



Pelleting with Puperabsorbent, Chitosan, and Phosphorus Fertilizer as a New Method to Improve Growth, Yield, and Physiological Attributes of Potato mini-tuber

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ABSTRACT

Seed pelleting is a technique of covering seeds with adhesive agents to improve seed performance and plant establishment while reducing production costs. To evaluate the responses of potato superelite minitubers to different pelleting treatments both qualitatively and quantitatively, a 2-year experiment was conducted based on randomized complete block design with three replications in Mohaghegh Ardabili University, Ardabil, Iran during 2018 and 2019. Experimental treatments included 17 different combinations of zeolite (ZE) or cocopeat (CO) fillers, coating (SC) or soil application (SS) of triple superphosphate, superabsorbent polymer (SP), and chitosan (CH). The results showed a significant effect of pelleting on yield, physiological, and qualitative traits. The highest fresh and dry tuber weight belonged to SS + ZE treatment, which showed 50.6 and 49.0% increase compared to the control treatment (without pelleting), respectively. Pelleting minitubers

by ZE + SS had the highest fresh tuber and biological yield (23.6 and 28.0 ton.ha⁻¹, respectively), which showed an increase of 156.5% and 145.6%, compared to the no pelleting treatment. Most pelleting treatments increased the leaf proline content and activity of the antioxidant enzymes. The highest peroxidase activity and lowest catalase activity (16.1 and 29.6 U.mg protein⁻¹.min, respectively) were observed in the CO+SC+CH treatment. Co-application of ZE, SC, and SP resulted in the highest protein, nitrate, and starch content. In addition, minituber pelleting with ZE+SS+SP increased methionine content significantly by 52.94% compared to the control. In general, the co-application of triple superphosphate, SP, and ZE increased the tuber yield and quality traits. Finally, the use of CH with these compounds resulted in improving physiological characteristics.

Keywords: Antioxidant activity, Methionine amino acid, Proline, Tuber yield, Zeolite

1. Introduction

Potato (*Solanum tuberosum* L.) is a non-grain crop widely cultivated worldwide due to its easy cultivation, nutrient richness, and high production. It is considered as the fourth largest crop after wheat, rice, and corn (Pawelzik & Möller 2014; Wang et al. 2020). Potato is the most crucial tuberous plant which is rich in carbohydrates, proteins, and essential amino acids for humans. Lysine and methionine are essential amino acids for humans not synthesized in the body and should be obtained from other sources. Potatoes are one of the rich sources of these amino acids (Kandi et al. 2012). The area under cultivation, production, and yield of potatoes in Iran was recorded as 148 441 ha, 5142 891 tons, and 34 646 kg/ha, respectively. Ardabil province is the second largest producer of this product after Hamadan province (Anonymous 2018). Among the superior characteristics of Ardabil province, low temperature, sufficient light, and a large difference between day and night temperatures are considered as necessary for the optimal growth in this plant (Song et al. 2014; Khan et al. 2015). The production of potatoes depends on the dynamics of nutrients in the soil and the capacity of the product to absorb nutrients in the soil (Martinet al. 2018) as well as genotype-environment interaction (Mohammadinia et al. 2021). The increasing population has led to an increase in demand for this product around the world. Thus, more attention should be paid to innovation in potato production to increase its quantitative and qualitative characteristics (Glover & Poole 2019; Hacıyusufoğlu 2020).

Seed pelleting is considered as the most useful methods used for improving seed germination and increasing seedling growth, especially under environmental stress (Rocha et al. 2019). Changing the shape of the seed or placing chemical compounds on the seed coat improves and regulates germination and growth, as well as increasing yield (Shinde et al. 2020). Seed pelleting and coating are two types of seed coatings used for commercial application (Taghizoghi et al. 2018). In the seed coating method, a thin layer of various substances such as pesticides, growth regulators, fertilizers, and nutrients and binders was added to the outer surface of the seed without improving the seed shape. Pelleting is one of the methods in which other required materials are used in addition to disinfecting the seeds such as adding nutrients to the seeds and their rounding (Mandal et al. 2015). Seed pelleting

improves the external structure of the seed and makes it uniform, which results in sowing seeds accurately. Moreover, seed priming and pelleting can increase the quality of seed, leading to an improvement in seedling growth power and reducing production costs (Szerement et al. 2014; Karagoz & Yucel 2020).

Potato minituber was produced *in vitro* conditions, ranging from 0.1 to 10 g or more, 4 to 7 mm diameter, and 10 to 12 mm length. Therefore, the cost of these tubers for planting is higher than that of the typical seed (Çalışkan et al. 2020). Pelleting those using nutrients, growth regulators, and inert substances is considered as a method for increasing the size of this minituber to be acceptable for cultivation in field conditions (Ravichandran et al. 2015). This method increases the weight and volume of tuber and standardizes the size of seed (Rykaczewska 2016). Some studies focused on pelleting potato seed and developed countries refused to release useful compounds or methods. In one study, two small potato tuber cultivars were pelleted using different combinations. Based on the results, the use of Acacia leaf powder increased germination, growth, and yield indices, including the number of tubers (Ravichandran et al. 2015). Pelleting with chitosan (CH) increased the chlorophyll content and catalase and peroxidase enzyme activities in peppers (Kheiri et al. 2016). Peanut seed pelleting with salicylic acid composition increased the quantitative and qualitative traits of this plant by increasing the activities of various enzymes (Dong et al. 2019). The use of salicylic acid and ethylene for pelleting and granulation improved the germination and growth indices of sugar beet (Pirasteh-Anosheh & Emam 2019).

Due to an increase in food per capita and calorie consumption, attempts were made to produce more agricultural products globally, especially in developing countries (Aksoy & Beghin 2004). Potatoes are the most critical nutrient source in nutritional importance due to the presence of essential amino acids, vitamins, and minerals in human nutrition. Due to the diverse distribution pattern of potatoes, this product has provided food security as a strategic plant in the world and Iran, especially in deprived areas. Therefore, innovations based on potato planting science leading to an increase in quantitative traits in line with qualitative characteristics are of considerable importance (Beumer et al. 2021). The present experiment aims to determine yield, physiological, and qualitative responses of potato minitubers to different pelleting treatments with zeolite (ZE), cocopeat (CO), triple superphosphate, CH, and superabsorbent (SP).

2. Material and Methods

2.1. Plant material and experimental design

To evaluate the growth, yield, physiological, and qualitative responses of potato minituber to different pelleting treatments under field conditions, an experimental randomized complete block design with three replications in the field of agricultural research and Natural resources of Mohagheh Ardabili University located North-West of Iran (38°15' N and 48°20' E and altitude of 1350 m above sea level) were conducted during 2017-2018. Figure 1 displays monthly climatic parameters recorded at a weather station located adjacent to the experimental site for two years.

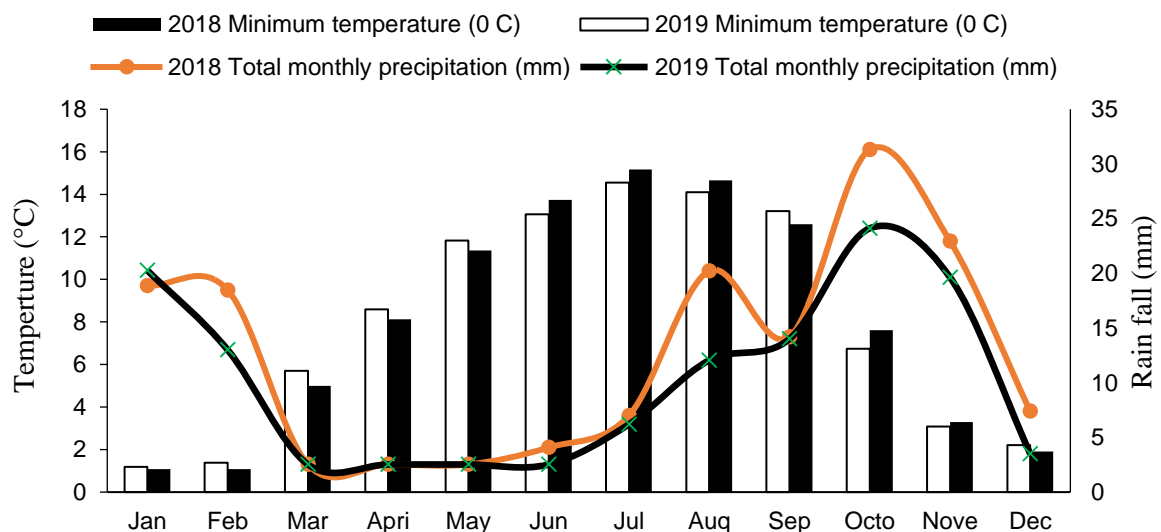


Figure 1-Climatic conditions (temperature and precipitation) during the growing season 2018 and 2019

Experimental treatment included 17 treatments (using triple superphosphate fertilizer as a coating (SC) or soil application (SS, ZE, CO, SP, and CH) were tested in combination with the control treatment (without pelleting) (Table 1). In this study, Agria cultivar was used. This cultivar is obtained from a cross between Quarta × Semlo cultivars, which has some characteristics such as medium late maturity, high yield, tuber elongated, high dry matter percentage, rapid foliage development, high height, and susceptible to tuber tuberculosis and potato 'Y' virus. The growth period of the Agria cultivar is 120 to 130 days.

Table 1- Combination of experimental treatments in the present study

<i>Treatment</i>	<i>Combination</i>	<i>Treatment</i>	<i>Combination</i>
T1	SP + CH + SC + ZE	T10	CH + SC + CO
T2	CH + SC + ZE	T11	SP + SC + CO
T3	SP + SC + ZE	T12	SC + CO
T4	SC + ZE	T13	SP + CH + SS + CO
T5	SP + CH + SS + ZE	T14	CH + SS + CO
T6	CH + SS + ZE	T15	SP + SS + CO
T7	SP + SS + ZE	T16	SS + CO
T8	SS + ZE	T17	Control (without pelleting)
T9	SP + CH + SC + CO		

ZE: Zeolite; CO: Cocopeat; SC: Superphosphate coating; SS: Soil application of superphosphate; SP: Superabsorbent; CH: Chitosan

2.2. Minutuber pelleting method

Potato minitubers were prepared from Dasht-e Zarrin Company in Ardabil province and placed in a warehouse with indirect light at 18-20 °C for one week to germinate. To prepare the CH solution (Sigma-Aldrich Company, USA), a 1% solution of acetic acid was designed and placed on a magnetic stirrer. Then, solutions of 5 g per liter of CH were prepared. In addition, chitosan powder was added slowly at 60 °C. After stirring for five to six hours when all of the CH particles were dissolved and the solution was clear, about 25% by the weight of the CH powder used glycerol (as Plasticizer) was added to the solution. In this study, Arabic gum was used as an adhesive for minitubers. To prepare a solution of Arabic gum, 1.5 l of water was exposed to a temperature of 70 °C, and then 50 g of Arabic gum was added to each liter of water was added, which was utterly powdered and sifted in a mortar to the solution. Further, it was placed on a magnetic stirrer (half an hour) (Shaddel et al. 2018). In the next stage, the pelleting material was applied. First, the tubers were impregnated with Arabic gum and then covered with ZE or CO and placed in a laboratory environment at the room temperature for about 25 °C for one day to be dried. After ensuring the tubers to dry, the tubers were covered with triple superphosphate powder in the next day using an electric mixer. Based on the experimental treatments, the tubers which were not covered with superphosphate were added to the soil. Finally, CH and SP were applied to the minitubers based on experimental treatments, and thus the pelleting process was completed. Pelleting with different compounds was done to create a layer of coating on the surface of the tuber and the whole surface was impregnated with the desired composition, which was easily done due to the use of Arabic gum as adhesive. After ensuring drying, the pelleted minitubers were transferred to the field for planting. The weight of each plate was considered to be about 0.1 to 0.2 g. Planting was conducted with a distance between plants of 25 cm, a distance between rows of 75 cm, and a planting depth of 5-10 cm in five rows of cultivation ($2 \times 3 \text{ m}^2$) was conducted on 30 April and 2 May for first and second years, respectively.

After planting, in addition to fertilizing based on soil test in Table 2, 180 kg nitrogen per ha in two stages from urea source, 120 kg phosphorus per ha from pentoxide phosphorus at planting, and 150 kg potassium per ha from potassium sulfate source), the operation included irrigation and weed control (manually three times), insects, and pests. Irrigation was conducted according to the needs of the plant and a total of eight irrigations. To control the deciduous pest of Colorado potato beetle, once spraying with Zolon herbicide at two liters per hectare rate was conducted 75 days after planting.

Table 2- Physico-chemical characteristics of experimental soil at depths of 0 to 30 cm (on average)

<i>Soil texture</i>	<i>Sand</i>	<i>Silt</i>	<i>Clay</i>	<i>Organic carbon (%)</i>	<i>Total Nitrogen (mg.kg^{-1})</i>	<i>Available Phosphorous (mg.kg^{-1})</i>	<i>Available Potassium (mg.kg^{-1})</i>	<i>Electrical conductivity (dS.m^{-1})</i>	<i>pH</i>
Sandy loam	52	22	26	1.17	1012	6.1	198	2.68	7.09

2.3. Yield and yield component parameters

Tuber yield and yield components such as the number of tubers, fresh and dry (70 °C for 48 h) tuber weight, and biological yield (shoots + roots + tubers weight) were calculated and measured from the middle three rows while ignoring border effects and ten plants were randomly selected. The harvest time was on 5 and 9 August in the first and second years, respectively.

2.4. Chlorophyll index and leaf area index (LAI)

After the flowering stage, the chlorophyll index was performed by manual chlorophyll meter (SPAD 502 PLUS model, England), and the LAI was measured by leaf area meter (CI202 model, USA) (Roosjen et al. 2018).

2.5. Proline content

Leaf-free proline content was determined by using Bates et al. (1973) method. First, 0.5 g of the fresh leaf was ground with quarts in porcelain pestle and mortar and treated with 10 mL sulfosalicylic acid. The homogenate was centrifuged at 13000 g for

10 min. The red surface layer and standard samples were simultaneously placed in a spectrophotometer, and the absorption of the samples was determined at the wavelength of 520 nm. The amount of proline content ($\mu\text{mol g}^{-1}$ FW) was calculated using regression equations and the standard curves.

2.6. Extraction and assay of enzymatic antioxidant activities

Antioxidant enzyme extraction was determined using the method of Sunohara & Matsumoto (2004) with slight modifications. First, 200 mg of leaves were sliced and homogenized in 10 mL of 25 mM K-P buffer (pH 7.8) containing one mM AsA, 0.4 mM EDTA, and 2% PVPP at four °C for 1 min. The homogenate solution was centrifuged at 15,000 g at 4 °C for 20 min, and the supernatant was filtered through filter paper (Whatman® No.1, England). The filtrate was collected to determine the activities of catalase (EC 1.11.1.6) and peroxidase (EC 1.11.1) enzymes (Ghahremani et al. 2019).

Catalase activity was determined based on Sunohara and Matsumoto's (2004) method with some modifications. First, it was assayed in a 2 mL reaction mixture including 1.9 mL of 50 mM K-P buffer (pH 7.0) containing 25 mM H_2O_2 and 0.1 mL of enzyme extract for 1 min. Then, CAT activity was measured in terms of the decomposition of H_2O_2 at 240 nm and activity calculated per leaf protein for 1 min.

In the next procedure, peroxidase activity was assayed by the method proposed by Chance & Maehly (1955). An alcoholic liquid of the tissue extract (100 μl) was added to 3 mL of assay solution including 3 mL of reaction mixture containing 13 mM guaiacol, five mM H_2O_2 , and 50 mM sodium (Na)-phosphate (pH 6.5). An increase in the optical density at 470 nm for 1 min at 25 °C was recorded using a spectrophotometer.

2.7. Tuber quality characteristics

The method of Kandi et al. (2012) was used for measuring qualitative traits including the percentage of protein, lysine, and methionine. Noda et al. (1995) method was applied for determining the tuber starch content using the phenol-sulfuric acid method. In addition, nitrate was measured by the proposed method of Errebhi et al. (1998).

2.8. Statistical analysis

After checking the normal distribution of the data by Kolmogorov-Smirnov and Shapiro-Wilk test, using Statistical Analysis System software (SAS Institute, Cary, NC, USA, ver. 9.2) was used for statistical analyses. When the result of ANOVA showed a significant treatment effect, the least significant difference (LSD) test was applied to compare the means at 0.05 probability level. Simple correlations between traits were performed using SAS software. Principal component analysis (PCA) and cluster analysis were performed by Ward method using Minitab statistical software (ver. 19).

3. Results and Discussion

3.1. Effect of minituber pelleting on yield and yield components

As shown in Table 3, mixed ANOVA of 2-year data indicated a significant effect of minituber pelleting on yield and yield components such as fresh and dry tuber weight, number of tuber per plant, tuber and biological yield ($P \leq 0.01$). The highest fresh and dry tuber weight (487.6 and 69.9 g, respectively) belonged to T8 (SS+ZE), which showed an increase of 102.6% and 96.3% compared to the control treatment (without pelleting). The lowest these traits were related to T17 (without pelleting) by 240.7 and 35.6 g, respectively (Table 3). In this regard, Bahador et al. (2015) reported that ZE application leads to an increase in the available moisture in the seeds, which results in increasing root moisture and uptake rate, and transferring nitrogenous substances. Bybordi (2016) indicated that the significant effect of ZE on plant height, leaf number, dry weight, and seed yield of rapeseed leading to an increase in the average of these traits.

Table 3- Comparison of two-year means of yield and yield components of potato minituber by different pelleting treatments

	<i>Fresh tuber weight (g.plant⁻¹)</i>	<i>Dry tuber weight (g.plant⁻¹)</i>	<i>Number of tuber per plant</i>	<i>Tuber yield (ton.ha⁻¹)</i>	<i>Biological yield (ton.ha⁻¹)</i>
Year					
First	366.45±91.47 a	51.96±11.52 a	7.35±1.78 b	17.21±4.52 a	20.45±5.08 a
Second	343.26±91.55 a	53.32±15.88 a	8.67±2.28 a	19.04±5.67 a	22.21±6.33 a
LSD ($\alpha=0.05$)	24.17	2.77	0.51	1.95	2.46
Treatments					
T1	319.7±13.1 ef	46.1±2.5 fg	7.3±0.4 de	16.3±0.6 de	19.1±0.8 de
T2	361.6±49.7 b-f	60.7±8.2 bc	7.1±0.5 ef	17.9±2.6 cde	21.7±3.1 b-e
T3	350.2±23.2 b-f	57.6±6.1 bcd	7.7±1.2 cde	17.8±1.6 cde	21.4±1.8 cde
T4	329.4±11.5 def	48.1±2.5 efg	7.6±0.8 de	16±1.6 de	19±1.7 de
T5	296.8±16.5 fg	42.3±2.7 gh	7.3±0.9 def	15.3±1.4 e	17.9±1.5 e
T6	340±25.6 c-f	52.7±4.8 c-f	8.7±1.0 bcd	18.6±2.2 b-e	21.9±2.5 b-e
T7	333.8±16.7 c-f	49.7±3.2 d-g	8.4±0.8 b-e	18.7±1.7 b-e	21.8±1.8 b-e
T8	487.6±35.1 a	69.9±7.6 a	8.2±0.7 b-e	23.6±2.9 a	28.0±3.4 a
T9	349.4±31 b-f	51±3.5 def	11.2±1.0 a	20.5±1.9 abc	23.7±2.0 bc
T10	344.2±39.6 c-f	46.8±3.9 fg	7.8±1.1 b-e	18.5±1.7 b-e	21.4±1.8 cde
T11	415.4±37.2 b	61.3±4.2 b	7±0.6 ef	22±1.7 ab	25.8±1.9 ab
T12	375.3±25.1 b-e	53.8±5.5 b-f	9.2±1.3 b	20.1±2.8 a-d	23.4±3.1 bc
T13	344.3±37.7 c-f	51±3.9 def	7.9±0.7 b-e	20±2.2 a-d	23.2±2.2 bcd
T14	396.7±51.3 bcd	59.3±2.8 bc	9.1±0.3 bc	18±1.8 b-e	21.7±1.9 b-e
T15	344±31.1 c-f	53.9±4.6 b-f	8.4±0.6 b-e	17.2±1.6 cde	20.5±1.8 cde
T16	403.6±65.1 bc	55.2±7.0 b-e	7.8±0.4 b-e	18.8±1.9 b-e	22.2±2.3 bcd
T17 (control)	240.7±15.8 g	35.6±2.8 h	5.8±0.3 f	9.2±0.7 f	11.4±0.8 f
LSD ($\alpha=0.05$)	70.4	8.07	1.49	4.09	4.25

Means followed by the same letter in each column are not significantly different according to the LSD test.

In addition, a considerable difference was observed in the number of tubers per plant under the effect of pelleting treatments, among which T9 (SP+CH+SC+CO) had the highest number of tubers per plant (11.2 number), which showed an increase of 93.1% compared to the control (Table 3). Further, most minituber pelleting treatments resulted in a significant increase in the fresh tuber yield and biological yield. The highest fresh tuber and biological yield was reported in T8 (ZE+SS) by 23.6 and 28.0 ton.ha⁻¹, respectively, which indicated an increase of 61.0 and 59.2% compared to the control treatment, respectively. The without pelleted minituber led to the lowest tuber and biological yield by 9.2 and 11.4 ton.ha⁻¹, respectively. Due to the developed root system, potatoes need a lot of nutrients to produce high yields and larger tubers, which the soil alone cannot provide the elements required by the plant (Fernandes et al. 2017). The use of organic and mineral fertilizers can have a significant impact on potato yield. Phosphorus is one of the most restricted biomass elements present in less than one percent soluble in the soils of different regions depending on climate (Alemayehu et al. 2020). Pelleting minituber with triple superphosphate increased the yield and yield components. A balanced supply of phosphorus led to an increase in yield and phosphorus uptake in the potato plant (Soratto & Fernandes 2016). Furthermore, phosphorus availability affects the uptake and concentration of other macro- and micro-elements which can affect yield (Fernandes et al. 2017). Generally, the seed pelleting by triple superphosphate has a significant advantage in yield traits and yield components compared to its soil application. It seems that more water uptake by seeds and access to elements are considered as the factors related to the superiority of pelleting compared to the soil application of superphosphate (Kataki et al. 2016).

In the present study, the application of ZE led to an increase in yield and yield components. Zeolites are among the minerals which are significantly present in Iran, the unique property is to increase cation exchange capacity and selective bonding for ammonium and potassium to improve soil structure (Alshameri et al. 2014; Ghasemi et al. 2018). Some researchers indicated that adding ZE to the soil by changing the soil permeability facilitates water movement in the soil improves vegetative growth and increases the secondary metabolite in Amaranths (Karami et al. 2020). Zeolites with a very porous structure and an extensive inner surface stabilize the nutrients in their structure, the gradual release of which provides these nutrients for the plant for a long time, which results in increasing efficiency and ability, and accordingly yield (Jakkula and Wani 2018). The application of ZE-based on the review of sources led to an increase in yields of many crops including potatoes (13%), canola (33%), rice (13%), and rapeseed (89%) (Aghaalikhani et al. 2012; Mohammadi & Rokhzadi 2012; Heydari et al. 2017). Some researchers reported that superabsorbent polymers affect the rate of water content in soil, specific gravity, and soil structure, and the evaporation rate from the soil surface leading to morpho-physiological changes in the plant (Khan et al. 2018; Bagherifard et al. 2020). It seems that the co-application of ZE and superabsorbent led to an improvement in water availability and soil physical characteristics, as well as increasing growth and yield parameters. The maximum use of resources and optimal growth conditions due to the availability of resources can be a significant factor in improving growth and yield parameters (Barnett & Morse 2013).

3.2. Effect of minituber pelleting on physiological characteristics

Physiological characteristics such as chlorophyll index, LAI, proline content, and activity of peroxidase and catalase enzymes are significantly affected by pelleting treatments. Increasing the chlorophyll content in the leaves can help researchers in the production of plants with higher photosynthetic power. The highest chlorophyll index is related to T7 (ZE+SS+SP) by 48.4, leading to an increase of 24.1% compared to the without pelleting treatment. Further, T1, T2, T3, T4, T6, T9, T10, T11, T14, and T16 treatments were in the same statistical group with treatment T7, indicating the highest means of this trait. Control treatment (without pelleting) had the lowest chlorophyll index by 39.0 (Table 4). The chlorophyll content is closely related to nitrogen availability and synthesis chlorophyll increased in the use of triple superphosphate fertilizer, ZE, and SP due to the key role of phosphorus in the structure of enzymes and the role of ZE in reducing the leaching of elements, especially nitrogen as one of the main elements in chlorophyll structure (Heydari et al. 2017).

Table 4- Comparison of two-year means of physiological traits of potato by different pelleting treatments

	<i>Chlorophyll index</i>	<i>LAI</i>	<i>Proline content</i> ($\mu\text{mol g}^{-1}$ FW)	<i>Peroxidase activity</i> (U mg protein ⁻¹ min)	<i>Catalase activity</i> (U mg protein ⁻¹ min)
Year					
First	44.48±3.18 b	2.79±0.21 a	237.86±20.31 b	14.48±2.26 a	37.26±10.53 a
Second	47.55±3.53 a	2.95±0.24 a	255.78±25.13 a	14.66±2.30 a	49.51±9.94 a
LSD ($\alpha=0.05$)	0.95	0.54	6.88	0.66	2.05
Treatments					
T1	47.7±1.8abc	2.92±0.1 a-d	257.7±6.9 ab	12.0±0.3gh	43.7±2.9bc
T2	47.8±1.0 abc	2.93±0.11 a-d	259.3±8.1 a	16.2±0.6 a	36.4±2.0 def
T3	48.1±1.6 ab	2.90±0.09 a-d	268.0±8.0 a	14.3±0.7 a-f	33.0±2.2 e-h
T4	46.7±0.8 a-f	2.89±0.16 a-d	262.8±7.1 a	11.5±0.4 h	52.4±2.6 a
T5	45.2±0.9 c-f	2.99±0.07abc	268.6±7.8 a	13±0.6 d-h	30.3±1.0 gh
T6	46.7±1.8 a-f	2.76±0.08 d	255.4±10.1 ab	11.9±0.4gh	29.4±2.2 h
T7	48.4±1.7 a	2.96±0.12 a-d	257±7.5 ab	13.6±0.8 b-g	36.3±1.6 d-g
T8	44.1±1.4ef	3.02±0.1 ab	262.8±9.6 a	15.9±0.6 a	30.9±2.4fgh
T9	47.6±1.0 abc	2.95±0.08 a-d	267.6±9.3 a	13.2±0.4 c-h	41.2±1.9bcd
T10	46.6±1.4 a-f	2.93±0.04 a-d	254.7±6.4 ab	16.1±0.3 a	29.6±1.4 h
T11	47.4±1.5 a-d	2.81±0.08 cd	251.4±6.6 ab	14.6±0.9 a-e	42.5±1.1bc
T12	44.0±1.9 f	2.88±0.1 a-d	238.4±7.1bc	12.7±0.4 e-h	44.5±2.4 b
T13	45.4±1.3 b-f	3.06±0.1 a	223.7±4.9 cd	15.5±0.7 ab	50.9±2.8 a
T14	46.8±1.0 a-d	2.84±0.07bcd	257.3±10.3 ab	15.0±0.9abc	41.9±3.4bcd
T15	44.5±0.9 def	2.83±0.06bcd	238.1±5.9bc	12.5±0.7fgh	40.3±1.9bcd
T16	46.9±1.0 a-d	2.91±0.07 a-d	259.1±7.1 a	14.8±0.8 a-d	38.3±1.6cde
T17 (control)	39.0±0.8 g	2.38±0.14 e	210.6±4.2 d	15.3±0.5 ab	32.5±0.8 e-h
LSD ($\alpha=0.05$)	2.78	0.21	20.07	1.94	6.00

Means followed by the same letter in each column are not significantly different according to the LSD test.

As shown in Table 4, the results indicated that the pelleting treatments significantly increased LAI. The average LAI was observed from 2.38 to 3.06. The highest LAI was related to T13 (CO+SS+CH+SP) by 3.06 and the lowest mean (2.38) was related to without pelleting treatment. The results showed no significant difference between the applications of ZE and CO in terms of LAI. In addition, the presence of triple superphosphate is necessary for increasing the LAI. Further, CH plays a significant role in increasing the average physiological traits such as LAI, proline content, and antioxidant enzymes. Chitosan, as a polysaccharide-based edible coating, has been successfully used to coat seed or fresh-cut fruits (Olawuyi et al. 2018). Chitosan action mechanism is most likely done by sending signals to synthesize plant hormones such as gibberellin and auxin, leading to some changes in physiological traits (Jiao et al. 2012; Katiyar et al. 2015). In this regard, some reported that the use of CH led to an increase in growth parameters of the potato plant by strengthening the defense mechanisms of enzymatic (antioxidant enzymes) and non-enzymatic (proline, carotenoids, etc.) (Muley et al. 2019).

Based on the results, the pelleting treatments increased the leaf proline content. The average proline content was observed from 210.6 to 268.6 $\mu\text{mol.g}^{-1}$ FW. As a result, the highest free proline content was obtained from T1-T11, T14, and T16. Moreover, the lowest proline content was T17 (control) by 210.6 $\mu\text{mol.g}^{-1}$ FW. In addition to increasing cellular potential, proline amino acid is involved in stabilizing the intracellular structure, redox potential of the cellular buffer, and eliminating free radicals (Muley et al. 2019). Further, proline may act as a protein-compatible hydrotrope, which results in reducing cytoplasmic acidity while maintaining the NADP⁺/NADPH ratios specified for cellular metabolic pathways (Ashraf and Foolad 2007). In another study, CH led to an increase in proline content in the potato plant (Muley et al. 2019), which is consistent with the findings of the present study.

Regarding the means, the peroxidase activity is more affected by pelleting treatments than the catalase enzyme. Further, different results were found in these two enzymes response to pelleting treatments so that the highest peroxidase activity (16.2 U.mg protein⁻¹.min) were observed in T2 (ZE+SC+CH) treatment and the lowest catalase activity (29.4 U.mg protein⁻¹.min)

were observed in T6 (ZE+SS+CH) treatment. The mean of peroxidase and catalase activities increased from 11.5 to 16.2 and 29.4 to 52.4 U.mg protein⁻¹.min, respectively. The highest activity of peroxidase and catalase enzymes showed an increase of 5.88% and 61.23% compared to the treatment without pelleting. The highest catalase activity was related to T4 (ZE+SC) and T13 (CO+SS+CH+SP) by 52.4 and 50.9 U.mg protein⁻¹.min, respectively (Table 4). The results of the present study indicated the increasing effect of CH on the activity of antioxidant enzyme peroxidase and catalase. In this regard, Loy et al. (2019) reported that CH pelleting treatment activated antioxidant enzyme activities of Thompson seedless.

3.3. Effect of minituber pelleting on tuber quality traits

Table 5 shows minituber quality characteristics such as protein, methionine, lysine, soluble carbohydrate, nitrate, and starch contents significantly affected by pelleting treatments ($p < 0.01$). The highest and lowest protein content (5.33 and 3.18%) was observed in T3 (ZE+SC+SP) and T13 (CO+SS+CH+SP) treatments, respectively. In the T3 treatment, an increase of 50.14% protein content occurred compared to the treatment without pelleting. Regarding the mean comparison of pelleting treatments, the highest and lowest methionine amino acid content was found in T1 and T15 (0.26 and 0.16%), respectively. The application of T1 (SP+CH+SC+ZE) treatment significantly increased methionine content by 52.94% compared to the control treatment (without pelleting) (Table 5). The average of tuber lysine content ranged from 0.37 to 0.57%. The highest lysine content was related to T1 and T12 (0.56 and 0.57%). T12 treatment significantly increased lysine content by 42.5%, compared to the treatment without pelleting (Table 5). Co-application of ZE, SC, and SP (T3 treatment) resulted in the highest average nitrate and starch content (239.7 mg.kg⁻¹ and 17.2%), respectively, which showed an increase of 16.52% and 32.30% compared to the control treatment. The lowest nitrate and starch content were related to T13 (146.6 mg.kg⁻¹ and 12.4%), respectively (Table 5). Any change in nitrogen uptake and metabolism directly affects the rate of nucleic acid and protein synthesis. Taheri-Soudejani et al. (2019) reported that the availability of phosphorus in the soil is adequate on nitrogen uptake and plant use and ZE can reduce nitrogen leaching and increase plant availability. This mechanism increased the minituber protein and the content of lysine and methionine amino acids using triple superphosphate and ZE. In this regard, some reported similar results for other plant species (Gholamhoseini et al. 2013).

Table 5- Comparison of two-year means of the quality of potato minituber by different pelleting treatments

	<i>Protein content (%)</i>	<i>Methionine content (%)</i>	<i>Lysine content (%)</i>	<i>Soluble carbohydrate (mg.g⁻¹ FW)</i>	<i>Nitrate content (mg.kg⁻¹)</i>	<i>Starch content (%)</i>
Year						
First	4.23±0.75 a	0.18±0.03 b	0.44±0.08 b	11.52±1.80 a	196.8±30.5 a	14.4±2.05 a
Second	4.09±1.0 a	0.25±0.04 a	0.47±0.06 a	11.81±1.37 a	201.6±39.9 a	14.3±2.82 a
LSD ($\alpha=0.05$)	0.22	0.009	0.021	0.38	6.62	0.60
Treatments						
T1	4.78±0.16 ab	0.26±0.02 a	0.56±0.03 a	12.8±0.3abc	215.0±7.4 b	12.4±0.4 g
T2	4.67±0.30 b	0.2±0.02 e-h	0.43±0.02 def	10.7±0.5ef	210.2±13.9 b	16.1±0.8 ab
T3	5.33±0.45 a	0.21±0.02 c-g	0.50±0.03abc	10.4±0.4 f	239.7±15.7 a	17.2±1.8 a
T4	4.76±0.25 ab	0.23±0.02 b-e	0.48±0.03bcd	12.2±0.3bcd	214.4±6.7 b	14.9±0.8bcd
T5	4.64±0.30 b	0.23±0.02bcd	0.49±0.03bcd	12.6±0.3 a-d	208.9±7.5 b	15.2±0.7bcd
T6	4.18±0.23 b-e	0.19±0.01ghi	0.43±0.02 d-g	11.7±0.5cde	188.1±8.5cde	13.6±0.8 d-g
T7	4.14±0.17 b-e	0.21±0.02 c-g	0.47±0.02 b-e	11.6±0.5 de	186.1±5.4 de	13.1±0.6efg
T8	4.53±0.15bc	0.22±0.02 b-f	0.48±0.01bcd	10.8±0.4ef	203.8±3.9bcd	15.3±0.4bcd
T9	4.46±0.28bcd	0.21±0.02 d-g	0.44±0.02 c-f	12.6±0.6 a-d	199.0±6.4 b-e	15.5±0.7abc
T10	4.63±0.24bc	0.21±0.02 c-g	0.41±0.01efg	13.0±0.3 ab	208.1±11.2 b	15.9±1.0 abc
T11	3.88±0.24 de	0.19±0.01ghi	0.37±0.02 g	12.2±0.5bcd	182.9±9.3 e	14.1±0.5 c-g
T12	3.99±0.50cde	0.24±0.02 ab	0.57±0.01 a	13.3±0.5 ab	188.1±18.4cde	14.4±1.6 b-f
T13	3.18±0.34 f	0.19±0.02fgh	0.42±0.02efg	10.1±0.3 f	146.6±13.6 f	12.4±0.5 g
T14	4.57±0.28bc	0.24±0.02abc	0.51±0.02 ab	9.8±0.4 f	205.5±8.7bc	15.7±0.9abc
T15	4.38±0.44bcd	0.16±0.01i	0.41±0.02fg	13.7±0.5 a	197.1±19 b-e	16.0±1.2 ab
T16	4.44±0.27bcd	0.22±0.02 b-f	0.47±0.02 b-e	13±0.2 ab	201.3±9.3 b-e	14.8±0.8 b-e
T17						
(control)	3.55±0.35ef	0.17±0.02 hi	0.40±0.01fg	10.4±0.4 f	205.7±8.6bc	13.0±0.6fg
LSD ($\alpha=0.05$)	0.67	0.028	0.062	1.12	19.30	1.76

Means followed by the same letter in each column are not significantly different according to the LSD test.

As shown in Tables 3, 4, and 5, the number of tubers, chlorophyll index, proline content, methionine and lysine content increased significantly during the second year by 17.96%, 6.90%, 7.53%, 38.8%, and 6.82% compared to the first year. It seems

that favorable climatic conditions in the second year, especially in terms of temperature and rainfall, led to an increase in the average growth, yield, and quality traits (Porter & Semenov 2005).

3.4. Simple correlation, cluster, and principal component analysis

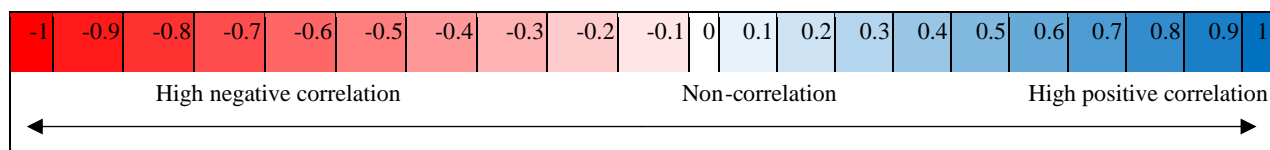
Table 6 indicates the results of simple correlations between yield, physiological, and qualitative traits. The tuber yield was positively and significantly correlated with fresh and dry tuber weight, number of tubers, biological yield, and LAI, while it was negatively correlated with methionine content. Lysine content as important qualitative traits in potatoes was positively correlated with soluble carbohydrate and starch content. In addition, positive and negative correlations were observed between the yield and morpho-physiological traits of potato and tuber yield had a positive correlation with yield components and tuber quality traits (Petros & Zelleke 2013).

Table 6- Simple correlation coefficients (Pearson) between yield, physiological, and qualitative traits of potato minituber under the influence of different seed pelleting treatments

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2	0.99**														
3	-0.67**	-0.67**													
4	0.59**	0.59**	0.31*												
5	0.59**	0.59**	0.08 ns	0.98**											
6	0.08 ns	0.09 ns	0.07 ns	0.09 ns	0.09 ns										
7	0.28*	0.28*	-0.07 ns	0.29*	0.29*	-0.03 ns									
8	0.23 ns	0.24 ns	-0.05 ns	0.25 ns	0.25 ns	0.28*	0.16 ns								
9	0.10 ns	0.10 ns	-0.01 ns	0.13 ns	0.14 ns	-0.16 ns	-0.31*	0.31*							
10	0.20 ns	0.21 ns	-0.40*	-0.10 ns	-0.10 ns	-0.22 ns	-0.11 ns	-0.20 ns	-0.34**						
11	-0.04 ns	-0.04 ns	-0.03 ns	-0.08 ns	-0.07 ns	0.29*	0.12 ns	0.03 ns	-0.09 ns	0.12 ns					
12	-0.33*	-0.33*	0.08 ns	-0.30*	-0.31*	-0.03 ns	0.02 ns	0.08 ns	0.43*	-0.30*	-0.45**				
13	-0.21 ns	-0.21 ns	-0.04 ns	-0.19 ns	-0.20 ns	-0.09 ns	0.15 ns	-0.09 ns	0.25 ns	0.13 ns	-0.25 ns	0.15 ns			
14	0.03 ns	0.03 ns	-0.02 ns	-0.08 ns	-0.09 ns	-0.04 ns	0.13 ns	0.06 ns	0.31*	-0.06 ns	-0.21 ns	0.13 ns	0.82**		
15	-0.04 ns	-0.04 ns	-0.03 ns	-0.08 ns	-0.07 ns	0.29*	0.12 ns	0.01 ns	-0.13 ns	0.01 ns	-0.32*	0.52**	-0.03 ns	-0.12 ns	
16	-0.15 ns	0.22 ns	0.33*	-0.10 ns	-0.09 ns	-0.10 ns	0.08 ns	0.07 ns	0.09 ns	0.09 ns	0.71**	0.31*	0.29*	0.86**	-0.32*

ns: non-significant; * and **: significant at the 5 and 1% probability level, respectively

1: fresh tuber weight; 2: dry tuber weight, 3: number of tuber per plant; 4: tuber yield; 5: biological yield; 6: chlorophyll index; 7: LAI; 8: proline content; 9: peroxidase activity; 10: catalase activity; 11: protein content; 12: methionine content; 13: lysine content; 14: soluble carbohydrate; 15: nitrate content; 16: starch content.



The treatment (cluster analysis) was classified for decreasing the number of experimental treatments and determining the most effective treatment. As shown in in Table 7 and Figure 2, 33 treatments were classified into three clusters, the first cluster included 11 treatments (T1, T2, T3, T4, T5, T6, T7, T9, T10, T13, and T15), the second had 5 treatments (T8, T11, T12, T14, and T16), and the control (T17) as the third cluster. The first cluster in terms of physiological characteristics such as chlorophyll index, LAI, proline, protein and soluble content, and the second cluster in terms of yield and yield components and tuber quality characteristics including fresh and dry tuber weight, tuber and biological yield, methionine and lysine content had the highest coefficients. A principal component analysis was performed to understand the correlation between characteristic and important traits (PCA) better (Figure 3). Based on the results, the first component could be explained by about 30% of the changes including proline, starch, nitrate, and protein content. Regarding the second component, 22% of the changes could be explained by the number of tubers, methionine, and lysine content. Further, the first component is called the physiological component, and the second is the qualitative component, which justifies 52% of the changes. Similar results were reported for the potato plant by other researchers (Deperi et al. 2018).

Table 7- Cluster analysis and grouping of studied variables of potato minitubers under the influence different pelleting treatments (Highest values are underlined)

Variable	Cluster1	Cluster2	Cluster3	Grand centroid
Fresh tuber weight	337.576	<u>415.709</u>	240.720	354.859
Dry tuber weight	50.889	<u>59.898</u>	35.632	52.641
Number of tubers	8.121	<u>8.239</u>	5.806	8.020
Tuber yield	17.875	<u>20.480</u>	9.213	18.132
Biological yield	21.056	<u>24.223</u>	11.440	21.422
Chlorophyll index	<u>46.792</u>	45.814	39.008	46.047
LAI	<u>2.919</u>	2.890	2.380	2.879
Proline	<u>255.715</u>	253.796	210.600	252.497
Peroxidase	13.613	14.597	<u>15.282</u>	14.001
Catalase	38.512	<u>39.639</u>	32.507	38.491
Protein	<u>4.468</u>	4.281	3.551	4.359
Methionine	0.210	<u>0.222</u>	0.173	0.211
Lysine	0.460	<u>0.480</u>	0.399	0.462
Soluble carbohydrate	<u>11.941</u>	11.836	10.420	11.821
Nitrate	201.219	196.330	<u>205.740</u>	200.047
Starch	14.748	<u>14.850</u>	12.969	14.674
Number of treatments	11	5	1	-

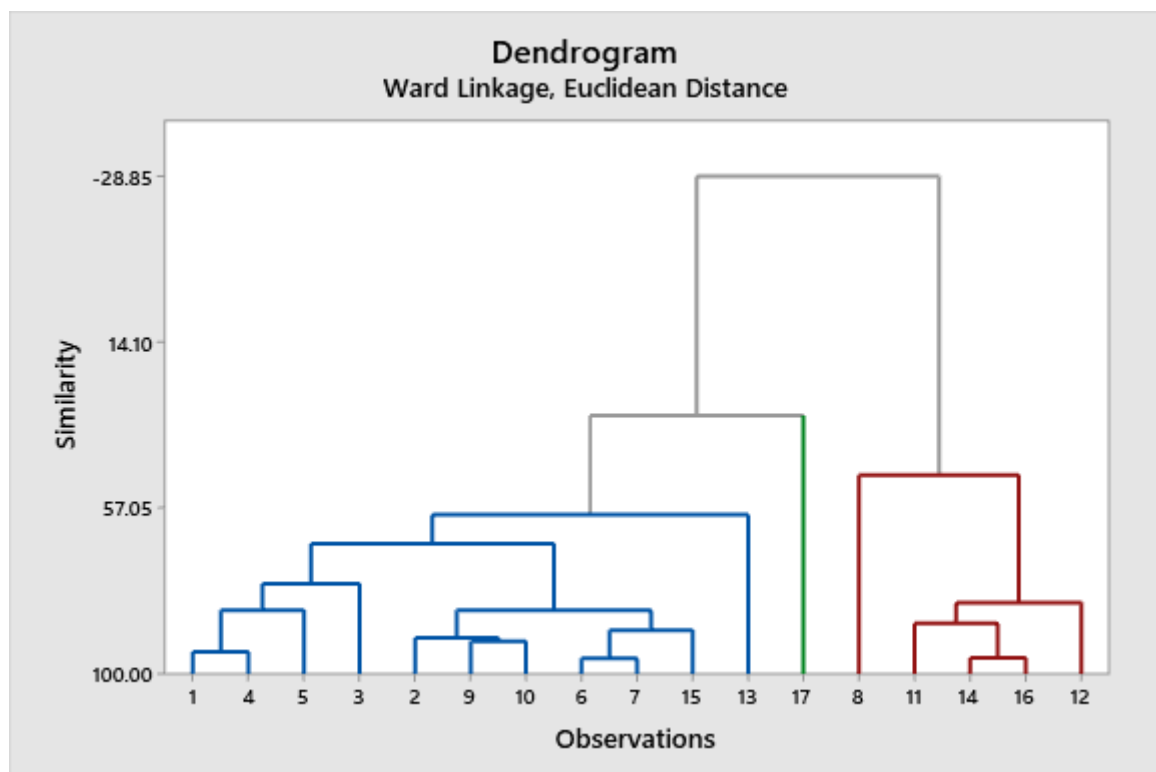


Figure 2- Grouping the combination of potato minituber pelleting treatments

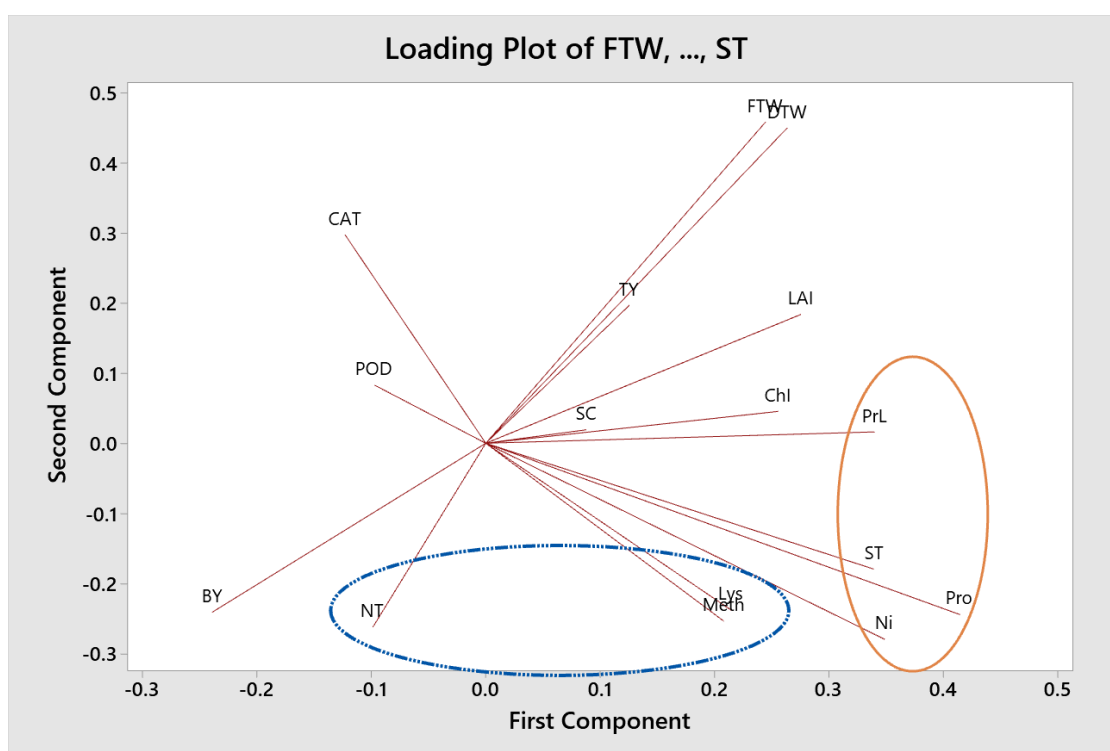


Figure 3- Principal component analysis (PCA) of yield, physiological, and qualitative traits of potato minituber

4. Conclusions

From the previous results it could be concluded that, the pelleting of potato minituber with triple superphosphate, ZE, CO, CH, and SP leads to an increase in growth, yield, and qualitative parameters of minitubers and changes in physiological traits. Therefore, the success of seed pelleting technology depends upon the selection of inexpensive and readily available pelleting agents with low cost. Collectively, cost effective, simple materials and methods are needed for use in third world countries.

Compliance with ethical standards

Conflict of interest: The authors declare that there is no conflict of interest concerning the publication of this article.

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