UNIVERSIDAD NACIONAL DE COLOMBIA

Acta Agronómica (2020) 69 (1) p 26-31 ISSN 0120-2812 | e-ISSN 2323-0118 doi: https://doi.org/10.15446/acag.v69n1.73832

Effect of *Bacillus subtilis* and *Bacillus amyloliquefaciens* culture on the growth and yield of off-season potato (*Solanum tuberosum* L.)

Efecto de la aplicación de *Bacillus subtilis* y *Bacillus amyloliquefaciens* en el crecimiento y rendimiento de patata (*Solanum tuberosum* L.) cultivada fuera de temporada

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Rec.: 2018-07-31 Acep.: 2019-12-28

Abstract

A study was carried out in order to investigate the effect of microbial fertilizer application on off-season potato (*Solanum tuberosum* L. cv.Universia) under field conditions in early spring and late autumn in 2016. The experiment included Control (C, no application), Standard Fertilizer Application (SFA), Microbial Fertilizer Application (MFA*)* and SFA+MFA treatments. An equal mixture of three strains of *Bacillus subtilis* VKPM B-10641(DSM 24613), *Bacillus amyloliquefaciens* VKPM B-10642 (DSM 24614) and *Bacillus* amyloliquefaciens B-10643 (DSM 24615) was applied at 1x10^{9}cfu/ml to tubers before planting and to plants at flowering stage in MFA and SFA+MFA plots. Treatments affected the plant growth parameters. SFA plots produced the highest tuber yields in both seasons. MFA plots had earlier emergence time (40 days), higher number of stems per plant (3), higher number of leaves per plant (37.2), thicker stem diameter (10.15 mm), higher above ground biomass yields (5.42 t/ha), higher single tuber weight (104.21 g) and higher tuber yields (22.06 t/ha) compared with control plots (42 days, 2.2, 31.2, 9.15 mm, 4.40 t/ha, 90.88 g and 20.14 t/ha respectively). Tuber yield in MFA plots (26.56 t/ha) was equal to SFA plots (26.81 t/ha) in warmer autumn planting. Combination of SFA and MFA produced lower tuber yields (21.72 t/ha) than SFA treatment (26.81 t/ha). In conclusion, *Bacillus subtilis* and *Bacillus amyloliquefaciens* gave comparable tuber yields to chemical fertilizer application in warmer autumn plantings, but lower tuber yields in colder spring conditions warranting further experiments with cold tolerant psychrophilic bacterial strains for off-season potato production.

Key words: Microbial fertilizer,potato, tuber yield

Resumen

Se estudió el efecto de la aplicación fuera de temporada de fertilizantes microbianos en patata (*Solanum tuberosum* L. cv. Universia) en condiciones de campo al comienzo de primavera y finales de otoño de 2016. El experimento incluyó tratamientos de control (C, sin aplicación), aplicación de fertilizante estándar (SFA), aplicación de fertilizante microbiano (MFA) y la combinación de SFA + MFA. Se aplicó la misma mezcla de tres cepas de *Bacillus subtilis* VKPM B-10641 (DSM 24613), *B. amyloliquefaciens* VKPM B-10642 (DSM 24614) y *B. amyloliquefaciens* B-10643 (DSM 24615) en concentración1x10^9 ufc/ml a tubérculos antes de plantar y plantas en floración en las parcelas MFA y SFA + MFA. Las parcelas MFA presentaron un tiempo de emergencia más temprano (40 días), un mayor número de tallos (3.0) y de hojas (37.2) por planta, un mayor diámetro de tallo (10.15 mm), una mayor biomasa aérea (5.42 t/ha), un mayor peso de tubérculo (104.21 g) y una mayor producción (22.06 t/ha) en comparación con las parcelas control (42 días, 2.2, 31.2, 9.15 mm, 4.40 t/ha, 90.88 g y 20.14 t/ha, respectivamente). El rendimiento en las parcelas MFA (26.56 t/ha) fue similar al de las parcelas SFA (26.81 t/ha) en el experimento de otoño. La combinación de SFA y MFA produjo rendimientos más bajos (21.72 t/ha) que el tratamiento con SFA (26.81 t / Ha). En conclusión, *Bacillus subtilis* y *Bacillus amyloliquefaciens* dieron rendimientos comparables al fertilizante químico en otoño, estación más cálida, pero no en condiciones de primavera (más frías).

Palabras clave: fertilizante microbiano, patata, rendimiento de tubérculos.

Introduction

Potato (*Solanumtuberosum* L.) is one of the most commonly cultivated crop plants in the world. As a food source it includes a good amino acid balance, vitamins C, B1, B3 and B6, folate and minerals such as potassium, phosphorus, calcium, magnesium and micronutrients iron and zinc. Potatoes are globally grown on 19.4 million hectares and its production exceeds 368 million tons (FAO, 2015). It is grown in all continents excepting Antarctica (Rowe and Powelson, 2002). Despite being a cool temperate climate crop plant, it adapted also to the areas with subtropical climate characteristics. Turkey has a suitable climate for potato production with an acreage of 130.200ha and a production exceeding 4.2 million tons (Turkstat, 2016). Most of the production is carried out as main crop in April-November period. In some regions it is grown as second crop after cereals (İlisulu, 1986; Samancı et al., 1998; Arıoğlu and Çalışkan, 1999; Arıoğlu et al., 2002). Mediterranean climatic conditions (Mauromicale et al., 2003; Günel et al., 2002) allow off season production of potatoes during winter in coastal belt of Mediterranean and Aegean Regions (Arıoğlu and Çalışkan, 1999; Arıoğlu, et al., 2002).

Off-season potato cultivation allows for earlier product introduction to the market and attracts premium prices and higher income from unit area. Off season crop cultivation has the advantage of low input sustainable production. However, under the effect of low air and soil temperatures, emergence takes longer and plants may suffer from cold stress limiting yield and quality (Çalışkan et al., 2002; Foti, 1999). Use of early maturing and cold tolerant cultivars (Arıoğlu and Çalışkan, 1999; Beukema and Van der Zaag, 1990; Mauromicale et al., 2003) and application of special cultivation techniques (Turgut, 1988; Logan and Turnbull, 2002; Samancı, et al., 1998) may increase the success of off season production.

There is well established information and technology for the production and fertilization of main potato production. But, off season potato production requires a new approach for fertilization program because of cold soil conditions prevailing during plant growth. The use of bio-fertilizers for off season potato production may offer environment friendly, cheap and sustainable alternativeplant nutrition. An important part of soil microflora consists of aerobes and facultative anaerobe and gram positive *Bacillus* sp.(Berkeley and Logan, 1997; Berkeley et al., 1984). *Bacillus subtilis* grows and survives under adverse mesophilic temperature conditions (below an optimal temperature of 25-35 ^{O}C) owing to the formation of stress-resistant endospores and genetic adaptation (Bandow et al., 2002). *Bacillus* based bio-fertilizers promoted plant growth hormone synthesis (Chabot et al.,1996; Amer andUtkheda, 2000) and released soil organic matter decomposing amyloclastic, celluloselytic, proteoclastic enzymes, enriching the plant tissue N and P concentrations and consequently increasing biomass production (Toro et al., 1997). Bacillius based microbial agents may play important role under sub-optimal air and soil temperatures prevailing in the production of off season potatoes in the field conditions.

This study investigated the possibility of using microbial fertilisers for off season potato (*Solanumtuberosum* L.) production under cold conditions of late autumn and early spring season plantings in comparison and in combination with mineral fertilizers under field conditions.

Materials and methods

An experiment was set up in order to investigate the effect of microbial fertilizer application on plant development and tuber yield of off season potato cv. Universiain the late autumn and early spring sowings in the field conditions. There were four treatments as follows: Control (C, no microbial and commercial fertilizer application), Standard Fertilizer Application (SFA), Microbial Fertilizer Application (MFA, a commercial mixture of *B. subtilis* and *B. amyloliquefaciens)* and SFA+MFA. The experiment was set up in a Randomised Blocks Design with four replicates on the experimental farm of Akdeniz University, Faculty of Agriculture in Antalya in the southwest of Turkey in the growth seasons of early spring and late autumn in 2016. Tubers of 40-50 days old stored under cool conditions (+4^{0}C) were planted by hand on 09 January 2016 in spring and on 15 August 2016 in autumn in the plots of two rows with 30 cm intra and 70 cm inter row spacing in 10-18 cm soil depth. Each plot had 20 plants in an area of 4.2 M^{2} (1.4 M x 3 M) giving 47619.04 plants/ha. Standard Fertilizer Application (SFA) plots were given commercial fertilizers as 50 kg/ha P_2O_5 and 50 kg/ha N_2 prior to sowing and 50 kg/ha N_2+50 kg/ha K_2O at first hoeing (Samancı, et al., 1998). In MFA and SFA+MFA plots, tubers were sprayed thoroughly and saturated with *B. subtilis* and *B. amyloliquefaciens* culture at the rate of 1 ml/ 10 L water immediately before planting. Plants in MFA and SFA+MFA plots were further sprayed with the bacterial culture of 1 ml/100 L water / ha at the beginning of flowering. Bacterial culture used included three strains of *B. subtilis*VKPM B-10641(DSM 24613),*B. amyloliquefaciens* VKPM B-10642 (DSM 24614) and *B. amyloliquefaciens* B-10643 (DSM 24615) in equal parts at a rate of 1x10^{9}cfu/ml in 10 ml commercially available sealed glass vials. Control and SFA plots were planted first by hand in order to prevent unintentional contamination. Potato cv.Universia used was early type cultivar grown commercially in the region (Anonymous, 2017). Experimental soil analysis on the samples taken at sowing from 20 cm soil depth on the experimental site showed it was clay loam with relatively low organic matter content (Table 1). Soil temperature at 20 cm soil depth at the experimental site was daily measured (Figure 1). Climatic data for the duration of experiment were obtained from Meteorological Observation and Record Station of Antalya Directorate of Turkish State Meteorological Service 3 km away from the experimental site (Table 2). Soil from experimental site was analyzed (Anonymous, 1985) in Laben Agricultural Analysis Laboratory (Antalya).

Plants were irrigated in drip irrigation and weeding was done by hand as required. Plant growth were observed and measurements of seedling emergence time, number of stems per plant, plant height, number of leaves per plantand stem diameterwere made on selected 5 plants at full flowering stage on each plot. Leaf chlorophyll content were measured also on 5 plants with a SPAD meter (SPAD 502 Chlorophyll Meter, Spectrum Technologies). Harvest was made on 29 April 2016 and 12December2016 for spring and autumn sowings respectively. Data were collected on above ground biomass yield, tuber number per plant, single tuber weight and tuber yield at harvest on each plot (Samancı, et al., 1998). Data were subjected to analysis of variance using statistical package of SPSS and means were separated by LSD test.

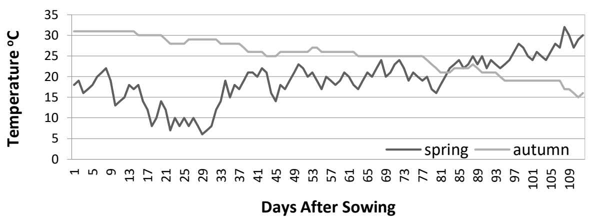

Figure 1. The soil temperature at 20 cm depth at the experimental site during the plant growth period.

Table 1. Chemical and physical properties of the soil sample from 20 cm depth on the experimental site.

Analysis Parameters	Analysis Results	Remarks
pH	7,9	Light Alkali
Lime Content (%)	42,3	Limy
Salt (%)	0,012	Without salt
Saturation (%)	50	Clayic Loam
Organic Matter (%)	1,19	Poor OM content
Total N (%)	0,100	Medium
Available P (kg/ha)	3,88	Poor
Available K (kg/ha)	70,3	Adequate
Extractable Ca (kg/ha)	1940,8	High
Extractable Mg (kg/ha)	88,5	Adequate
Plant Available Fe (ppm)	3,92	Adequate
Plant AvailableMn (ppm)	8,45	Adequate
Plant Available Zn (ppm)	0,78	Adequate

Results

Treatments investigated significantly (P<0.05) affected the plant growth parameters in both spring and autumn planting dates depending on the application (Tables 3, 4). Emergence time ranged between 38 - 42 days (Table 3). Emergence was 4 days earlier in SFA+MFA plots and 2 days earlier in MFA plots in spring period in colder soil temperatures (Figure 1, Table 3) whereas there was no advantage in SFA and MFA plots in warmer soil temperatures in autumn planting (Table 3).In the SFA+MFA applied plots, emergence time 3.7 days earlier in both seasons compared with control plots (Table 3, 4). The number of stems did not differ significantly in the spring but it was higher in SFA, MFA and SFA+MFA applied plots than control in autumn planting (Tables 3, 4). No statistically significant difference was recorded for plant height in relation to treatments in both seasons. But on average of both seasons, SFA and SFA+MFA plots produced taller plantsby 3.4-5.0 cm than control plots (Table 3). Leaf number was higher in SFA (47.4), MFA (41.6) and SFA+MFA (52.0) plots than in control (38.6) plots in autumn planting but no statisticallysignificant difference was recorded in spring sowing (Tables 3, 4). On Average of both seasons, number of leaves was higher in SFA (35.8), MFA (37.2) and SFA+MFA (42.3) than control (31.2) plots (Table 3). Leaf SPAD readings were higher in SFA-MFA (40.2), MFA (34.2) and SFA (38.7) than in control plots (33.6). Treatments investigated did

Table 2. Climatic data for long term average (LTA, 1929-2015) and growth period (2016) on the experimental site

Month	Monthly Total Precipitation (mm)		Average Air Temperature (°C)		Maximum Air Temperature (°C)		Minimum Air Temperature (°C)	
	2016	LTA	2016	LTA	2016	LTA	2016	LTA
January	85	231.9	10.4	9.9	19.5	23,9	1.0	-3.4
February	67.4	150.2	14.5	10.4	25.2	25.9	6.1	-4.6
March	54.4	103.2	15.2	12.7	22.4	28.8	9.1	-1.6
April	14.6	55.5	19.1	16.2	29.1	36.4	11.9	1.4
May	25.9	31.4	20.4	20.5	29.2	38.0	12.9	5.7
June	23.4	7.7	26.9	25.3	42.3	44.8	18.9	11.1
July	1.1	2.8	29.9	28.4	41.4	45.0	25.2	14.6
August	0.0	3.1	29.5	28.2	40.7	44.6	24.2	15.3
September	32.3	15.8	26.4	24.8	39.5	42.1	19.0	10.6
October	0	80.1	23.3	20.0	32.2	37.7	14.9	4.9
November	99.2	135.0	17.5	14.9	23.9	33.0	10.9	0.8
December	76.3	257.9	11.2	11.4	21.3	25.4	2.7	-1.9

Table 3. Effect of commercial of *B. subtilis* and *B. amyloliquefaciens* culture broth on the plant growth and yield of potato (*S. tuberosum* L. var. Universia).

Treat.	SE (days)	S (no.)	PH	Leaf (no.)	Leaf SPAD	SD (mm)	AG (t/ha)	Tuber (no.)	ST (g)	TY
					Spring Season					
Control	42.0	2.1	49.5	23.9	33.6	8.5	3.82	5.2	69.89	16.07
SFA	40.0	2.0	54.9	24.2	38.7	9.4	7.08	6.4	90.71	29.75
MFA	40.0	2.8	50.2	32.8	34.2	9.6	4.18	5.1	76.36	17.55
SFA+MFA	38.0	2.8	51.4	32.7	40.2	10.5	5.70	6.1	91.32	23.94
Aver.	40.0	2.4	51.5	28.4	36.6	9.5	5.2	5.7	82.07	21.83
LSD	1.15*	0.47ns	3.21ns	5.54ns	1.58*	0.50**	0.10ns	0.69ns	5.90*	0.38**
					Autumn Season					
Control	42.7	2.4	58.2	38.6	43.7	9.8	4.98	5.1	111.86	24.21
SFA	42.2	3.2	62.8	47.4	49.5	10.2	8.06	4.3	139.53	26.81
MFA	43.5	3.3	56.8	41.6	44.5	10.7	6.66	4.7	132.06	26.56
SFA+MFA	39.2	3.3	63.0	52.0	47.9	12.1	7.42	4.1	130.07	21.72
Aver.	41.9	3.05	60.2	44.9	46.4	10.7	6.78	4.55	128.38	24.825
LSD	1.16**	1.19*	3.75ns	2.73*	0.10ns	0.72*	0.10*	0.58ns	16.72*	0.29*
					Mean of Both Seasons					
Control	42.3	2.2	53.8	31.2	38.6	9.15	4.40	5.1	90.88	20.14
SFA	41.1	2.6	58.8	35.8	44.1	9.80	7.57	5.3	115.12	28.28
MFA	43.5	3.0	53.5	37.2	39.3	10.15	5.42	4.9	104.21	22.06
SFA+MFA	38.6	3.0	57.2	42.3	44.0	11.30	6.56	5.1	110.70	22.83
Aver.	42.3	2.2	3.8	31.2	38.6	9.15	4.40	5.1	90.88	20.14
LSD	0.55*	0.17*	51.70**	2.50**	1.39**	0.384*	2.939NS	0.37*	11.130*	3.148 NS

*Statistically significant at (P<0.05), ** Statistically significant at (P<0.01), NS Statistically not significant.

SE (days): Seedling emergence time; S (no.): Number of stems per plant; PH (cm): Plant height; Leaf (no.): Number of leaves per plant; Leaf SPAD: Leaf SPAD values; SD: Stem diameter (mm); AG (t/ha): Above ground biomass yield; Tuber (no.): Tuber number per plant; ST (g): Single tuber weigh; TY (t/ha): Tuber yield.

Table 4. Percent Advantage (+) or disadvantage (-) of Standard Fertilizer Application (SFA), Microbial Fertilizer Application (*B.subtilis* and*B. amyloliquefaciens)* (MFA) and SFA+MFA over control (no application).

Plant Growth Parameters	Growing Season					
	Spring			Autumn		
	SFA	MFA	SFA+ MFA	SFA	MFA	SFA+ MFA
Seedling Emergence time (day)	5	5	10.52	1.18	-1.73	8.92
Number of stems per plant	-4.77	35.71	33.33	32.65	36.73	36.73
Plant Height (cm)	10.91	1.41	3.83	7.99	-2.41	8.33
Number of Leaves Per Plant	1.04	36.95	36.53	22.77	7.63	34.54
Leaf SPAD values	15.21	1.73	19.61	13.37	1.99	9.7
Stem Diameter (mm)	11.45	13.46	23.73	3.67	9.58	23.24
Above Ground Bio-mass Yield (T/ha)	85.34	9.42	49.21	61.85	33.73	48.99
Tuber Number Per Plant	23.56	-3.07	16.09	-16.12	-9.13	-20.98
Single Tuber Weight (g)	62.03	6.83	52.73	3.25	6.92	-11.42
Tuber yield (t/ha)	85.13	9.21	48.97	10.74	9.71	-10.29

not significantly affect SPAD values in autumn planting. On average of both seasons, the highest SPAD readings were recorded in SFA+MFA plots (40.2) in spring and SFA applied plots (47.9) in autumn planting whereas the lowest values were obtained in control plots (Table 3). Stem diameter was higher only in SFA+MFA plots in both seasons. The above ground biomass yield did not differ significantly between treatments in spring period but all MFA and SFA+MFA plots produced 33.73 and 48.99 % higher above ground biomass yields than control plots in autumn sowing (Table 4). Tuber number per plant was significantly affected by treatments in both seasons (Tables 3, 4). Treatments significantly increased also tuber weight in both seasons (Tables 3, 4).Single tuber weight in MFA plots was higher (76.36 g in spring and 132.06 g in autumn)than in control plots(69.89 g and 111.86 g, respectively), but the highest single tuber weights were obtained in SFA+MFA plots in spring(90.71 g)and SFA plots in autumn (139.53 g)(Tables 3, 4).

Tuber yields ranged between 16.07t/ha and 29.75 t/ha depending on season and treatments (Table 3). Control plots alwaysproduced the lowest tuber yields (Table 3, 4). In spring sowing, SFA plots produced the highest tuber yields (29.75 t/ha) followed by SFA+MFA (23.94 t/ha) and MFA (17.55 t/ha) plots. SFA+MFA treatment yields (23.94 t/ha) were even lower than SFA plots (29.75 t/ha). In autumn sowing, SFA (26.81 t/ha) and MFA (26.56 t/ha) yields were higher than control (24.21 t/ha) and SFA+MFA (21.27 t/ha). SFA+MFA plots produced lower yields than control plots. On average of both seasons, MFA applied plots produced 22.06 t/ha tuber yield compared with control (20.14 t/ha) and SFA plots (28.28 t/ha). SFA+MFA plots produced even lower tuber yields (22.83 t/ha) than SFA plots. MFA applied plots produced more tuber yields by1.48 t/hain spring, 2.35 t/hain autumnand by 1.92 t/ha on average of both seasons when compared with control plots (Table 3).

Discussion

Data obtained from off season field experiments in spring and autumn period showed that the application of *B. subtilis* and *B. amyloliquefaciens* culture shortened emergence time, increased plant growth, produced fewer but larger tubers and increased tuber yield over control treatment. Microbial fertilizer application produced 9.2% more tuber yields in spring and 9.7% in autumn period over control plots. Tuber yield was higher in autumn (26.56 t/ha) than in spring (17.55 t/ha) in MFA plots probably due to warmer soil temperatures in autumn sowing favorable for shoot growth and probably proliferation of bacteria inoculated on tubers in the soil. Although emergence time was the same in spring and autumn planting, average above ground biomass, plant height, number of leaves and stem diameter were higher in autumn than in spring plantings. Warmer soil and air temperatures recorded for autumn were favorable for plant growth and development. Colder soil and air temperatures during January, February and March period as minimum temperatures of 1.0 °C, 6.1 °C and 9.1 °C limited plant growth and probably activity of bacterial growth in spring. Higher tuber yields obtained from microbial fertilizer applied (MFA) plots (26.56 t/ha) in warmer autumn period, which were equal to SFA plots (26.81 t/ha), as opposed to colder spring period (17.55 t/ha), may support the above assumption. MFA plots produced higher tuber yields than control plots in both seasons although spring yields were lower. In this experiment, an equal mixture of *Bacillus* strains were used in order to investigate the possibility of employing environmental friendly alternative fertilization for off-season potato production in coastal areas where relatively warmertemperatures allow plant growth during winter. Further experiment was warranted with more cold tolerant *Bacillus* bacterial strains alone or in mixtures for off season potato production. Selection of bacterial strains that maintain their activity and survive in cold soils may offer new avenue for the use of associative Rhizobacteria in off season potatoproduction. Data here with two seasons indicate that under favorable conditions microbial fertilizers alone produced tuber yields equal to chemical fertilizers. Microbial fertilizers may be also used for organic potato production. SFA ensured higher tuber yields even in colder spring period. Tuber yields were higher in SFA plots in both seasons.Tuber yield was evenhigher in fertilizer applied (SF) plots in spring than in autumn planting.Soil conditions in spring probably favored the slow, effective and longer utilization of nitrogen, phosphorus and potassium applied with chemical fertilizersas opposed to warmer soil temperatures recorded in August planting.This mayalso belinked with lower tuber yields in SFA+MFA plots both in spring and autumn period. Supplementation of chemical fertilizers with microbial fertilizers did not ensure higher tuber yields. Although not measuredin this experiment, competition of bacteria inoculated on tubers for nitrogen, phosphorus and other plant nutrients probably resulted in lower yields in SFA + MFA plots (Madigan et al., 1997; Herrero et al., 2001). In further experiments, survivability of strains and availably of macro nutrients in soil may be followed in order to explain this case.

Plant growth promoting rhizobacteria including *B. subtilis* and *B. amyloliquefaciens* had functions of bioprotection, phytostimulation and biofertilization on potato enhancing plant growth and tuber yields (Aloo et al., 2019). There were plenty of evidence that PGPR had positive effects on potato growth. In our experiment, *B. subtilis* and *B. amyloliquefaciens* culture applied plots had equal yields compared with chemical fertilizer applied plots in warmer August conditions.However, advantage of *B. subtilis* and *B. amyloliquefaciens* application was not seen in colder January plantings. Colder conditions probably restricted activities of *B. subtilis* and *B. amyloliquefaciens*. Psychrophilic bacteria collected from Andean mountains at 4500 above sea level exhibited plant growth promoting properties (Balcazar et al., 2015). Hence, isolation and use of cold tolerant psychrophilic bacteria may offer an alternative strategy for off season potato production during winter period.

Conclusions

A two season experiment with an equal mixture of *B. subtilis* and *B. amyloliquefaciens* strains produced consistently higher tuber yields in off-season potato. Tuber yields in microbial fertilizer applied plots were equal to chemical fertilizer applied plots under more favorable autumn planting, but lower in colder spring planting. Further experiments are warranted with selection and use of psychrophilic bacterial strains that maintain their activity in cold soils for the production of off-season potato production.

Acknowledgements

This work was part of a study presented to Akdeniz University for M.Sc. dissertation thesis by the first author.

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