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Microbes-Coated Urea for Reducing Urea Dose of Strawberry Early Growth in Soilless Media

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Abstract

Strawberry is a high-value fruit in Indonesia. During the growth phase for transplant production, farmers applied conventional urea that is easy to volatile and leach. Coated Urea has proven to reduce nitrogen (N) losses from urea fertilizer. Microbial-coated urea application is a reliable way to limit the loss of N from urea and at the same time increase the use of biofertilizer. Azotobacter and Bacillus are widely used as a biofertilizer formulation. This experimental objective was to determine the effect of two formulations and doses of urea coated with solid organic inoculant of Azotobacter and Bacillus on the growth parameters of strawberry seedling as well as reducing urea fertilizer. The green house experiment was carried out in randomized completely block design (RCBD) with five treatments and five replications. One-month old strawberry cv Festival seedlings were grown in coco peat based organic substrate. The seedlings were treated with four combinations of two doses and formulation of microbial coated urea (MCU). Control seedlings received a dose of conventional prilled urea. The results showed that MCU affected root dry weight, root volume, root to shoot ratio, SPAD value, and N uptake but did not significantly affect shoot parameters compared to controls. The best composition of urea coated material was compost-based inoculant enriched with 5% zeolite and 5% liquid inoculant. Moreover, this experiment explained that microbial-coated urea might replace 50% of conventional urea.

Keyword: Bacteria coated Urea, *Azotobacter* sp., *Bacillus* sp., Zeolite, Fertilizers doses, Strawberry growth

1. Introduction

Strawberries (Fragaria × Ananassa Duch) grow well in Indonesian mountainous area with good physical soil properties. Farmers in high land Bandung and Bandung Barat Regency cultivate the strawberries since decade ago with significant economic benefit. Strawberry productivity and quality in Indonesian high land are limited by the nutrients management. In general, farmers propagate the strawberry from runner, well known as stolon, that grow above the ground. The new clone will grow and can be separated from the mother plant once the stolon roots touch the soil.

Some strawberry producers have carried out strawberry nurseries to produce strawberry using soilless growth media composed of coco peat and manure (Ameri *et al*., 2012; Raja *et al*., 2018). Compared to soil, this medium contains only a few nutrients but its physical properties are good for rooting. Farmers in Bandung Regency applied chemical fertilizer, urea and NPK compound as well, to provide nutrient during bare-root strawberry transplant production.

The disadvantage of using urea is ammonia volatilization at high temperatures environment (Fan *et al*., 2011; Jadon *et al.,* 2018). Increasing temperature from 20 to 30 °C enhanced NH_3 volatilization with higher loss recorded in sandy soil than loamy soil (Fan *et al*., 2011). Urea can be easily leaching from root zone since the precipitation is higher over the water holding capacity (Burger and Jackson, 2003; Wang *et al*., 2015). To overcome the constraints, coated urea has been recommended as a reliable way to slow and control N release from urea (Bibi *et al*., 2016). Ground application of neem-and oleoresin-coated urea reported to reduce the ammonia volatilization and nitrate leaching significantly (Jadon *et al*., 2018).

We have limited information about fertilizer/urea coated with beneficial microbes. Researchers have shown the effectivity of microbes-coated urea (MCU) to reduce the level of chlorinated pesticide and the persistent organic pollutant in soil (Wahyuni *et al*., 2016). Ahmad *et al* (2017) stated that bacterial-impregnated ammonium phosphate enhancing nitrogen (N) and phosphorus (P) use efficiency of wheat. Coating urea with soil beneficial microbes such as the N-fixing *Azotobacter* and the phosphate solubilizing *Bacillus* is also a way to enhance the beneficial microbe application. *Azotobacter* and *Bacillus* are the active ingredients of biofertilizer suggested to provide nutrients and ensure plant growth through N fixation and phosphate solubilization respectively (Rubio *et al*., 2013; Saeid *et al*., 2018). Both

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rhizobacteria produced phytohormones of auxins cytokinins, gibberellins (Fitriatin *et al*. 2020; Hindersah *et al*., 2019; Hindersah *et al*. 2020a) which is beneficial to stimulate root and subsequent plant growth (Bhattacharyya and Jha. 2012). The *Azotobacter* and *Bacillus* form cysts and spores (Rodriguez-Salazar *et al.*, 2017; Tan and Rammurthi, 2014) as a response to drought stress. However, farmers in Indonesia are rarely including the biofertilizer in their nutrient management since the labor cost will increase when the biofertilizer is applied separately from the urea. Coating urea with the microbes might overcome this constraint.

Nitrogen and P are essential macronutrients and determine plant productivity. The advantage of N-fixer Azotobacter and P-solubilizer Bacillus inoculation in strawberry cultivation have been documented to increase significant growth and yield in field and greenhouse as well (Mishra and Tripathi, 2011; Shternshis *et al.*, 2015; Reddy and Goyal, 2021). Moreover, Bacillus can control the diseases and induce plant resistance to the strawberry diseases (Shternshis *et al*., 2015; Wei *et al*., 2016).

We have already developed a mixed liquid biofertilizer containing *A. chroococcum, A. vinelandii, B. subtilis* and *B. megaterium* with the equal composition to maintain each bacterial population up 10^8 CFU/ml (Hindersah *et al*., 2020b). For coating the urea, a solid inoculant is needed to avoid direct intact with urea and ensure the bacterial viability since the water content of urea is as low as 0.5%.

Based on previous research, an effective carrier for maintaining the population of both microbes was 200-mesh compost enriched with 100-mesh zeolite at 15% moisture content (Hindersah *et al*., 2020a). The level zeolite and liquid inoculant in solid inoculant formultion is also essential prior to urea coating. Furthermore, compost-based solid inoculant with 5% Zeolite + 5% Liquid as well as 1% zeolite and 10% liquid Inoculant supported Azotobacter and Bacillus count at 10^9 and 10^{11} CFU/g respectively during 4 weeks of storage (Hindersah *et al*., 2021). In the formulation described above, the molecule structure of zeolites functions in adsorbing water (Tatlier *et al*, 2018) to maintain low water content in carrier and further coated urea fertilizer.

Biofertilizers are now integrated in horticultural crops production for decreasing the level of chemical fertilizer and increasing the soil health. However, the price of microbial-coated urea might be higher over the conventional urea. The use of coated urea will be efficient for high value horticultural products such as strawberry in Bandung Regency, and then research to optimize the application of these newer coated urea is needed. The effectiveness of the urea coated with Azotobacter-Bacillus consortium on the growth of strawberry seedlings, especially the morphological parameters, needs to be verified prior to the wider use by the farmers. The objective of this greenhouse experiment was to determine the effect of solid inoculant-coated urea on the growth properties of strawberry seedlings grown in soilless media for 4 weeks during bare-roots strawberry seedling production.

2. Material and Methods

Greenhouse trials were conducted from October 2019 to March 2020 at Bumi Agrotechnology Farm in Cisarua, Bandung Barat Regency at the altitude of 1,225 m above sea level. The location situated in tropics with average annual temperature 17-26°C and humidity 70-90%. Urea was coated with solid inoculant of N-fixing *A. vinelandii* and *A. chrococcum,* and phosphate-solubilizing *B. subtilis* and *B. megaterium* consortia developed by the Soil Biology Laboratory Faculty of Agriculture, Universitas Padjadjaran. Liquid inoculant of all bacteria was prepared in molasses based broth enriched with N source (Hindersah *et al*., 2020b). The Azotobacter and Bacillus produced phytohormone of indole acetic acid (IAA), cytokinins (CK) and gibberellins (GA) in the in-vitro test (Hindersah *et al*., 2020b). The seedlings of strawberry cv. Festival were provided by Bumi Agrotechnology Farm. Four-week old daughter plants of strawberry growing on the tip of stolon have been separated from the mother plant at planting time (Fig 1).

Figure 1. One-month old strawberry seedlings before being cutting from the mother plant and transplant.

2.1. Experimental Establishment

The experiment was setup in completely randomized block design with 5 treatments consisting of 4 combinations of doses and composition of MCU and one control treatment (Fig 2); all treatments were replicated 5 times. Based on previous experiment, two best formulations of solid bacterial inoculant for coating the urea are:

1. 200-mesh cow manure compost enriched with 5% of 100-mesh zeolite and 5% of mixed liquid Inoculant of *Bacillus* and *Azotobacter* (composition I) and
2. 200-mesh cow manure compost enriched with 1% zeolite + 10% liquid inoculant (composition II).

The MCU treatments were the combination of each formula with full and half application doses, so that we have 4 combination treatments of Microbial coated urea (MCU). The doses of MCU were based on the recommended urea dose for strawberry released by Indonesian Agricultural Research and Development Institute, i.e. 200 kg/ha equal to 2 g/plant. Plant with full and half doses received 1 g and 2 g of urea respectively. The control treatment was 2 g of conventional prilled urea.

The 14-cm height of strawberry seedling cv Festival with 5 leaves and 18-20 cm in crown diameter were grown in the substrate contained mixed of coco peat, chicken and sheep manure at volume ratio of 8:2:1. The substrates were average in N (0.57%), and very low in P_2O_5 (0.035 mg/kg) and K_2O (0.26 mg/100 g) with the C/N 39.74. The media were put in 40x40 cm polyethylene bag and placed in the greenhouse for a week prior to transplant with two strawberry seedlings. One week after transplanting, strawberry seedlings received the MCU that incorporated with the first two 2-cm depth at 10 cm next to the base crown. Inorganic NPK fertilizer (16:16:16) at the rate of 2 g/plant (Palupi *et al*., 2017) was applied two weeks after planting to all treatments. The fertilizer was placing on the circle about 10 cm away from the base crown and covering with the growth medium.

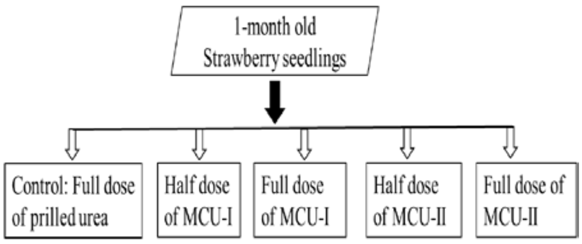

Figure 2. The experimental treatments of microbial coated urea (MCU) application in strawberry seedlings cultivation

2.2. Parameters Measurement

All plants were maintained in the greenhouse for 4 weeks. The data of plant height, root length, crown diameter, root dry weight, root volume, shoot dry weight, root to shoot ratio, chlorophyll unit, as well as N content and N uptake of shoot were taken at the end of trial. Nitrogen uptake represent their accumulation in crops as well as N availability in soil (Gastal and Lemaire, 2002). Root and shoot biomass were dried separately in oven at 70 ^{0}C for two days until constant weight prior to dry weight measurement. Root volume was determined by Water Displacement Method (Pang et al., 2011); roots were immersed in 100 mL water in graduated cylinder with 0.1 ml accuracy measurement. The water volume increment after root immersion was suggested as the roots volume. The crown (thickened stem) diameter of strawberry seedlings was measured from the upper end of the crown using a caliper with 0.1 ml accuracy.

Chlorophyll value of strawberry foliage were estimated using a Soil Plant Analysis Development (SPAD-502) chlorophyll meter (Güler et al., 2006) for the six points in five leaves below the fully opening leaves on the top of shoot. The N content of shoots was analyzed by Kjeldahl Method based on Association of Official Analytical Chemists (AOAC) methods for proximate analysis (AOAC, 2012). The N uptake of shoots was calculated by multiplying the N content of shoot with the shoot dry weight.

2.3. Statistical Analysis

All data were subjected to analysis of variance (F test with $p \leq 0.05$) to verify the significant of sum square on the parameters. The Duncan's multiple range test ($p \leq 0.05$) was then performed when F test was significant. The data were analyzed with IBM SPSS statistics version 25 (Mustafa and Hayder, 2021)

3. Results

MCU-I refers to coated urea with compost-based biofertilizer formulated enriched with 5% zeolite enrichment and inoculated with 5% mixed Azotobacter-Bacillus liquid Inoculant, whereas MCU-II is coated urea with compost-based biofertilizer with 1% zeolite and 10% liquid inoculant.

3.1. Root Parameters

The different dose of MCU integrated with different composition of solid inoculant for coating the urea has not affected the roots length but some treatments increased root dry weight and volume as compared with the control (Table 1). Strawberry seedling received 1 g of urea coated with solid inoculant contained 5% zeolite and 5% liquid inoculant (MCU-I) have highest root dry weight compared to the control and other treatments after 4 weeks in soilless substrate. The root volume of seedling received 1 g urea coated with solid inoculant contained 1% zeolite and 10% liquid inoculant (MCU-II) was higher than other treatments but their root dry weight was slightly lower than seedling with 1 g MCU-I.

Table 1. The effect of dose and microbes-coated urea on roots length dry weight and volume of four-week old strawberry grown in the greenhouse.

Coated Urea Treatments	Root Parameters		
	Lenght (cm)	Dry weight (g)	Volume (ml)
2 g of Prilled Urea (control)	25.3 a	2.7 b	103.2 c
1 g of Microbial Coated Urea -I[a]	25.8 a	5.7 a	110.5 b
2 g of Microbial Coated Urea -I	25.0 a	5.1 a	109.3 b
1 g of Microbial Coated Urea -II[b]	25.2 a	4.4 ab	114.2 a
2 g of Microbial Coated Urea -II	27.8 a	2.9 b	107.5 b

Numbers followed by the same letter didn't significantly differ based on Least Significant Test ($p<0.05$). aCoated urea with compost-based biofertilizer contained 5% zeolite + 5% liquid inoculant; bCoated urea with compost-based biofertilizer contained 1% zeolite + 10% liquid inoculant.

3.2. Shoot Parameters

The results verified that the dose of urea coated with different composition of solid bacterial inoculant has not affected all measured shoot parameter (Table 2). However, reducing the dose of MCU to 50% resulted in equal shoot height and dry weight as well as crown diameter.

Tabel 2. The effect of dose and microbes-coated urea on shoot height and dry weight, and crown diameter of four-week old strawberry grown in the greenhouse

Coated Urea Treatments	Shoot Parameters		
	Dry Weight (gr)	Height (cm)	Crown Diameter (cm)
2 g of Prilled Urea (control)	2.7 a	22.2 a	2.4 a
1 g of Microbial Coated Urea -I[a]	3.1 a	21.3 a	2.4 a
2 g of Microbial Coated Urea -I	2.5 a	20.5 a	2.9 a
1 g of Microbial Coated Urea -II[b]	2.6 a	20.6 a	2.4 a
2 g of Microbial Coated Urea -II	2.8 a	20.2 a	2.5 a

Numbers followed by the same letter didn't significantly differ based on Least Significant Test ($p<0.05$). [a]Coated urea with compost-based biofertilizer contained 5% zeolite + 5% liquid inoculant; [b]Coated urea with compost-based biofertilizer contained 1% zeolite + 10% liquid inoculant.

3.3. Root to shoot ratio

The ratios of root to shoot dry weight (R/S) of strawberry were significantly different among the treatments. Application of 1 g of MCU-I clearly resulted in higher R/S of the plant (Table 3). The R/S mostly > 1 revealed that the root growth was more rigorous than shoot growth. Nonetheless, seedlings treated with either recommended-dose urea or 2 g of MCU-II have R/S <1.

Tabel 3. Change in root to shoot ratio of 4-week old strawberry in the greenhouse after different dose and microbes-coated urea application

Coated Urea Treatments	Root to Shoot Ratio
2 g of Prilled Urea (control)	0.96 c
1 g of Microbial Coated Urea-I[a]	2.53 a
2 g of Microbial Coated Urea-I	1.86 b
1 g of Microbial Coated Urea -II[b]	1.74 b
2 g of Microbial Coated Urea -II	0.94 c

Numbers followed by the same letter didn't significantly differ based on Least Significant Test ($p<0.05$). [a]Coated urea with compost-based biofertilizer contained 5% zeolite + 5% liquid inoculant; [b]Coated urea with compost-based biofertilizer contained 1% zeolite + 10% liquid inoculant.

3.4. Chlorophyll content and N uptake

Chlorophyll unit, percentage of N in shoot and N uptake (mg/plant) of strawberry shoot were influenced by MCU doses and types (Table 4). The result showed that 2 g of MCU-II and 1 g of MCU-I increased the SPAD value compared to the control treatment. The plants treated with 2 g of MCU had the highest SPAD value.

Tabel 4. The effect of dose and microbes-coated urea on SPAD value, and N content and N uptake of 4-week old strawberry shoots

Coated Urea Treatments	SPAD value	Shoot N content (%)	Shoot N uptake (g/plant)
2 g of Urea (control)	26.1 c	2.38 b	0.07 b
1 g of Microbial Coated Urea -I[a]	25.9 cd	2.47 a	0.06 b
2 g of Microbial Coated Urea -I	29.1 b	2.41 a	0.09 a
1 g of Microbial Coated Urea -II[b]	24.8 d	2.19 c	0.07 b
2 g of Microbial Coated Urea -II	31.1 a	2.23 b	0.07 b

Numbers followed by the same letter didn't significantly differ based on Least Significant Test ($p<0.05$). [a]Coated urea with compost-based biofertilizer contained 5% zeolite + 5% liquid inoculant; [b]Coated urea with compost-based biofertilizer contained 1% zeolite + 10% liquid inoculant.

4. Discussion

The experiment found that the effect of MCU was mostly significant for root growth compared to the shoots. Increased root dry weight of MCU-treated plants compared to the control plants was possibly caused by root volume increment. Both Bacillus and Azotobacter produce phytohormones IAA, GA and CK (Hindersah et al., 2020a) which stimulate root growth. Plant treated with lower doses of MCU-I showed the intensive rooting compared to the control or higher doses of MCU.

Phytohormones play a central role on root growth. Plant has endogenous phytohormones, then the balance composition of the three phytohormones associated with well performance of shoot and root growth. Small quantities of IAA produced by soil microbes have been reported to increase root but high concentration inhibit root elongation (Kurepin et al., 2014). The better root growth might relate to the ability of all bacteria to synthesize the CK and GA. The CK is involved in the regulation of many processes in plant development (Kulaeva et al., 2002). The IAA positively interacts with GA in growth regulation, in which the concentration of GA is enhanced in the presence of IAA. The GA also plays an essential role in the normal development of roots, keeping the root long and slender (Tanimoto, 2005). In common, normal roots enable to uptake the water and nutrients optimally.

The MCU supports root growth indicated by higher R/S (Table 3) due to available N slow released from urea and Azotobacter as well. The effect of MCU on the delay of N release has not been yet reported, but coating the urea is already known as controlled-released way to slow down the N release (Bibi *et al., 2016*). The main ingredients of solid inoculant in this experiment were composted cow waste. Organic matter in the surface of urea has a function to prevent high temperature exposure to urea and hence reduce the ammonia volatilization. Microbial solid inoculant can minimize direct contact of water to urea and further reduce nitrate leaving the root zone. This agrees with the reduction of 27.5% in ammonia volatilization and 18.3% in nitrate leaching of neem-coated urea (Jadon *et al*., 2018).

The coco peat-based growth substrate used in this experiment contained average level of total N due to enrichment with animal manure, but Shanmugasundaram *et al*. (2013) state that coco peat contains very low N, P and potassium (K); then N, P and K supplement is considered to be applied. However, mixing coco peat in organic media enables improving the physical properties of the potted substrate and hence supports root growth in limited area of a pot (Singh *et al*., 2016). The good physical properties induce the growth of rhizobacteria utilized in coating the urea. Low N in coco peat induce nitrogenase activity to fix the dinitrogen (N_2) since the abundance of N limited the N fixation (Hoffman et al. 2014). On the other hand, high porosity of coco peat-based substrate cause urea leaching when excessive watering had taken place (Burger and Jackson, 2003; Wang *et al*., 2015).

Strawberry shoot parameters did not influence by MCU at any doses and composition compared to the control. The duration of our experiment was only one month since after that the seedling will be transplanted for strawberry production. A one-month experiment might be too short to demonstrate the effect of MCU on shoot parameters**.** Contrary to our results, positive effect of urea application combined with biofertilizer on plant height was reported for potted and hydroponic strawberry during 60-120 days (Rueda et al., 2016; Beer et al., 2017; Reddy and Goyal, 2020). In their study, application of biofertilizers and N fertilizer increased the plant height, plant spread, number of leaves per plant and crown diameter significantly. Our results indicated that the reduced dose of MCU maintains the crown diameter. In strawberry, crown as well as roots have an important role as carbohydrate reserve (Menzel and Smith, 2012). The crown size clearly affected strawberry yield under Florida conditions in two-year consecutive seasons (Torres-Quezada et al., 2015). Our strawberry seedling will be utilized in strawberry production; the crown size >10 mm ensures total fruit number compared to < 10 mm (Torres-Quezada et al., 2015).

Only half and full doses of MCU-I increased shoot N content compared to control plant but SPAD value of full dose MCU treatment was higher than the control (Table 4). The Azotobacter fix N_2 to ammonia which is then nitrify to nitrate by nitrifying bacteria (Fiencke et al. 2005). Mostly terrestrial plant uptake the nitrate as N source in the metabolisms (Chapin et al., 2002); with involving of specific transporter of nitrate, NRT (Nacry *et al*., 2013). Highest N content usually related to chlorophyll-a since the chlorophyll-a is substituted tetrapyrrole that contained four N atoms (Berg et al, 2002). The chlorophyll is a central photoreceptor for electron transport in photoautotrophic metabolisms (Berg et al, 2002) in order to generate the energy for plant growth. High N content of shoot of strawberry with MCU-I was due to constant supply on N and phosphate from rhizobacteria for roots uptake. Reducing urea fertilizer to 50% in lower dose of MCU-I apparently induced N fixation that needs a lot of ATP molecules since nitrogenase is sensitive to high available N of substrate (Hoffman et al., 2014). The presence of available P by phosphate solubilizing Bacillus may contribute the P supply for ATP formation. Both Bacillus species in this experiment produced extracellular phosphatase (Hindersah et al., 2020b) as a prominent mechanism to solubilize the organic P in growth substrate (Guang *et al.*, 2008; Ambreen *et al*.,2020).

Although the strawberry has grown only for a month, Our experiment showed that half dose of MCU resulted in the similar value of root and shoot parameter (Table 1 and Table 2). This indicated that urea fertilizer dose can be saved up to 50%. Delaying N released from coated urea might lead to increase the N efficiency used from fertilizer based on shoot N uptake (Mesquita et al., 2017).

The result showed that seedlings received half dose MCU-I and half dose of MCU-II have higher R/S at early growth compared to the control. Biofertilizer application integrated with urea play a significant role to increase strawberry rooting compared to the control**.** In early vegetative, good rooting and crown size of bare roots strawberry transplant plant ensure the strawberry biomass due to optimal N uptake (Tagliavini *et al*., 2005; Cocco *et al*., 2011). In Bandung Regency, the first harvest of strawberry fruit is commonly no later than 10 weeks after transplanting the bare-root strawberry transplant.

In general, lower doses of MCU-I was more effective to replace conventional urea in early growth of strawberry. The MCU was the urea coated with solid inoculant with compost as the main ingredients of carrier enriched with zeolite. The MCU-I contained 5% zeolite while the MCU-II contained only 1 % zeolite. Higher content of zeolite in coating material of MCU-I can protect the urea from the humidity as well as slower urea hydrolysis and N release to soil. However, the result indicated that the N uptake of one individual plant was very low compared to applied urea and NPK fertilizer. This verifiy that N use efficiency (NUE) by seedling in soilless substrate might be low. Further experiment is needed to assess the NUE value.

Strawberry is an important horticultural product of Bandung and Bandung Barat Regency. Nowadays, the strawberry productivity is not as high as years before due to fertilization and seedling problem. The results of this greenhouse trial are the first information concerning the response of strawberry seedlings to reduced dose of chemical fertilizer application in Indonesia. However, next experiment is needed to verify the long-term effect of MCU doses and application method on NUE and plant growth during strawberry transplant production.

5. Conclusion

Urea fertilizer coated with solid biofertilizer composed of composted manure, 5% zeolite and 5% liquid bacterial inoculant increased root volume, root dry weight, root to shoot ratio and shoot N content significantly, but only full dose of that MCU formulation increased shoot N uptake and SPAD value. Compared to the control, MCU at any dose and formulation did not affect crown diameter, root length, plant height and shoot dry weight at 4 weeks after planting. The effect of urea coated with solid inoculant of Azotobacter and Bacillus was mostly increased root parameters compared to the shoots. However, MCU application resulted in > 20 mm of crown diameter which ensures the growth of transplant in strawberry production. Utilizing half dose of urea fertilizer coated with composted manure with 5% zeolite and 5% liquid inoculant is considered resulted in the increment of certain growth parameter of strawberry seeding until 4 weeks after treatment compared to a dose of conventional. This result indicated that utilizing microbial coated urea might lower the doses of urea applications up to 50%.

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