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Inulin evaluation of Jerusalem artichoke (*Helianthus tuberosus*) from organic cultivation areas, Phayao, Thailand

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Abstract The results showed that Jerusalem artichoke (*Helianthus tuberosus* L.) experienced normal growth in an organic farm plot. The highest inulin content of Jerusalem artichoke in the plots was 81.5%, and soil phosphorus exhibited a positive correlation with inulin content. The organic farm at Ban Bua is suitable for Jerusalem artichoke cultivation in northern Thailand.

Keywords: Jerusalem artichoke, Inulin, Organic farm, Extraction

Introduction

Jerusalem artichoke (*Helianthus tuberosus* L.) is the property of Asteraceae encompassing both Chrysanthemum and sunflowers. Jerusalem artichoke is a native plant found in North America and can be cultivated into an edible plant. Currently, in countries such as America, France, and other European countries, artichoke can be commonly reffered to as "Sunchoke" (Vorasoot and Jogloy, 2006) Jerusalem artichoke is a plant having a storage bulb, and the important compound in their bulbs is inulin (Kays and Nottingham, 2008). Inulin is believed to have significant benefits for the human body and has been widely used to prevent obesity, reducing the risk of diabetes, cholesterol in blood and the risk of heart disease (Kays and Nottingham, 2008; Yang *et al*., 2015; Saengkanuk *et al*., 2011). In addition to its potential for humans, inulin is a prebiotic for microorganisms in the intestine and especially benefits bacteria. For example, inulin reduces foodborne pathogens and stimulates the human immune system (Yang *et al*., 2015; Gholami *et al*., 2018; Punha *et al*., 2019). Therefore, Jerusalem artichoke is a novel plant used in the production of healthy food. Properties of this particular artichoke can be

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compared to chemical synthetic substances found in Jerusalem artichoke and these substances can be expanded into raw materials that generate fructans and inulin because it is a natural and a nontoxic compound to human and environment (Hanč *et al*., 2008).

The average yield of Jerusalem artichoke cultivation in Thailand is not sufficient enough to meet global standards. Bulb sizes used to cultivate the artichoke were either small or medium. Big bulbs were rare during this stage of production (Khuenpet *et al*., 2015). The low fertile soil leads to low yield which resulted to the farmers used large amount of chemical fertilizer to increase yield. Consequently, the soil becomes unsuitable for Jerusalem artichoke root growth and bulb construction (Filipović *et al*., 2016; Starovoitova *et al*., 2019). The Jerusalem artichoke is a salt-tolerant and drought-tolerant species and can be cultivated with a relatively small amount of N fertilization (90 kg/ha, 2 g/plant) (Zhao *et al*., 2008). In Thailand, most Jerusalem artichoke plants grow in the north-east, middle and lower part of north areas, however, farmers in these areas have had to apply both chemical and organic fertilizers (Vorasoot and Jogloy, 2006; Aduldecha *et al*., 2016).

There is currently limited research relating to the process of organic farming focusing specifically on Jerusalem artichoke in northern Thailand. Since organic farming has gained more notoriety and people are beginning to plant more edible plants and herbs. The system management is operated in a natural way that does not generate either toxic compounds or non-residual toxins. Also, this system safes any potential harmful chemical contaminants entering into the environment either through the soil, water or the air. The particular organic system also supports biodiversity in ecological habitats, recovers balanced environments, and organizes production to achieve the highest yield whilst also following and maintaining good manufacturing practice (GMP), this ensures improved nutrition and health for consumers (Vorasoot and Jogloy, 2006).

Ban Bua, Ban Tun sub-strict, Mueang district, Phayao province is located in the upper part of northern Thailand. Farmers in Phayao province brought Jerusalem artichoke and planted seeds in the nearby Ban Bua Village. These seeds were planted and used in organic agriculture. Ban Bua Village is located in the midst of some 400 acres (160 hectares) for organic rice fields. The farmers at Ban Bua use organic fertilizers to grow their rice naturally and free of toxic chemicals. The community has currently planted around 200 acres (80 hectares) of bamboo forest, outside the rice cultivation season (Pinmongkhonkul *et al*., 2019). Farmers in this region have decided to adopt an organic system in order to grow and nourish Jerusalem artichoke as an alternative plant.

In this study, we analyzed the plant growth of Jerusalem artichoke in terms of organic farming and the main nutrient amounts in the soil affecting inulin contents in root blubs. The information is possible to be a guideline for growing Jerusalem artichoke in an organic system with qualified inulin content.

Materials and methods

Experimental design

Three field experiments were conducted during the 2018 field seasons between June 2018 and September 2018 to determine the influence of Nitrogen (N), Phosphorus (P) and Potassium (K) of soil in an organic farm on the inulin performance of Jerusalem artichoke. The experimental design was a split-plot with three replications.

Location, plant material and crop management

The experiments were conducted at the experimental organic farm of Ban Bua village, Ban Tun Subdistrict, Phayao Province located in northern Thailand (latitude 19°08.659' N and longitude 099°49.390' E). The hybrid plants with Jerusalem artichoke were planted. The crop was planted by hand, and Jerusalem artichoke was planted in late March in an ideal plant spacing of 0.40 m×1.00 m. Tubers were previously cut into segments of about 50 g (fresh matter). The experimental unit was a plot of 20.0 m long and 40.0 m apart (four rows 1.00 m apart and 0.40 m between plants). Organic fertilizer, including livestock manure composted with various residual plant materials (e.g., straw on farm processing wastes) was used as fertilizer in planting. Frequency of irrigation was about 1-2 days per week.

Soil samples were randomly collected from 3 plots in 3 stations, 1,000 grams per each station. The soil samples were analyzed to determine their pH, soil texture, N, P, and K. Data on monthly average temperatures and humidity, registered at the experimental site during the crop cycles, were reported form Phayao meteorological station.

Measurements

Plant growth was assessed 75 days after transplanting. Plant height and leaf width were measured. Aboveground biomass and tubers were harvested by hand at each harvest in September 2018. The sampling area was 3 m^2, corresponding to the two central rows of each plot. Afterwards, tubers were washed and weighed; about 2 kg of each plot was peeled and cut into 0.5 cm cubes, and the cubes were stored at -20 ℃ for further analysis.

Inulin determination

The Jerusalem artichokes were dried at 60 ℃ in an oven for 10 h and then milled and sieved into a powder. Inulin content was determined by the methods described by Saengkanuk *et al*. (2011) and Noori (2014). Inulin isolated from the JA powder (1.00 g) was put into a 150-mL conical flask, and water was added (ratio 1: 100). The inulin was extracted by using ultrasonic cleaning bath Model GT Sonic Model 2227QTS (Guangdong GT Ultrasonic Co., Ltd., Guangdong, china) at a frequency of 59 kHz with a temperature 70 ℃ for 25 min. Then, the extract was filtered through filter paper and stored in a refrigerator for further analysis using a UV/Vis spectrophotometer. The yield of inulin was determined. Total carbohydrate was determined by the phenolsulphuric acid method of Dubois *et al.* (1956) using inulin (Raftiline®GR) as a standard. The reducing sugar was determined by the dinitrosalicylic acid method using D (-)-Fructose (Mw = 180.16, Fluka) as a standard (Miller, 1959). The pH value was measured with a pH meter. The inulin content was measured with the difference between total carbohydrate and reducing sugars. Inulinextraction yield (%) = (inulin content × volume of extraction liquid/mass of artichoke powder) ×100.

Statistical analysis

All measured and derived data from each season were separately subjected to an analysis of variance (ANOVA). When the F ratio was significant ($p < 0.05$), Tukey's test was performed and used to compare means. Correlation between parameters was calculated by Pearson's correlation coefficient and evaluated at the $p<0.05$ significance level.

Results

Average annual rain fall, temperature and humidity

The average annual climatic data of January to December 2018 was collected from the monthly values of meteorological observation by the School of Energy and Environment, University of Phayao (Figure 1).

During the period, the total rainfall was 1289.25 mm, and there was less than 30 mm monthly rainfall from January to March 2018 and November to December 2018. The average annual temperature was 24.3 ℃ with the highest value in May at 26.0 ℃ and the lowest in January at 21.5 ℃. The average relative humidity was 70.56% with the highest and lowest value in August (79.3%) and March (53.7%), respectively.

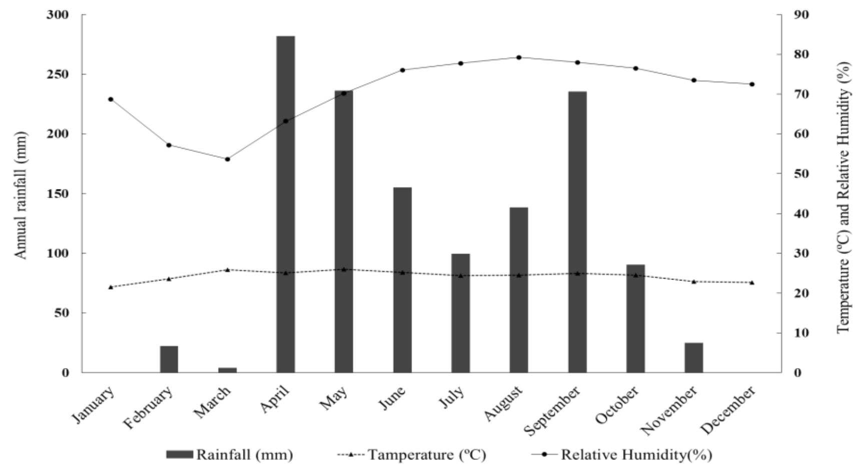

Figure 1. Monthly rainfall, temperature, and relative humidity from January to December 2018 in Phayao province

The mean leaf width of Jerusalem artichoke

An average growth rate of the leaf width of Jerusalem artichoke was 1.48 cm/week over the period. The mean and standard deviation (SD) of the leaf width started from 2.30±0.20 cm to 6.74±0.23 cm. The highest growth rate was found at 1.87 cm/week during month 3 to month 4 (Figure 2).

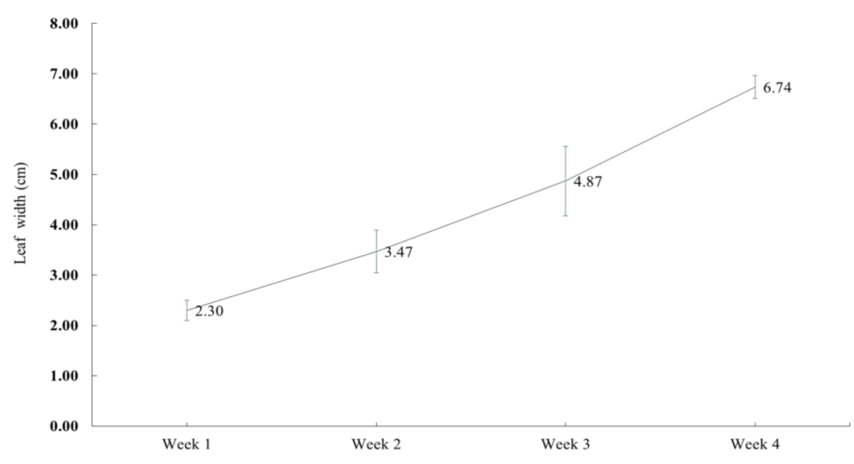

Figure 2. Change in growth of Jerusalem artichoke's leaf width during for 4 weeks (the growth phase)

Jerusalem artichoke mean height

The mean height ± SD of Jerusalem artichokes were 34.2±4.23 cm and 170.15±12.0 cm at the beginning and the end of the period, respectively (n=4). An increased rate of mean growth in height was 45.32 cm/month. After 30 days from the beginning of the study, the height of Jerusalem artichoke showed a dramatic increase in the rate at 77.29 cm/month from July to August 2018 (Figure 3).

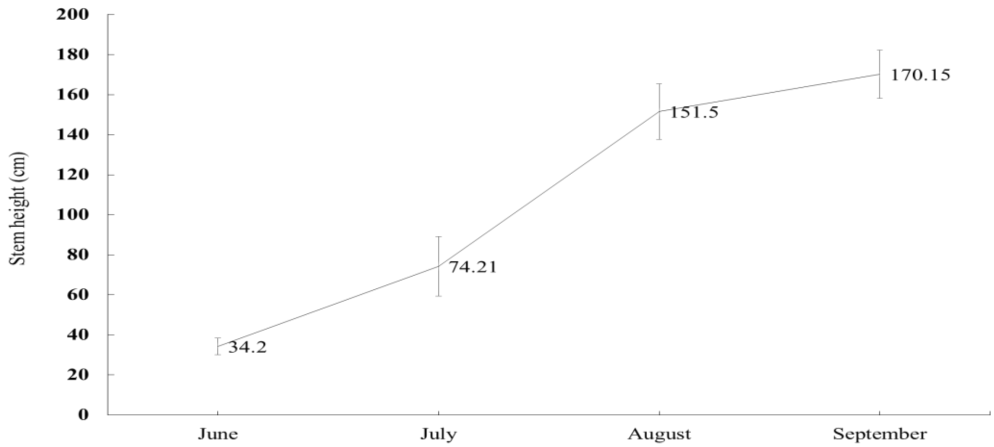

Figure 3. Change in height growth of Jerusalem artichokes during from June to September 2017

Soil texture characteristic

Based on the soil texture shown in Figure 4, the percentage of particle composition of sand (81%), silt (11%), and clay (8%) can be used to classify the soil as loamy sand.

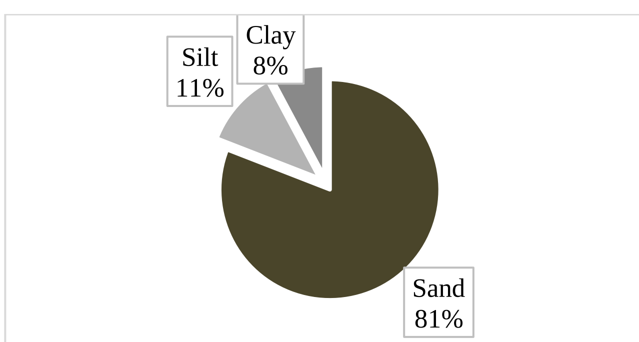

Figure 4. Percent of soil texture characteristics for the planted Jerusalem artichoke from organic areas of Ban Bua

Soil quantity

The most important nutrients are nitrogen (N), phosphorus (P), and potassium (K), which are required by plants and crops. As a result, the percentage proportion (±SD) of soil nutrients namely, N, P, and K contributed 4.53±1.91, 0.68±0.26, and 0.22±0.09 to the soil, respectively (Figure 5).

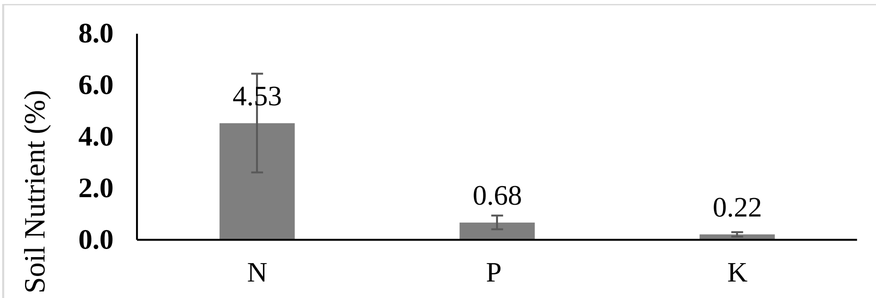

Figure 5. Percentage of soil nutrient composition

The graph displaying 490 nm light absorption values of standard glucose solutions using Phenol-sulfuric acid assays showed that the light absorption values and solution concentration exhibited a linear relationship, where an equation displaying the relationship between standard glucose solution concentrations and 490 nm light absorption values could be written as y= 0.0285x, where y is the value of 490 nm light absorption of standard glucose solutions, and x is the concentration of standard glucose solutions (g); the correlation coefficient (R^2) calculated for y= 0.0285x+0.088 displays the linear relationship between the 2 variables with a correlation of 0.9823 (Figure 6).

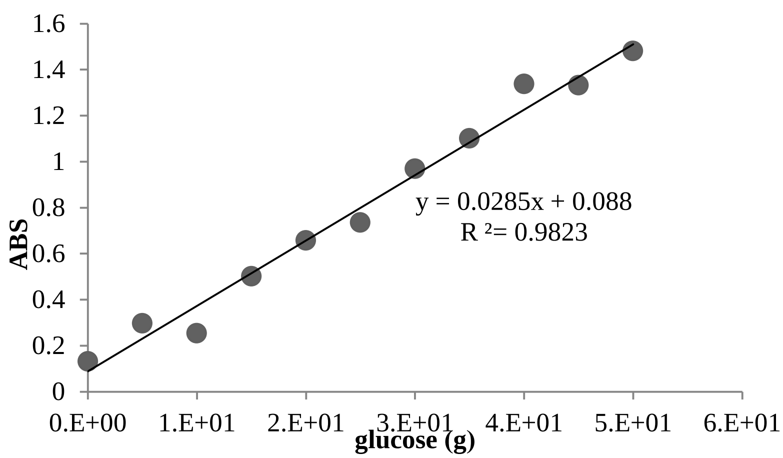

Figure 6. Calibration curve of total carbohydrate analysis using Phenol-sulfuric acid assays

The graph displaying 560 nm light absorption values of standard fructose solutions using 3,5-Dinitrosalicylic acid colorimetric assay showed that light absorption values and solution concentrations exhibited a linear relationship, where an equation displaying the relationship between standard fructose solution concentrations and 560 nm light absorption values could be written as y = 0.1173x, where y is the value of 560 nm light absorption of standard fructose solutions, and x is the concentration of standard glucose solutions (g); the correlation coefficient (R^2) for y = 0.1173x + 0.0243 was 0.9883 (Figure 7).

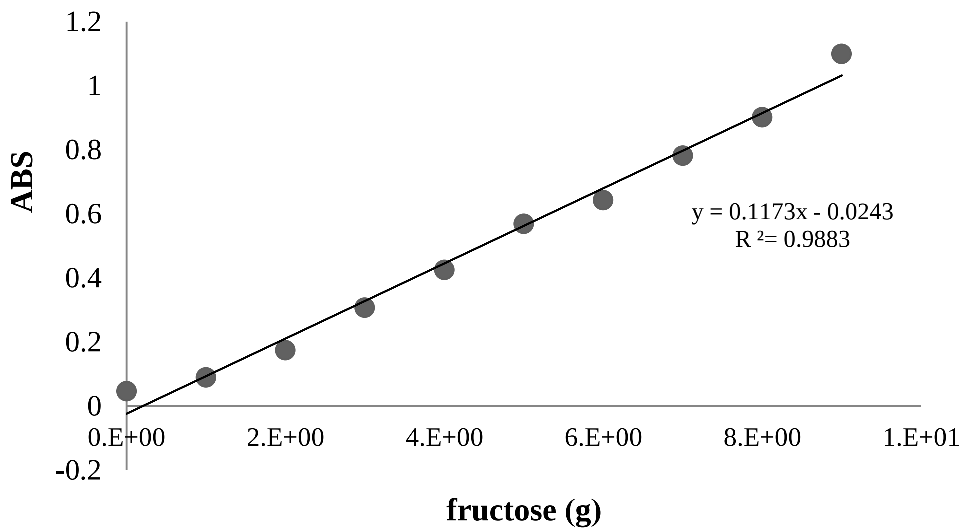

Figure 7. Calibration curve of the analysis of the quantity of reducing sugar using 3, 5-Dinitrosalicylic acid colorimetric assays

Inulin quantities in Jerusalem artichokes

Studies have found that plot 2 had light absorption values of 0.845 for standard glucose solutions and 0.0591 for standard fructose solutions, with a mean (±SD) quantity of inulin at 8.40±0.81%, which was the highest among Jerusalem artichokes. This was followed by plot 3, with light absorption values of 0.663 for standard glucose solutions and 0.016 for standard fructose solutions, with a mean quantity of inulin at 64.70±1.14, and plot 1 exhibited light absorption values of 0.0624 for standard glucose solutions and 0.025 for standard fructose solutions, with a mean quantity of inulin at 59.90±0.41% (Figure 8).

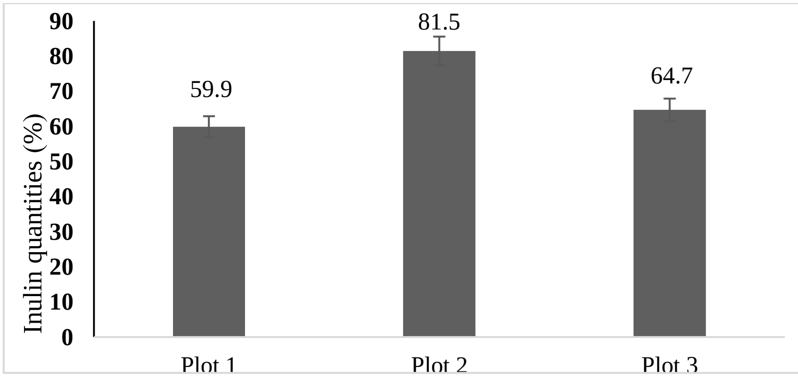

Figure 8. Graph displaying comparisons of inulin quantity of Jerusalem artichokes

The relationship between soil nutrients (N, P, K) and the inulin of Jerusalem artichokes found that soil nitrogen per Jerusalem artichoke inulin quantities exhibited a correlated relationship (R^2=0.5452). Soil phosphorus per Jerusalem artichoke inulin quantities exhibited a positively correlated relationship (R^2=0. 8462). Potassium quantities and Jerusalem artichoke inulin quantities were also positively correlated (R^2=0.652) (Table 1)

Table 1. Comparisons of soil nutrients (N, P, K) and Inulin quantities in Jerusalem artichokes

Sample	**Soil nutrients**			**Inulin (%)**
	N (%)	**P (%)**	**K (%)**	
Plot 1	7.22±1.88[a]	0.38±0.08[a]	0.33±0.09	59.90±0.41[a]
Plot 2	3.92±0.46[b]	1.00±0.08[b]	0.17±0.02	81.50±0.81[b]
Plot 3	4.26±0.50[b]	0.74±0.06[c]	0.21±0.01	64.70±1.14[c]

Discussion

Jerusalem artichoke from each plot exhibited a continuous growth rate (June to September) until the harvesting period (October to November), when the stem growth begins to stabilize. This is consistent with the study of growth and development quality as well as storage quality of the Jerusalem artichoke, which gradually grows during the first week and rapidly grows later on. This can be seen from the height and weight of the fresh stem, similar to the S shape or sigmoid curve, which is a characteristic of general plant growth (Denoroy, 1996). Furthermore, the statistical analysis of height and branches per stem of

Jerusalem artichoke showed that the application of fertilizer in different treatments resulted in no statistical difference in height (Jogloy *et al*., 2006). The average width from June to November (Harvesting period) is consistent with the study of Jogloy *et al*. (2006). Through harvesting 75, 90, and 105 days after planting, dry weight including leaves, leaf area, and leaf area index have no statistical difference. However, those values tend to decrease when harvesting late (or harvesting in November) with a growth period as long as 180 days.

In terms of soil nutrients, the analysis found that the amount of nitrogen in the plot is higher for general crop due to different amounts of organic fertilizer in cultivation. Relating to the study of Jogloy *et al*. (2006), the organic fertilizer at a ratio of 1,000 kg rai^{-1} yields the maximum average fresh weight of Jerusalem artichoke. Moreover, maturity compost contains an amount of nitrogen for the growth of trunk and leaves of Jerusalem artichoke plus other key nutrients (Poincelot, 1975). The amount of phosphorus is beneficial to the plants in the soil, and the plot carries a higher amount of phosphorus. This result is consistent with Kadoglidou *et al*. (2019), who indicated that the key element of the composting process such as phosphorus was increased after composting mixture of raw material for the production of a stable and mature end-product that is suitable to be applied as fertilizer or a soil amendment compared to the untreated control.

Finally, the soil after the experiment increased in pH, organic matter content, and the amount of beneficial phosphorus. Potassium content in the soil reveals that the plot holds a lower amount of potassium resulting from the use of different amounts of organic fertilizer. Alabadan *et al*. (2009) report that poultry manure increased the availability of P and K. This might be due to the reason that the application of organic manure secreted organic acid during the process of decomposition, which might lead to mineralization of the fixed potassium and an increase in the availability of potassium.

Jerusalem artichoke has been mentioned as a plant with responses to soil fertility, especially soil with high organic matter. In addition, it can efficiently absorb and utilize nutrients remaining in the soil from another season. Soil with high organic matter can double the yield of Jerusalem artichoke compared with low-organic soil. In addition, soil with higher amounts of organic matter produces better quality Jerusalem artichoke, such as increasing the proportion of large and medium heads (Kays and Nottingham, 2008). Supplementing organic materials to improve the soil generally directly appends nutrients to the soil, although less than chemical fertilizers, yet contain both primary and secondary nutrients for growth and productivity, which gradually release to be beneficial to plants in the long run. Jogloy *et al*. (2006) show that organic fertilizer at a ratio of 1000 kg rai^{-1} yields the maximum average fresh weight of Jerusalem artichoke. Besides, the maturity compost incorporates the amount of nitrogen for the growth of the trunk and leaves of the Jerusalem artichoke, including other key nutrients (Poincelot, 1975) Kulanit *et al*. (2014) investigated the effects of nutrients on the yield of Jerusalem artichoke and illustrated that the most influential nutrients are N, followed by P, K, Ca, Mg, B, Cu, and Zn. Moreover, Gosling and Shepherd (2005) state that adding various organic matter to the soil (e.g., organic fertilizer, bio-fertilizer, bio-organic fertilizer) results in the improvement of the soil's physical properties. As a result, the organic matter in the organic fertilizer can support soil aggregation, which can improve the physical properties such as soil structure, bulk density, water holding capacity, drainage, porosity, and permeability. Okwuagwu *et al*. (2003) observed increases of organic matter with the application of organic manures, and this could be explained on the basis of the addition of organic matter and enhancement of biological activity.

The study of inulin content in the Jerusalem artichoke shows the highest positive correlation with phosphorus in soil. This is consistent with the Nacoon *et al*. (2020) investigation that revealed beneficial effects of dual inoculation of a PSB strain (*Klebsiella variicola*) and AMF (especially *Rhizophagus intraradices*), when combined with addition of rock phosphate, in the growth promotion and inulin production of Jerusalem artichoke. Phosphorus was subsequently taken up and transported to the root of Jerusalem artichoke by AMF. Therefore, dual inoculation may be a promising strategy to both reduce expensive synthetic fertilizers and to enhance inulin production. This is different from the investigation by Michalska-Ciechanowska *et al*. (2019) that found potassium content correlates with the amount of inulin, potassium fertilizer on inulin, and polyphenols content in three species of Jerusalem artichoke and suggests that potassium correlates with an inulin content and the use of potassium fertilizer will result in a higher inulin content. Therefore, potassium affects inulin content since potassium plays an important role in the activation of an enzyme for the integration of the substance, which is necessary for protein synthesis, starch, and sugar formation as well as the transfer of photosynthesis from leaves to the tubers, an important food storage source (Menzel *et al*., 1992). Ultimately, phosphorus and potassium are essential for transporting nutrients to the tuber, which can be absorbed for utilization.

Inulin quality in Jerusalem artichoke planted with organic methods was affected by the different organic manures used and their combinations, which might be due to the role of decomposition micro-organisms to enhance one or more of physical, chemical, and biological properties of the soil. For example, the stimulated effect may be related to the good equilibrium of nutrients and water in the root medium (Abdelaziz *et al*., 2007) or to the beneficial effects of bacteria on vital enzymes and hormonal, stimulating effects on plant growth. Furthermore, these organisms may affect their host plant by one or more mechanisms, such as nitrogen fixation, production of organic acids, enhancing nutrients uptake, synthesis of vitamins, amino acids, auxins, and gibberellins, which stimulate growth. Moreover, El-Ghadban *et al*. (2002) mentioned that organic composts led to an increase in carbohydrate percentage and some macronutrients. These increases might be related to the positive effect of compost and microorganisms in increasing the root surface area of soil volume water-use efficiency and photosynthetic activity, which directly affects the physiological processes and utilization of carbohydrates.

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