatching (8 tons per hectare). The experiment was set up as a completely randomized block design (CRBD) with 7 treatments and 3 replications. The treatments were: Normal N similar to the region (T1, control), Normal N+25 mg/L Nanosilver (T2), Normal N+50 mg/L Nanosilver (T3), Normal N+75 mg/L Nanosilver (T4), No N+Nitroxin (T5), 1/2 N+Nitroxin (T6), 1/2 N+Nitroxin+50 mg/L Nanosilver (T7).

Nitroxin biofertilizer was bought from Mehr Asia Technology Company (MABCO), Tehran, Iran. For Nitroxin treatments, 4 L of Nitroxin was added in 20 L of water and mix thoroughly. The minitubers were then soaked in this solution for about 10 min and after mixing, they were spread in the shade until they were dried completely before culture. Nanosilver was provided by Pars Nano Nasb Company, Tehran, Iran. For nanosilver treatments, solutions containing the desired concentrations (25, 50 & 75 mg/L) were prepared and similar to Nitroxin application were used but no need to be spread in the shade. For combined application of nanosilver and Nitroxin, nanosilver was applied first and the same minitubers were treated in Nitroxin solution after one hour and allowed to dry in the shade. For control treatment, the minitubers were similarly socked in distilled water before culture.

In the field, each plot was 15 m^2 (5×3 m) with 4 rows of 75 cm inter row and 19 cm intra row spacing and minitubers were sown in the middle part of the row in a depth of about 6 cm. Before culture, soil samples were taken in a depth of 0-30 cm and analyzed (Table I). Based on soil analysis results, the recommended amount of nitrogen fertilizer (400 kg/ha urea) was determined as Normal N and used in related treatments in 3 time-installments including 1/3 at the time of culture, 1/3 at the time of 6-8 leaf stage and 1/3 before flowering stage. The irrigation method was sprinkler raining systems.

During growth season and at the time of harvesting, different parameters such as number of shoots/plant, number of tubers/plant, average tuber diameter, average weight of each tuber and the tuber yield of each plant were recorded and statistically analyzed using MSTATC program. Means were compared using Duncan’s multiple range test at 5% significant level.

RESULTS AND DISCUSSION

There were significant differences among treatments regarding the average weight of each tuber and the tuber yield of each plant but no significant differences were observed among treatments regarding the number of shoots per plant, the tuber diameter and the number of tubers produced from each plant (Table II). Nanosilver had its best effects in a concentration of 50 mg/L (T3) compared to the control. The greatest weight of each tuber and tuber yield of each plant were obtained in T7 (Table III & Fig. 1). This combination of Nanosilver and Nitroxin (T7) increased tuber yield 85% and 117% compared to T5 and T1, respectively.

Regarding the number of tubers per plant and the tuber diameter, although the analysis of variances was not significant (Table II) but the differences among treatments were considerable (e.g., T7 & T3, respectively 16.33 & 15.33 compared to others 11-12.66) and separated in different classes by Duncan multiple range test (Table III).

Table I: Soil analysis for potato experimental field in Ghorveh region, Sanadaj, Kurdistan province, Iran, 2009

Depth (cm)	Texture	Salinity (dS/m)	pH	% Lime	% Organic C	% Total N	P (mg/kg)	K (mg/kg)
0-30	Clay loam	0.236	8.02	18	0.4	0.04	2.9	570

Table II: Analysis of variances of the evaluated parameters

SOV	DF	MS				
		Tuber Diameter	No Tubers/Plant	Tuber yield/ Plant	Weight of Tuber	Stems/Plant
Block	2	0.185	6.619	35740.397	50.591	0.047
Treatment	6	0.498	12.158	195508.132**	322.231*	0.206
Error	12	0.234	5.063	21151.955	106.960	0.158
CV%	_	9.682	17.246	18.636	18.209	33.466

*and ** show significant difference at 5 and 1%, respectively

Table III: Effects of nanosilver and Nitroxin treatments on yield and yield components of potato minitubers grown in the field

No.	Treatment	Stems/Plant	Average Weight of Tuber (g)	Tuber yield/Plant (g)	No Tubers/Plant	Tuber Diameter (mm)
T1	Normal N similar to the region	1.0 a	52.28 ab	565.8 b	11.0 b	4.72 ab
T2	Normal N + 25 mg/L Nanosilver	1.33 a	55.65 ab	658.1 b	11.66 b	5.21 ab
T3	Normal N + 50 mg/L Nanosilver	1.0 a	69.13 a	1057.7 a	15.33 a	5.28 ab
T4	Normal N + 75 mg/L Nanosilver	1.0 a	52.25 ab	607.4 b	11.66 b	4.44 b
T5	No N + Nitroxin	1.0 a	42.15 b	663.3 b	12.66 ab	5.04 ab
T6	1/2 N + Nitroxin	1.66 a	53.84 ab	682.8 b	12.66 ab	4.71 ab
T7	1/2 N+Nitroxin+50 mg/L Nanosilver	1.33 a	71.94 a	1227.6 a	16.33 a	5.63 a

Means with the same letters in each column are not significantly different (Duncan's multiple range test)

Even though, T7 and T3 had the highest records regarding these two parameters, respectively. It seems that nanosilver particularly in a concentration of 50 mg/L had a significant and positive effect on the efficiency of tuber production and addition of Nitroxin has improved the production of tuber, considerably.

Biofertilizers are good tools to reduce environmental damages and enhance the yield (Lévai *et al.*, 2006). The efficiency of Azotobacter, Azospirillium and phosphate soloubilizing bacteria on growth and essential oil of marjoram (*Majorana hortensis* L.) plants were studied by Fatma *et al.* (2006). They suggested that the mineral N and P fertilizers can be replaced by bio-fertilizers, which can reduce both the production costs and the damages to the environment, particularly the nitrate form of nitrogen. Sharifi and Haghnia (2006) showed that the application of Nitroxin biofertilizer increased grain yield of wheat (cv Sabalan). Rasipour and Asgharzadeh (2006) also obtained similar results in Soybean.

Fallahi *et al.* (2008) studied the effects of Nitroxin biofertilizer, phosphate suloblizing bacteria and Nitroxin biofertilizer+phosphate suloblizing bacteria on quantity and quality yield of Chamomile. Their results showed that the treatments had significant effects on main shoot, number of flower per plant, diameter of flower, fresh flower yield, dry flower yield, seed yield, essential oil and kamauzolen yield. The Highest fresh and dry flower yield was observed when Nitroxin and phosphate suloblizing bacteria were applied together. They concluded that this biofertilizers can be considered as a replacement for chemical fertilizers in chamomile medicinal plant production.

Fig. 1: Effects of different treatments on number of tubers and tuber yield per plant

In the present work, significant differences were observed among Nitroxin and nanosilver treatments regarding the average weight of each tuber and tuber yield per each plant, but number of tubers per plant, tuber diameter and number of stems (shoots) per each plant was less affected. Application of nanosilver on its own increased number of tuber and tuber yield per plant especially in 50 mg/L concentration (T3). However, the differences were not significant regarding the number of shoots, weight of each tuber and tuber diameter compared to control (T1). It was also observed that increasing nanosilver concentration to 75 mg/L was not beneficial (Table III).

When the total mineral nitrogen fertilizer was replaced by Nitroxin (T5), all recorded parameters showed no significant differences compared to control (T1). This means that mineral N fertilized can be replaced by Nitroxin with no considerable reduction in yield and yield components. When 1/2 N fertilizer was replaced by Nitroxin (T6), number of shoots, average weight of tuber and tuber yield were slightly increased but number of tubers per plant and tuber diameter showed no change or slightly reduced (Table III). However, when 1/2 N fertilizer was replaced with combination of Nitroxin and 50 mg/L of nanosilver (T7), the results were the best and more, larger and heavier tubers and higher final tuber yield was obtained in this treatment.

Chandrasekar *et al.* (2005) studied the influence of biofertilizers and nitrogen source level on the growth and yield of *Echinochloa frumentacea* (Roxb.) Link and found that both morphology and yield parameters were better when the combination of biofertilizers and chemical N fertilizer was applied compared with using either source alone. Biofertilizers with 100% urea treatment produced highest yields compared to control. Maheshwari *et al.* (1991) also showed that by using biofertilizer (*Azotobacter chroococcum*) alone on palmarosa (*Cymbopogon martini* var. Motia), yield increased by 16% and when applied together with 80 kg nitrogen the yield increased by 29%. Kandeel *et al.* (2002) found that dual inoculation with symbiotic N2 fixers (*Azotobacter* & *Azospirillum*) with half or full doses of inorganic N fertilizer increased plant height, number of branches per plant, and fresh and dry weights of leaves and roots.

Nanosilver has shown to have antibacterial, antifungal and antiviral effects (Nomiya *et al.*, 2004; Sondi & Salopek-Sondi, 2004). Studies have demonstrated that silver ions interact with sulfydryl (–SH) groups of proteins as well as with the bases of DNA leading either to the inhibition of respiratory processes (Bragg & Rannie, 1974) or DNA unwinding (Batarseh, 2004). Inhibition of cell division and damage to bacterial cell envelopes are also recorded (Richards *et al.*, 1984) and interaction with hydrogen bonding processes has been demonstrated to occur (Russell & Hugo, 1994). Interruption of cell wall synthesis resulting in loss of essential nutrients has been shown to occur in yeasts (Wells *et al.*, 1995) and may well occur in other fungi. Antiviral activity of silver ions has been recorded and interaction with –SH groups has been implicated in the mode of action (Thurmann & Gerba, 1989). Contact time and temperature can have impact on both the rate and extent of antimicrobial activity (Dibrov *et al.*, 2002).

The antibacterial efficiency of the nanoparticles was investigated by introducing the particles into a media containing *E. coli* and it was found that they exhibited antibacterial effect at low concentrations (Baker *et al.*, 2005). Kim *et al.* (2009) investigated antifungal activity of three different forms of silver nanoparticles against the unidentified ambrosia fungus Raffaelea sp., which has been responsible for the mortality of a large number of oak trees in Korea. They have found that fungi growth in the presence of silver nanoparticles was inhibited significantly in a dose dependent manner. Microscopic observation revealed that silver nanoparticles had detrimental effects not only on fungal hyphae but also on conidial germination.

The potential of nanosilver for removing bacterial contaminants from nodal explants of valerian was evaluated in vitro by Abdi *et al.* (2008). Nanosilver at two stages (before & after surface sterilization along with control) with three rates (25, 50 & 100 mg/L) at three times of soaking (30, 60 & 180 min) was applied. It was found that using 100 mg/L of nanosilver solution after surface sterilization resulted in the highest percentage (89%) of disinfected explants. Nanosilver solution did not affect the other characters measured. It was concluded that nanosilver had a good potential for removing of the bacterial contaminants in plant tissue culture procedures.

According to the results, it can be concluded that by application of Nitroxin the amount of mineral nitrogen fertilizer can be reduced to half. This would have positive environmental impacts. Interestingly, the application of nanosilver in combination with Nitroxin caused significantly higher tuber yield probably due to its antimicrobial effect. This antimicrobial effect of nanosilver might have helped seed tubers to stay healthier for longer time in the soil and subsequently produced more vigorous plants. On the other hand, the production of various antibiotics by the bacteria present in Nitroxin in rhizophores of roots may prevent the invasion of the root and seed tuber by infectious soil-borne organisms and nematodes and increase the resistance of plants to these destructive agents. To the best of our knowledge, effects of nanosilver and Nitroxin® biofertilizer on the efficiency of potato seed tuber production from minitubers have not been evaluated in the field before.

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